

Fabrication and Characterization of Copper Nanoparticles by Green Synthesis Approach Using *Plectranthus amboinicus* Leaves Extract

Srividya P¹, Sanjana J^{1*},
Sudestna C¹, Prathyusha S¹,
Darshani J¹, Kaumudi G¹,
Astha B¹, PandimaDevi M²

Abstract

The field of nanotechnology is gaining interest among the researchers towards the eco-friendly way of synthesis of nanoparticles. In this project, green synthesis technique was employed to induce the synthesis of copper nanoparticles using *Plectranthus amboinicus*, i.e., Mexican mint, identified as *Coleus amboinicus* leaf extract. We report an eco-friendly synthesis of copper nanoparticle using *Plectranthus amboinicus* leaf extract, which is a simple and an ostentatiously rapid method which produces stable nanoparticles. The copper sulphate solution was naturally employed as a precursor for synthesizing the copper nanoparticles. The extract of the plant *Plectranthus amboinicus* was found to showcase excellent reducing and stabilizing properties. By using Ultraviolet-Visible spectroscopy, Zeta Potential, and X-Ray Diffraction (XRD) studies, it was confirmed that copper nanoparticles have been synthesized. The UV-Spectrometer analysis shows the characteristic peak indicating the synthesis of copper nanoparticles. The pattern of XRD analysis showed particle size of 16 - 25 nm and it reveals high crystallinity of the copper nanoparticles. Zeta potential was done to find the charge of the nanoparticles and size distribution which showed to have significant stability. This method proves to be cost-effective, can be performed at ease, and it's also free of pollutants.

Keywords: Green synthesis; *Plectranthus amboinicus*; Copper nanoparticles; Leaf extract; Ultraviolet-Visible spectroscopy; X-Ray diffraction; Zeta potential.

Department of Biotechnology, School of Bioengineering, SRM Institute of Science and Technology, Kattankulathur, Tamil Nadu, India

*Corresponding author:

Sanjana Jayacumar

✉ sanjanajayacumar@gmail.com

Sanjana Jayacumar, Department of Biotechnology, School of Bioengineering, SRM Institute of Science and Technology, Kattankulathur-603203, Tamil Nadu, India.

Citation: Srividya P, Sanjana J, Sudestna C, Prathyusha S, Darshani J, et al. (2021) Fabrication and Characterization of Copper Nanoparticles by Green Synthesis Approach Using *Plectranthus amboinicus* Leaves Extract. Nano Res Appl Vol.7 No.4:16

Received: December 11, 2020; **Accepted:** April 19, 2021; **Published:** April 23, 2021

Introduction

Copper is a cost-effective material and has gathered attention due to its extensive applications in nanoparticle synthesis for novel catalytic antimicrobial and other optical properties [1]. To gain the complete benefits of copper nanoparticles, an eco-friendly and economically suitable, synthesis is desired. Copper is less toxic and; it is an important element in human health [2]. Copper is a less-expensive metal than other metals which are often utilized as Nano-sized particles for increasing antibiotic effectiveness [3]. The ratio between area and volume is high for copper nanoparticles. The antibacterial activity, catalytic potency, magnetic and optical characteristics are higher for copper when compared to other metals like gold and silver [4]. The important applications of copper nanoparticles are to act as an antibacterial compound [5]. The application of copper nanoparticles towards health-related processes is important because of its antifungal

and antibacterial activities [6]. The applications of copper nanoparticles are wide, as it is used in many fields like cosmetics for nano pigment purpose and in pharmaceutical industries for drug-eluting [7]. In food packages, to reinforce the antibacterial system, the laser-generated copper nanoparticles were used [8]. By the reduction process using CuSO_4 as the precursor, the copper nanoparticles are synthesized [9]. For synthesizing copper nanoparticles there are three methods, namely chemical, physical, and biological methods [10].

