IJPSR (2021), Volume 12, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 13 January 2020; received in revised form, 06 May 2020; accepted, 10 May 2020; published 01 January 2021

GREEN SYNTHESIS AND CHARACTERIZATION OF COPPER NANOPARTICLES SYNTHESIZED FROM *LAWSONIA INERMIS* LEAF EXTRACT

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Keywords:

Biosynthesis, Copper nanoparticles, UV-Vis spectroscopy, SEM, TEM, FTIR, XRD

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ABSTRACT: Plant diseases caused by microbial pathogen leads to severe economic loss. Attempt to synthesize nanoparticles for this purpose is an economical and effective means to control these pathogens. For this purpose, nanoparticles synthesized *via* biological means are not only a safer choice but also a big step towards conservation of the integrity of environmental elements. In the present investigation, a successful attempt has been made to synthesize and characterize copper nanoparticles biologically synthesized from *Lawsonia inermis* leaf extract by an eco-friendly method. Formation of copper nanoparticles is confirmed by UV–Vis absorption spectroscopy, shape, and size studies were carried out Scanning electron microscopy (SEM), Transmission electron microscopy (TEM) analysis. TEM analysis revealed that copper nanoparticles were 2-5 nm in size and X-ray Diffraction studies confirmed the crystal structure of the nanoparticles. Functional groups and the chemical composition of associated molecules were studied by FTIR analysis. Further research involves the implementation of this research to control some plant diseases caused by pathogens that have developed resistance against present available bio-control agents.

INTRODUCTION: Nanoparticles are particles between 1 and 100 nanometers in size. Nano-particle research is currently an area of intense scientific interest due to a wide variety of potential applications in biomedical, optical, electronic, and agricultural fields. In recent years nanoparticles and nanomaterials have been used for a range of purposes, which includes targeted drug delivery, imaging, diagnosis, cosmetics, and biosensors [1].

QUICK RESPONSE CODE	DOI: 10.13040/IJPSR.0975-8232.12(1).477-81
	The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(1).477-81

Owing to their special biophysical properties, researchers are trying to employ nano-particles find use in fields as diverse as looking at the recent research trends it can be concluded that metal nanoparticles are gaining much importance in biological fields due to their Physiochemical properties [2, 3].

A number of approaches are available for the synthesis of metal nano-particles, which include physical, chemical, and biological. Among these methods, biological synthesis is a seemingly convenient way to fabricate benign nanostructure materials, also to reduce the use of or generation of hazardous substances to human health and the environment. Biosynthesis of metal nanoparticles using microorganisms like bacteria, fungi, and yeast has been extensively reported [4, 5, 6]. Investigation of the plant systems as the potential nanofactoriesis the emerging and seemingly promising field in the biological synthesis of nanoparticles. Current research findings reveal that metal nanoparticles synthesized from plants are more reliable, safe, and eco-friendly when compared with those synthesized using physical and chemical methods. The plant-mediated nanoparticles synthesis is a cost-effective method and, therefore, a forthcoming commercial alternative for large-scale production [7, 8].

There are plenty of reports on the biosynthesis of nanoparticles of silver, gold, and other metals, but not much work has been done on the synthesis of copper nanoparticles. Also, it has been found difficult to synthesize copper nanoparticles because of the strong tendency of copper to form oxides resulting in formation of copper oxide nano-particles. This investigation reports the green synthesis of copper nanoparticles using *Lawsonia inermis* leaf extract. Leaf extract of *Lawsonia inermis* not only reduces ionic copper to metallic nano-particulate form but also stabilizes them by preventing their oxidation.

MATERIALS AND METHODS: All chemical reagents were used without any purification. Copper Sulphate (CuSO4) was purchased From Hi-Media (USA), and Milli-Q water was used in all experiments.

