

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

Srividya Parthasarathy

Department of Biotechnology, School of Bio-Engineering, SRM Institute of Science and Technology, Kattankulathur, 603203, Tamil Nadu, India.

Sanjana Jayacumar (✉ sanjanajayacumar@gmail.com)

Department of Biotechnology, School of Bio-Engineering, SRM Institute of Science and Technology, Kattankulathur, 603203, Tamil Nadu, India.

Sudestna Chakraborty

Department of Biotechnology, School of Bio-Engineering, SRM Institute of Science and Technology, Kattankulathur, 603203, Tamil Nadu, India.

Prathyusha Soundararajan

Department of Biotechnology, School of Bio-Engineering, SRM Institute of Science and Technology, Kattankulathur, 603 203, Tamil Nadu, India.

Darshani Joshi

Department of Biotechnology, School of Bio-Engineering, SRM Institute of Science and Technology, Kattankulathur, 603203, Tamil Nadu, India.

Kaumudi Gangwar

Department of Biotechnology, School of Bio-Engineering, SRM Institute of Science and Technology, Kattankulathur, 603203, Tamil Nadu, India.

Astha Bhattacharjee

Department of Biotechnology, School of Bio-Engineering, SRM Institute of Science and Technology, Kattankulathur, 603203, Tamil Nadu, India.

M.Pandima Devi Venkatesh

Department of Biotechnology, School of Bio-Engineering, SRM Institute of Science and Technology, Kattankulathur, 603203, Tamil Nadu, India.

Research

Keywords: Green Synthesis, *Plectranthus amboinicus*, Copper nanoparticles, Leaf extract, Ultraviolet-Visible spectroscopy, X-Ray Diffraction, Zeta potential.

Posted Date: August 17th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-55984/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

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.

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:

3.1 Materials required:

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

3.2 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.

3.3 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 CuSO₄ solution with concentration 1mM was prepared and the leaf extract of *Plectranthus amboinicus* that was prepared and was mixed in 1:5 ratio (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 Fig 1(a) using distilled water and plant extract was prepared and a negative control solution as shown in the Fig 1(b) that contained only copper sulphate solution was also prepared. 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 Fig 2(a) and 2(b) respectively. 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 PANalytical instrument with 15 mA, 40 kV using Cu - K α 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 using HORIBA.

Results And Discussion:

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

5.2 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].

Table 1
XRD DATA CALCULATION:

Peak (2 θ)	θ	$\text{Sin}^2\theta$	$\frac{\text{sin}^2\theta}{\text{sin}^2\theta_{\text{min}}}$	3^* $\frac{\text{sin}^2\theta}{\text{sin}^2\theta_{\text{min}}}$	$h^2+k^2+l^2$	hkl	a(Å)	FWHM (°2 θ)	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

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 in Fig 4. The obtained results illustrate that CuSO₄ has indeed been reduced to Copper nanoparticles by *Plectranthus amboinicus* plant extract under the appropriate reaction medium and conditions.

5.3 Zeta Potential:

The Z-average was estimated as 5.3 nm as shown in the Fig 5(a) using DLS. The charge obtained using zeta analysis was -25.2mV as shown in the Fig 5(b). 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].

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.

Declarations

Ethics approval & Consent to Participate:

Not Applicable

Consent for Publication:

Not Applicable

Availability of Data & Materials:

All data generated or analyzed during this study are included in this manuscript.

Competing Interest:

The authors declare that they have no competing interests.

Funding:

Not Applicable

Author's Contribution:

SP & PDM designed the research. SJ, PS & AB carried out synthesis of copper nanoparticles. SP, SJ, SC, DJ & KG carried out characterization of copper nanoparticles. SP & SJ analyzed the data and wrote the paper. All authors read & approved the final manuscript.

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.