The biological method using extracts of the plant has been put forward for consideration as a possible environmental-friendly method than physical and chemical methods [11]. The advantage of plants concentrate on their biosynthetic reaction is one of the important factors in the biosynthesis of nanoparticles [12]. Synthesis of copper nanoparticles in solutions showed the absorption peak at 574 nm [13]. Copper nanoparticles synthesized by the electro-reduction process showed XRD patterns peaks very

sharply due to high nano-crystalline property of the metal where 2θ values are 43.4° , 50.5° and 74.2° [14]. Zeta potential was used to evaluate the morphological properties of nanoparticles. A study revealed that the zeta potential for copper nanoparticle at a concentration of 20 mg/L was -9.25 mV [15].

Materials and Methods

Materials required

Plectranthus amboinicus leaves, analytical grade copper sulphate, double distilled water, sterile beaker, pipette, tips, filter cloth, magnetic stirrer, hot water bath.

Preparation of *Plectranthus amboinicus* leaf extract

Leaves of *Plectranthus amboinicus* were washed and cleaned thoroughly with sterile water, finely cut and soaked in 100 ml of distilled water, and boiled on a hot plate. The leaves were strained using a filter cloth and the leaf extract was cooled at room temperature and was stored in the deep freezer.

Synthesis of Copper nanoparticles using *Plectranthus amboinicus* leaf extract

The leaf extract of *Plectranthus amboinicus* has inherent reducing properties, thereby aiding in the reduction of aqueous copper sulphate into copper nanoparticles. The aqueous CuSO_4 solution with concentration 1mM was prepared and the leaf extract of *Plectranthus amboinicus* that was prepared and was mixed in 1:5 ratios (v/v), i.e., 2ml of the plant extract was added to 10 ml of copper sulphate solution, dropwise on a magnetic stirrer. A positive control solution as shown in the figure using distilled water and plant extract was prepared and a negative control solution as shown in the figure that contained only copper sulphate solution was also prepared (**Figures 1a and 1b**). The solutions were incubated overnight in the darkness at room temperature and monitored constantly. After 24 hours, the change in colour of the solution from light green to dark green was observed. By centrifuging the solution at 12000 rpm for 10 – 15 min, the copper nanoparticle was obtained. The pellet was stored at 4°C for the characterization and analysis [16].

Characterization of copper nanoparticles

The important factor of metal nanoparticles is that their optical properties change due to the morphological characteristics of the nanoparticles [17]. The change in color from light green to dark green denoted the copper nanoparticles formation as shown in the figures respectively (**Figures 2a and 2b**). UV-Vis spectral analysis was done by using Shimadzu UV-Visible spectrophotometer with a resolution operating at 1 nm used and the UV spectrum was recorded. The powder X-Ray Diffraction analysis was done using Malvern PAN analytical instrument with 15 mA, 40 kV using $\text{Cu} - \text{K}\alpha$ radiation in $\theta - 2\theta$ configurations. Dynamic Light Scattering analysis was deployed to decode the mean grain size of the nanoparticles by zeta potential analysis (**Table 1**).

Results and Discussion

Ultraviolet Visible Spectrophotometer

The copper nanoparticles formation is usually confirmed by UV-Vis spectroscopy studies of colored solution [18]. The concept of obtaining the peak in the UV-Vis spectrum is that, on the surface of metal nanoparticles the free electrons will be present which undergoes strong repulsion with the light of specific wavelength [19]. Higher the conversion of copper sulphate solution to copper nanoparticles, higher will be the absorbance which corresponds to higher concentration of copper nanoparticles [20]. The spectroscopy studies of the solution were recorded after 24

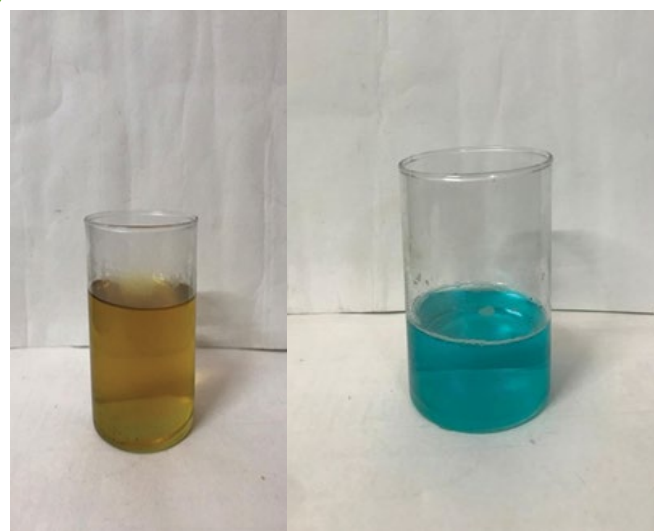


Figure 1 A positive control solution as shown in (a) using distilled water and plant extract was prepared and a negative control solution as shown in (b) that contained only copper sulphates.