Preparation of Plant Extract: Lawsonia leaves were collected from college campus. The collected leaves (25 g) were washed thoroughly with Milli-Q water to remove dirt and other impurities. Care was taken that no impurities in any form remain that may hinder the further process. Cleaned leaves were chopped finely and were kept in 250 ml Erlenmeyer flask containing 100 ml of deionized water for 24 h. Due to the extraction of plant metabolites the color of the solution changes to orange. This solution is then filtered using Whatman filter paper No. 1 (pore size 11 µm) and filtered again through vacuum filtration using cellulose nitrate membrane filters (0.45 µm pore size) to remove all suspended impurities (organic and inorganic). The filtrate was collected in a 200 ml Erlenmeyer flask, sealed, and stored at 4 °C for further use. This filtrate was typically used within 24 h.

Synthesis of Nanoparticles: For green synthesis, 100 ml of Lawsonia filtrate was boiled until only half the volume remained (50 ml). This hot Lawsonia extract solution was added slowly to 450ml of 1mM copper sulphate solution. 1N NaOH was added dropwise to adjust the pH to 11 with continuous stirring. The color of the solution begins to change rapidly and eventually turns opaque, indicating the formation of copper nanoparticles. The progress of the reaction was monitored by studying the UV- Vis absorption peak in the wavelength range of 200- 700 nm (using ELICO SL- 159 UV-Visible spectrophotometer) by periodic sampling of aliquots (2 ml) of the reaction mixture and measuring the UV- Vis spectra of the same with deionized water as a reference.

Characterization of Nanoparticles: Copper nano-particle solution thus obtained was subjected to repeated centrifugation at 15,000 rpm for 20 min followed by redispersion of the pellet in deionized water to remove all impurities. This procedure was repeated thrice. The shiny precipitate was collected and dried in hot air oven for 24 h at 80 °C. The dried crystalline powder was subjected to characterization studies. The size and shape of nano-particle was determined by SEM and TEM analysis. TEM analysis was carried out on a TEM, Phillip Holland Technai 20 model operating at 200 kV.

The sample for TEM was prepared by putting one drop of the suspension onto standard carbon-coated copper grids and then drying under an IR lamp for 30 min. For crystal structure and particle size determination, XRD study was carried out using Rigaku ultima 4 power diffractor, operating at a voltage and current of 40 kv and 40 mA respectively in the 2θ range of 20° to 90°. The organic molecules and functional groups associated with the nanoparticle were identified by FTIR. FT-IR spectra of vacuum-dried CuNPs were recorded as KBr pellet on Perkin Elmer RX1 model in the range of 4000–400cm^{-1}.

Results:

UV-visible spectroscopy: Primary confirmation of nanoparticle synthesis is usually done by measuring the absorbance of the test sample in the UV-Visible region. The brick red color, characteristic of copper nanoparticles 7 is obtained within 15 min of mixing of copper sulphate with Lawsonia leaf extract. The UV-Vis spectroscopy shows the characteristic surface plasmon resonance (SPR) spectra peak at 578 nm, which can be attributed to the formation of copper nanoparticles [8, 9]. Peaks corresponding to copper oxides were absent in the spectra, indicating the formation of pure copper nanoparticles.

SEM and TEM Study: A representative TEM image **Fig. 2B** shows the particles observed are spherical with an average particle size of 2–5 nm. TEM image also reveals that the morphology of copper nanoparticles is nearly spherical in shape. The SEM and TEM images reveal that particles are well dispersed and crystalline in nature.

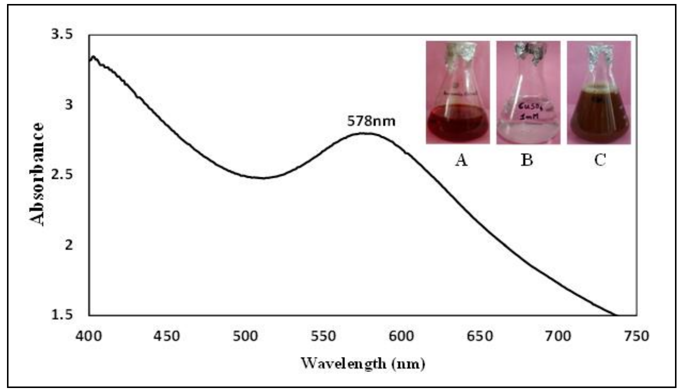

FIG. 1: UV-VIS ABSORPTION SPECTRA OF THE COPPER NANOPARTICLES A) LAWSONIA INERMIS LEAF EXTRACT B) 1 MM COPPER SULPHATE SOLUTION C) REACTION MIXTURE CONTAINING COPPER NANOPARTICLES