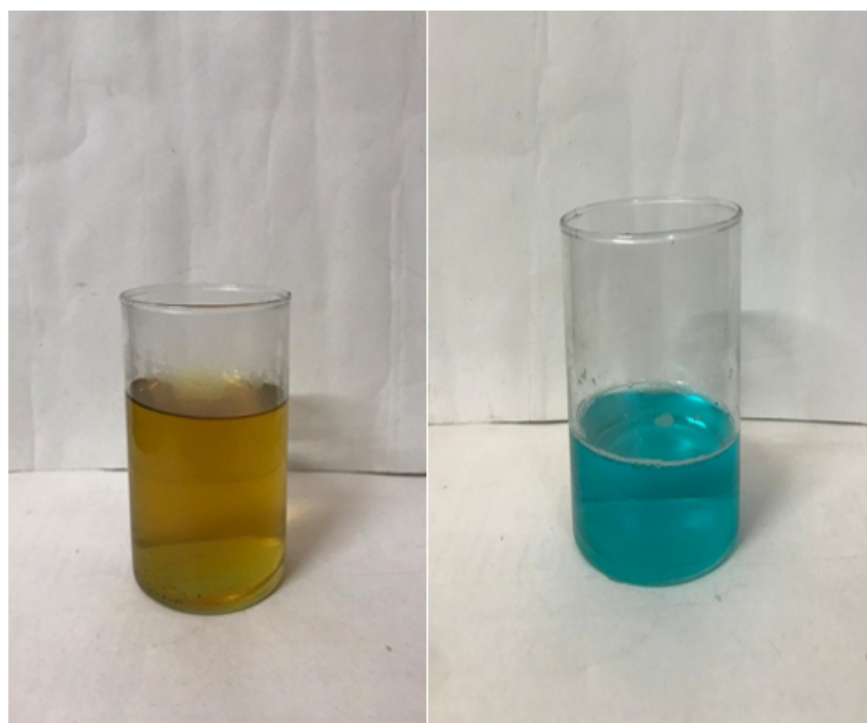
References

1. Naga Padma P, Banu ST, Chaitanya Kumari S (2018) Studies on Green Synthesis of Copper Nanoparticles Using Punica granatum. Annu Res Rev Biol 23:1–10. <https://doi.org/10.9734/ARRB/2018/38894>