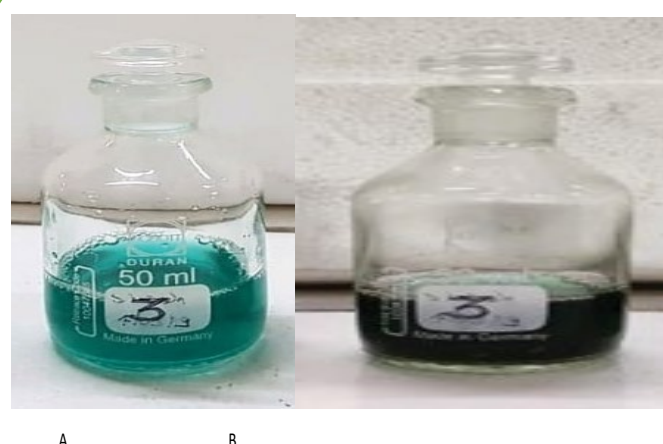


Figure 2 The change in color from light green to dark green denoted the copper nanoparticles formation as shown in the (a) and (b) respectively.

hours. The peak of absorbance from the absorption spectra was between the ranges of 230 nm - 270 nm as shown in figure pinpointing copper nanoparticles formation (Figure 3).

X-Ray Diffraction (XRD)

X-Ray Diffraction is an analytical method for determining the size and crystalline structure of the nanoparticles [21]. The high degree of crystallinity of nanoparticles is reflected by the intensity and sharpness of peaks [22]. There is an undeniable role played by the secondary metabolites in the plant extract even if the clear mechanism is unknown [23]. The average size of the copper nanoparticles was obtained using Scherrer's Formula:

$$D_p = \frac{K\lambda}{B\cos\theta}$$

Where D_p depicts the average grain size, K is Scherrer's constant which is 1.54060, λ denotes the X-Ray diffraction wavelength which has a value of 1.54178 nm, B is FWHM i.e., Full Width at Half Maximum derived from the peak of XRD. The observed XRD peak broadening is said to come from the crystalline size. θ is the XRD peak position i.e., the Bragg's angle obtained from the half of 2θ , where 2θ is in the range of 30 – 80° [24].

The average grain size of the copper nanoparticles was estimated as 21.47 nm. From the XRD curves, the intense and sharp peaks of the copper nanoparticles were observed at 34.96°, 41.50°, 58.37°, 69.34°, and 75.57°. It has the plane values as (111), (200), (220), (311), and (222) respectively. Therefore, all the diffraction peaks can be defined and indexed using face-centered cubic cell. The average lattice parameter value is determined to be 4.4195 Å. The XRD pattern proves the crystalline nature of the copper nanoparticles as shown (Figure 4). The obtained results illustrate that CuSO_4 has indeed been reduced to Copper nanoparticles

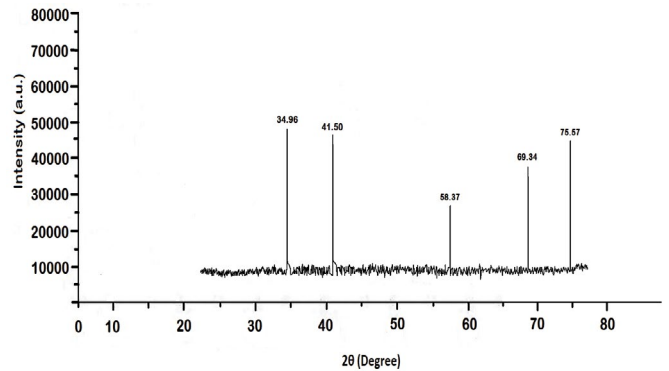


Figure 4 XRD analyses of the Copper nanoparticles.