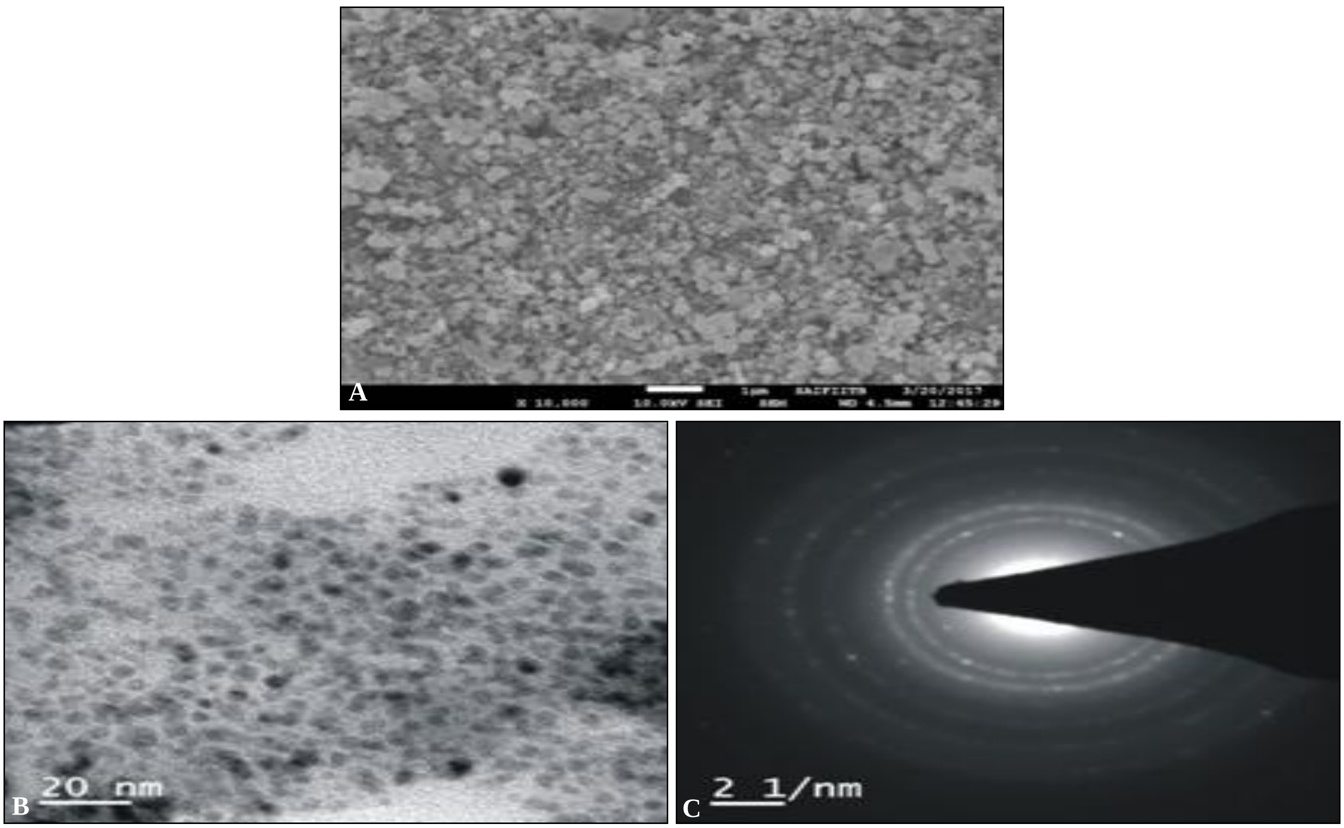

FIG. 2: A. SEM, B. TEM, C. SAED

XRD analysis: The XRD pattern as obtained is in agreement with earlier reports. Different peak positions were 43.6°, 50.7° and 74.45° of 2θ representing (1 1 1), (2 0 0) and (2 2 0) planes of copper, depicting Face Centered Cubic structure of copper nanoparticles (JCPDS copper: 04-0836) [10, 11, 12, 13]. The size of the crystallites estimated from Debye–Scherrer formula is about 6 nm.