2. Thanh- Dong Pham, Byeong- Kyu Lee (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. <https://doi.org/10.1016/j.apsusc.2014.01.006>
3. Nurit Byeth Y, Hourri-Haddad A, Domb W, Khan R, Hazan (2015) Alternative antimicrobial approach: Nano-antimicrobial materials, Evidence-Based Complement. *Altern Med*. <https://doi.org/10.1155/2015/246012>
4. Asim Umer S, Naveed N, Ramzan MS, Rafique, Muhammad Imran (2014) A green method for the synthesis of copper nanoparticles using l-ascorbic acid. *Rev Mater* 19(3):197–203. <https://doi.org/10.1590/S1517-70762014000300002>
5. Prateek Khare A, Sharma N Verma (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. <https://doi.org/10.1016/j.jcis.2013.12.026>
6. Morales-Luckie L, Argueta-Figueroa, R, Rogelio Scougall- Vilchis A, Oscar J, Olea- Mejia F (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. <https://doi.org/10.1016/j.pnsc.2014.07.002>
7. Adam Schröfel G, Kratošová I, Šafařík M, Šafaříková I, Raška, Leslie Shor M (2014) Applications of biosynthesized metallic nanoparticles. A review. *Acta Biomater* 10:4023–4042. <https://doi.org/10.1016/j.actbio.2014.05.022>
8. Longano D, Ditaranto N, Cioffi N, Di Niso F, Sibillano T, Ancona A, Conte A, Del Nobile MA, Sabbatini L, Torsi L (2012) Analytical characterization of laser-generated copper nanoparticles for antibacterial composite food packaging. *Anal Bioanal Chem* 403:1179–1186. <https://doi.org/10.1007/s00216-011-5689-5>
9. Ayesha Khan A, Rashid R, Younas R, Chong (2016) A chemical reduction approach to the synthesis of copper nanoparticles. *Int Nano Lett* 6:21–26. <https://doi.org/10.1007/s40089-015-0163-6>
10. Tamilvanan A, Balamurugan K, Ponappa K, Madhan Kumar B (2014) Copper nanoparticles: Synthetic strategies, properties and multifunctional application. *Int J Nanosci* 13. <https://doi.org/10.1142/S0219581X14300016>
11. Lee H-J, Song jaeyong, Beom Soo Kim (2013) Biological synthesis of copper nanoparticles using Magnolia kobus leaf extract and their antibacterial activity. *J Chem Technol Biotechnol* 88:1971–1977. <https://doi.org/10.1002/jctb.4052>
12. [10.1016/B978-0-08-102579-6.00009-5](https://doi.org/10.1016/B978-0-08-102579-6.00009-5)
Santhoshkumar J, Happy Agarwal S, Menon, Rajeshkumar S, Venkat Kumar S (2019) A biological synthesis of copper nanoparticles and its potential applications *Green Synthesis, Characterization and Applications of Nanoparticles* 199–221. <https://doi.org/10.1016/B978-0-08-102579-6.00009-5>
13. Wu S-H, Dong- Hwang Chen (2004) Synthesis of high-concentration Cu nanoparticles in aqueous CTAB solutions. *J Colloid Interface Sci* 273:165–169. <https://doi.org/10.1016/j.jcis.2004.01.071>
14. Mohan Raja, Shuba J, Ali FB, Sung Hun Ryu (2008) Synthesis of copper nanoparticles by electroreduction process. *Mater Manuf Process* 23:782–785. <https://doi.org/10.1080/10426910802382080>
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. Joy Sarkar N, Chakraborty A, Chatterjee A, Bhattacharjee D, Dasgupta K Acharya (2020) Green synthesized copper oxide nanoparticles ameliorate defence and antioxidant enzymes in *Lens culinaris*. *Nanomaterials* 10:1–27. <https://doi.org/10.3390/nano10020312>
17. Mahmoodreza Behravan AH, Panahi A, Naghizadeh M, Ziaee R, Mahdavi A Mirzapour (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. <https://doi.org/10.1016/j.ijbiomac.2018.11.101>
18. Sravanthi M, Muni Kumar D, Usha B, Ravichandra M, Mahendra Rao M, Hemalatha KPJ, Ravichandra M (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. DOI:10.21474/IJAR01/1251
19. Ashtaputrey SD, Ashtaputrey PD, Gunjan R (2017) Eco-friendly green synthesis and characterization of silver nanoparticles derived from *Murraya koenigii* leaves extract. *Asian J Chem* 29:1966–1968. <https://doi.org/10.14233/ajchem.2017.20680>

20. Abhishek Mathur A, Kushwaha V, Dalakoti G, Dalakoti DS, Singh (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. <https://doi.org/10.1016/j.saa.2012.08.072>
22. Irshad Wani A, Aparna Ganguly J, Ahmed T Ahmad (2011) Silver nanoparticles: Ultrasonic wave assisted synthesis, optical characterization and surface area studies. *Mater Lett* 65:520–522. <https://doi.org/10.1016/j.matlet.2010.11.003>
23. Saba Pirtarighat M, Ghannadnia S Baghshahi (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. <https://doi.org/10.1007/s40097-018-0291-4>
24. Ruben Sierra-Ávila, Marissa Pérez-Alvarez, Gregorio Cadenas-Pliego, Víctor Comparán Padilla, Carlos Ávila-Orta, Odilia Pérez Camacho, Enrique Jiménez-Regalado, Ernesto Hernández-Hernández, Rosa Martha Jiménez-Barrera(2015) Synthesis of Copper Nanoparticles Using Mixture of Allylamine and Polyallylamine. *J. Nanomater.* <https://doi.org/10.1155/2015/367341>
25. Rouhollah Heydari M, Rashidipour (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.* <https://doi.org/10.1155/2015/846743>
26. Mali SC, Raj S, Rohini Trivedi (2019) Biosynthesis of copper oxide nanoparticles using *Enicostemma axillare* (Lam.) leaf extract. *Biochem Biophys Reports* 20:100699