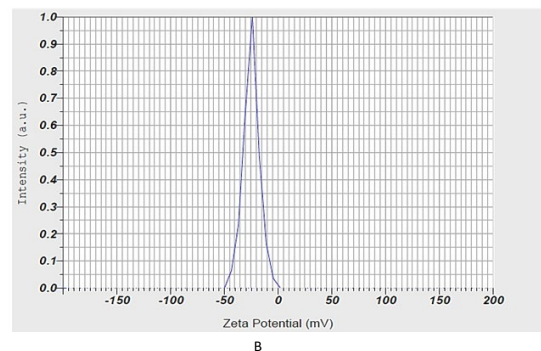
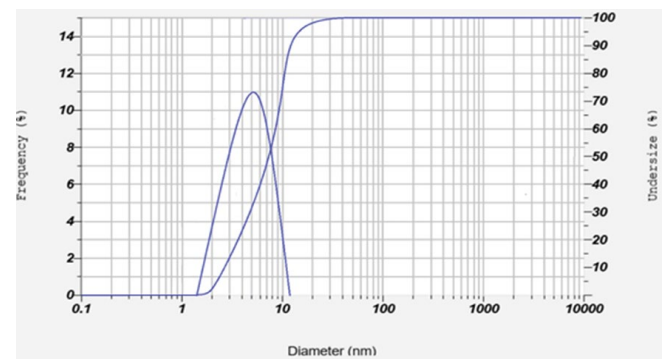


Figure 5 (a) - DLS graph for Zeta size of the Copper nanoparticles using *Plectranthus amboinicus* extract (b) - DLS graph for Charge of the copper nanoparticles using *Plectranthus amboinicus* extract.

by *Plectranthus amboinicus* plant extract under the appropriate reaction medium and conditions.

Zeta Potential

The Z-average was estimated as 5.3 nm as shown in the figure using DLS (Figure 5a). The charge obtained using zeta analysis was -25.2 mV as shown (Figure 5b). Particles that have charges of or above -25 and +25 tend to be more stable according to the previous research documents [25]. Particles that have negative charges tend to have a strong force of repulsion between them. This indicates the properties of stability and quality [26].

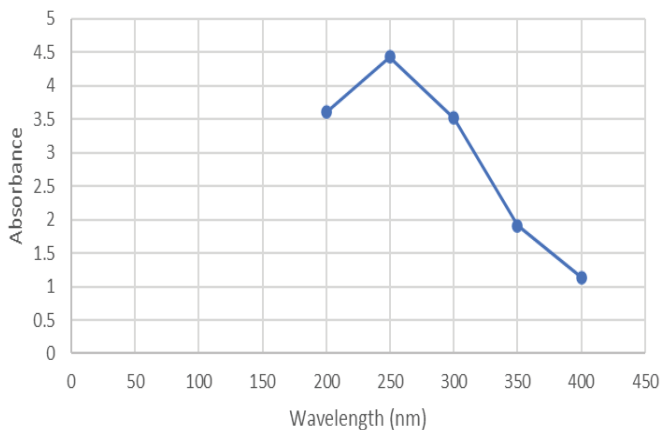


Figure 3 UV spectrophotometer results for Copper nanoparticles.

Table 1 XRD data calculation.

Peak (2θ)	θ	Sin ² θ	sin ² θ/ sin ² θ _{min}	3* sin ² θ/sinθ _{min}	h ² +k ² +l ²	hkl	a(Å)	FWHM (°2θ)	d-spacing (Å)	D _p (nm)
34.96	17.48	0.0902	1	3	3	111	4.4407	0.5077	16.5184	17.15
41.50	20.75	0.1255	1.3914	4.1742	4	200	4.3471	0.5077	7.7959	17.50
58.37	29.185	0.2377	2.6364	7.9092	8	220	4.4671	0.3385	5.9188	28.11
69.34	34.67	0.3236	3.5876	10.7628	11	311	4.4893	0.4231	2.1759	23.87
75.57	37.785	0.3754	4.1619	12.4857	12	222	4.3535	0.5077	1.6591	20.70
							Average a: 4.4195 Å			Average D _p : 21.47 nm