FTIR Analysis: FTIR Study was carried out to determine the functional groups associated with the synthesized nanoparticles. Various peak positions show the presence of organic molecules attached to the copper nanoparticles.

It is hypothesized that these molecules play an active role in the reduction of ionic copper to its nanoparticle form. As seen in **Fig. 4**, the FTIR peaks at around 3270 and 3230 cm^{-1} correspond to O–H stretching vibrations, whereas 2922.15 cm^{-1} attributed to the C–H stretching vibration of flavonoids/ phenolic, respectively [13, 14].

The peaks at 1408 and 1390 cm^{-1} correspond to the O–H bend of polyphenols, indicating the presence of an aromatic group [15]. The absorption peaks at 1013 and 1044 cm^{-1} are due to C–O–C and secondary –OH of the phenolic group. From this data it can be clearly stated that organic molecules from *Lawsonia inermis* leaf extract aid in the formation and stability of synthesized nanoparticles.

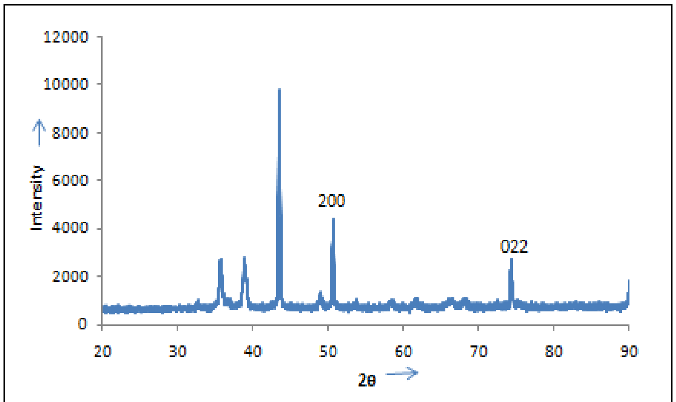

FIG. 3: XRD SPECTRUM

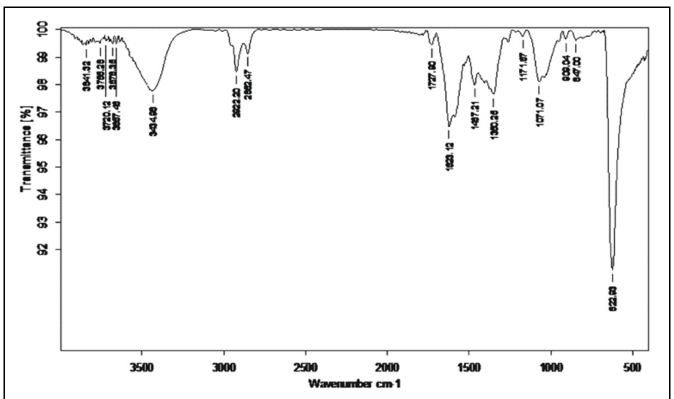

FIG. 4: FTIR SPECTRUM

CONCLUSION: In this report, a novel method for the fabrication of Cu NPs using non-toxic/biodegradable *Lawsonia inermis* leaf extract is presented. The formation of Cu-NPs was characterized by UV–Vis spectroscopy, SEM, TEM, XRD, and FTIR. It was found that the mean diameter of Cu-NPs, synthesized using Lawsonia Leaf extract was in size range of 2-5 nm. Maximum nanoparticles show particle size of 20 nm with distinct cap, which may be due to the flavonoids, proteins, and other functional groups present in the leaf broth of *Lawsonia inermis* and are likely to be responsible for the formation of copper nanoparticles. The explored biosynthetic route has potent applications in biomedical, biotechnological, and pharmaceutical applications with several advantages such as cost-effective, efficient, and environment friendly. This method is also suitable for large scale commercial production

ACKNOWLEDGEMENT: The authors are grateful to the CSIR, New Delhi, for financial assistance in carrying out the research work. IIT Bombay for carrying out SEM, TEM, and FTIR analysis. Professor N. Lakshmi, Department of physics, Mohanlal Sukhadia University for assisting in XRD analysis.