Figures



1(a)

1(b)

Figure 1

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



2(a)

2(b)

Figure 2

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

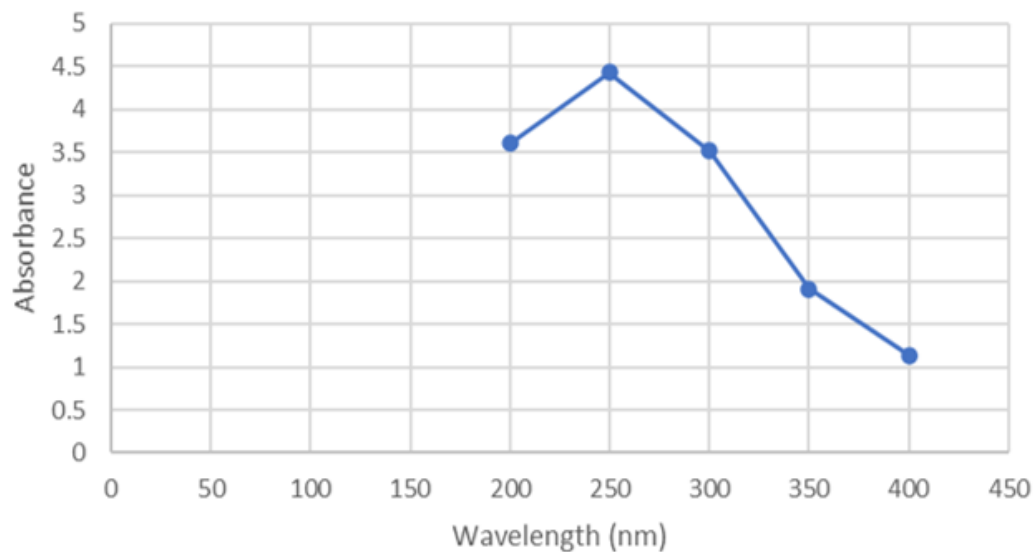


Figure 3

UV spectrophotometer results for Copper Nanoparticles.