Conclusion

The environment friendly methods for the fabrication of copper nanoparticles can be developed by applying knowledge from the areas of nanotechnology and incorporated into use with the plant extract of *Plectranthus amboinicus*. The copper nanoparticles were produced by the plant extract which functions both as a reducer and a stabilizer. Thereby, the copper sulphate solution was reduced into copper ions without employing harsh man-made chemicals. From this study, it is evident that the reaction is completely a green chemical process favorable for nanoparticle synthesis. We used UV-Visible spectroscopy, Zeta potential analysis, and XRD analysis techniques for characterization of formed copper nanoparticles. UV absorbance peaks were observed between 230 nm-270 nm, therefore confirming the presence of Copper nanoparticles. The copper nanoparticles were analyzed using XRD and the average grain size was calculated to be 21.47 nm. DLS assessment showed the average diameter i.e., Z-average of the Copper nanoparticles to be 5.3 nm and the

surface charge of -25.2 mV indicating its high stability. The use of *Plectranthus amboinicus* is beneficial for the production of copper nanoparticles because it is readily available, less expensive, has a simple method that makes it easy to perform, acts as reducing and stabilizing agents, contributes in producing nanoparticles that are stable and crystalline in structure. Thus, proves to be one of the best plants for the production of the copper nanoparticle by the eco-friendly green synthesis method.

Competing Interest

The authors declare that they have no competing interests.

Acknowledgement

The authors thank Dr. M. Pandima Devi, Bioprocess and Enzyme Technology Laboratory, Associate Professor, Department of Biotechnology, School of Bio-Engineering, SRM Institute of Science and Technology, Kattankulathur, 603203, Tamil Nadu, India, for her support and encouragement regarding this work.

References

- Naga Padma P, Thanveer Banu S, Chaitanya Kumari S (2018) Studies on Green Synthesis of Copper Nanoparticles Using *Punica granatum*. *Annu Res Rev Biol* 23: 1–10.
- Thanh-Dong P, Byeong-Kyu L (2014) Cu doped TiO₂/GF for photocatalytic disinfection of *Escherichia coli* in bioaerosols under visible light irradiation: Application and mechanism. *Appl Surf Sci* 296: 15–23.
- Byeth N, Hour-Haddad Y, Domb A, Khan W, Hazan R (2015) Alternative anti-microbial approach: Nano-antimicrobial materials, evidence-based complement. *Altern Med*.
- Umer A, Naveed S, Ramzan N, Rafique MS, Imran M (2014) A green method for the synthesis of copper nanoparticles using l-ascorbic acid. *Rev Mater* 19: 197–203.
- Khare P, Sharma A, Verma N (2014) Synthesis of phenolic precursor-based porous carbon beads in situ dispersed with copper-silver bimetal nanoparticles for antibacterial applications. *J. Colloid Interface Sci*. 418: 216–224.
- Argueta-Figueroa L, Morales-Luckie RA, Scougall-Vilchis RJ, Olea-Mejia OF (2014) Synthesis, characterization and antibacterial activity of copper, nickel and bimetallic Cu-Ni nanoparticles for potential use in dental materials. *Prog. Nat. Sci. Mater. Int.* 24: 321-328.
- Schröfel A, Kratošová G, Šafařík I, Šafaříková M (2014) Applications of biosynthesized metallic nanoparticles. A review. *Acta Biomater* 10: 4023–4042.
- Longano D, Ditaranto N, Cioffi N, Di-Niso F (2012) Analytical characterization of laser-generated copper nanoparticles for antibacterial composite food packaging. *Anal Bioanal Chem* 403: 1179–1186.
- Khan A, Rashid A, Younas R, Chong R (2016) A chemical reduction approach to the synthesis of copper nanoparticles. *Int Nano Lett* 6: 21–26.
- Tamilvanan A, Balamurugan K, Ponappa K, Madhan Kumar B (2014) Copper nanoparticles: Synthetic strategies, properties and multifunctional application. *Int J Nanosci* p. 13.
- Hyo-Jeoung L, Jae Yong S, Beom Soo K (2013) Biological synthesis of copper nanoparticles using *Magnolia kobus* leaf extract and their antibacterial activity. *J Chem Technol Biotechnol* 88: 1971–1977.
- Santhosh Kumar J, Agarwal H, Menon S, Rajesh Kumar S (2019) A biological synthesis of copper nanoparticles and its potential applications Green Synthesis, Characterization and Applications of Nanoparticles 199-221.
- Szu-Han W, Dong-Hwang C (2004) Synthesis of high-concentration Cu nanoparticles in aqueous CTAB solutions. *J Colloid Interface Sci* 273: 165–169.