CONFLICTS OF INTEREST: On behalf of all listed authors, the corresponding author declares that there is not any financial and non-financial conflict of interests in the subject materials mentioned in this manuscript

REFERENCES:

1. Patra JK, Das G and Fraceto LF: Nano based drug delivery systems: recent developments and future prospects. J Nanobiotechnol 2018; 16: 71.
2. Khan I, Saeed K and Khan I: Nanoparticles: Properties, applications and toxicities. Arabian Journal of Chemistry. 2019; 12 (7): 908-31.
3. Seqqat R, Blaney L, Quesada D, Kumar B and Cumbal L: Nanoparticles for environment, engineering, and nano-medicine. Journal of nanotechnology. 2019;ArticleID 2850723 https://doi.org/10.1155/2019/2850723
4. Gour A and Jain NK: Advances in green synthesis of nanoparticles. An international Journal of Artificial cells, Nanomedicine and Biotechnology 2019; 47(1): 844-851.
5. Ahmad F, Ashraf N and Ashraf T: Biological synthesis of metallic nanoparticles (MNPs) by plants and microbes: their cellular uptake, biocompatibility, and biomedical applications. Appl Microbiol Biotechnol 2019; 103: 2913-35.
6. Sharma D, Kanchi S and Bisetty K: Biogenic synthesis of nanoparticles: A review. Arabian Journal of Chemistry 2019; 12 (8): 3576-00.
7. Pirtarighat S, Ghannadnia M and Baghshahi S: Green synthesis of silver nanoparticles using the plant extract of Salvia spinosa grown *in-vitro* and their antibacterial activity assessment. J of Nanostru in Chem 2019; 9: 1-9.
8. Nayantara and Kaur P: Biosynthesis of nanoparticles using eco-friendly factories and their role in plant pathogenicity: a review. Biotech Res and Innovation 2018; 2(1): 63-73.
9. Laghari GN, Nafady A, Al-Saeedi SI, Sirajuddin, Sherazi STH, Nisar J, Shah MR, Abro MI, Arain M, Bhargava SK: Nanomaterials (Basel) 2019; 9(1): 83.
10. Thiruvengadam M, Chung I and Gomathi T: Synthesis, characterization and pharmacological potential of green synthesized copper nanoparticles. Bioprocess Biosyst Eng 2019; 42: 1769-77.
11. Hasheminya S and Dehghannya J: Green synthesis and characterization of copper nanoparticles using *Eryngium caucasicum* Trautv aqueous extracts and its antioxidant and antimicrobial properties. An international journal of particulate science and technology 2019https://doi.org/10.1080/02726351.2019.1658664

12. Shah R, Pathan A, Aaghela H, Ameta S and Parmar K: Green synthesis and characterization of copper nanoparticles using mixture (*Zingiber officinale*, *Piper nigrum* and *Piper longum*) extract and its antimicrobial activity. Chemical Science Transactions 2019; 8(1): 63-69.
13. Sarkar J, Chakraborty N, Chatterjee A, Bhattacharjee A, Dasgupta D and Acharya K: Green synthesized copper oxide nanoparticles ameliorate defence and antioxidant enzymes in lens culinaris. Nanomaterials 2019; 10, 312.
14. Długosz, O, Chwastowski J and Banach M: Hawthorn berries extract for the green synthesis of copper and silver nanoparticles. Chem Pap 2020; 74: 239-52.
15. Buazar F, Sweidi S and Badri M: Biofabrication of highly pure copper oxide nanoparticles using wheat seed extract and their catalytic activity: A mechanistic approach. Green Processing and Synthesis 2019; 8(1): 691-02.

How to cite this article:

Rathore K, Hada D and Sharma K: Green synthesis and characterization of copper nanoparticles synthesized from *Lawsonia inermis* leaf extract. Int J Pharm Sci & Res 2021; 12(1): 477-81. doi: 10.13040/IJPSR.0975-8232.12(1).477-81.