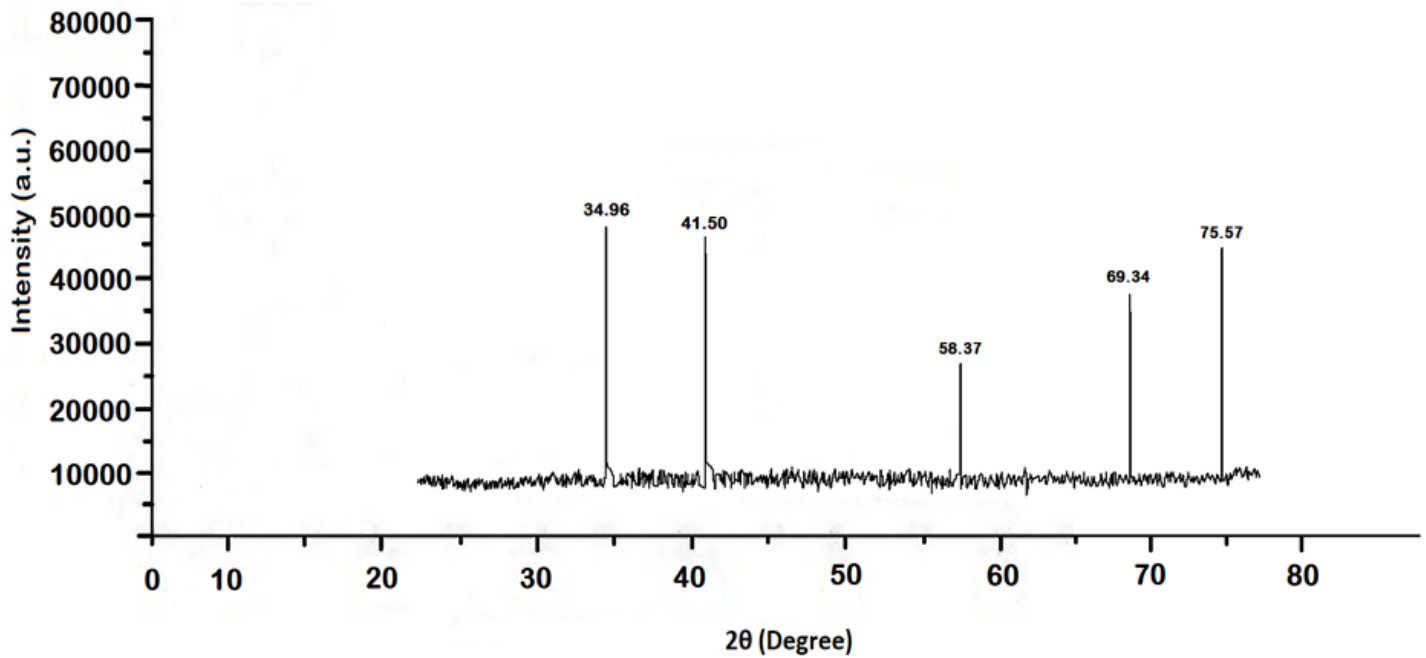


Fig 4: XRD Analysis of the Copper Nanoparticles

Figure 4

XRD Analysis of the Copper Nanoparticles

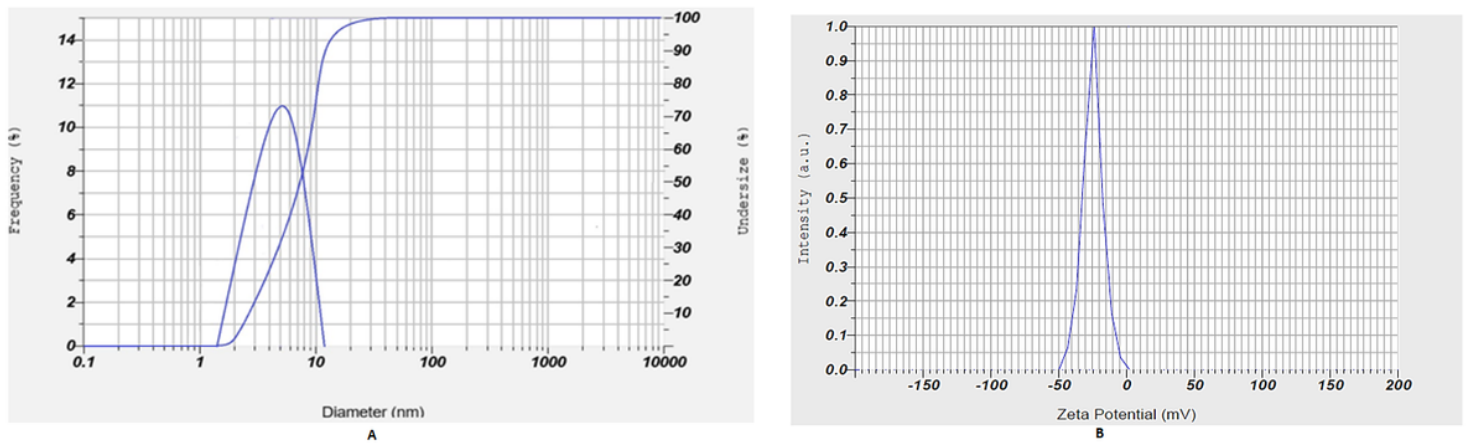


Figure 5

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.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [GRAPHICALABSTRACT.docx](#)