- 14 Raja M, Shuba J, Binti Ali F, Ryu SH (2008) Synthesis of copper nanoparticles by electroreduction process. *Mater Manuf Process* 23: 782–785.
- 15 Sadowski Z, Maliszewska IH, Grochowalska B, Polowczyk I, Koźlecki T (2008) Synthesis of silver nanoparticles using microorganisms. *Mater Sci Pol* 26: 419–424.
- 16 Sarkar J, Chakraborty N, Chatterjee A, Bhattacharjee A, Dasgupta D, et al. (2020) Green synthesized copper oxide nanoparticles ameliorate defence and antioxidant enzymes in *Lens culinaris*. *Nanomaterials* 10: 1–27.
- 17 Behravan M, Panahi AH, Naghizadeh A, Ziaee M, Mahdavi R, et al. (2019) Facile green synthesis of silver nanoparticles using *Berberis vulgaris* leaf and root aqueous extract and its antibacterial activity. *Int J Biol Macromol* 124: 148–154.
- 18 Sravanthi M, Muni Kumar D, Usha B, Ravichandra M, Mahendra Rao M, et al. (2016) Biological Synthesis and Characterization of Copper Oxide Nanoparticles Using *Antigonon leptopus* Leaf Extract and Their Antibacterial Activity. *Int J Adv Res* 4: 589–602.
- 19 Ashtaputrey SD, Ashtaputrey PD, Rathod G (2017) Eco-friendly green synthesis and characterization of silver nanoparticles derived from *Murraya koenigii* leaves extract. *Asian J Chem* 29: 1966–1968.
- 20 Mathur A, Kushwaha A, Dalakoti V, Dalakoti G, Singh DS (2014) Green synthesis of silver nanoparticles using medicinal plant and its characterization. *Der Pharm Sin* 5: 118–122.
- 21 Mervat-Zayed F, Wael-Eisa H, Shabaka AA (2012) *Malva parviflora* extract assisted green synthesis of silver nanoparticles. *Spectrochim Acta Part A Mol Biomol Spectrosc* 98: 423–428.
- 22 Irshad WA, Ganguly A, Ahmed J, Ahmad T (2011) Silver nanoparticles: Ultrasonic wave assisted synthesis, optical characterization and surface area studies. *Mater Lett* 65: 520–522.
- 23 Pirtarighat S, Ghannadnia M, Baghshahi S (2019) Green synthesis of silver nanoparticles using the plant extract of *Salvia spinosa* grown *in vitro* and their antibacterial activity assessment. *J Nanostructure Chem* 9: 1–9.
- 24 Sierra-Ávila R, Pérez-Alvarez M, Cadenas-Pliego G, Padilla VC, Ávila-Orta C, et al. (2015) Synthesis of copper nanoparticles using mixture of allylamine and polyallylamine. *J Nanomater.*
- 25 Heydari R, Rashidipour M (2015) Green synthesis of silver nanoparticles using extract of oak Fruit hull (Jaft): Synthesis and *in vitro* cytotoxic effect on MCF-7 cells. *Int J Breast Cancer*.
- 26 Mali SC, Raj S, Trivedi R (2019) Biosynthesis of copper oxide nanoparticles using *Encostemma axillare* (Lam.) leaf extract. *Biochem Biophys Reports* 20: 100699.