Plant Archives Vol. 25, Supplement 2, 2025 pp. 2727-2731 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.supplement-2.347

CHARACTERIZATION AND CYTOTOXIC ACTIVITY OF GREEN SYNTHESIZED COPPER NANOPARTICLES

Anjali Sharma* and Gunmala Gugalia
Sangam University, Atoon, Bhilwara, Rajasthan 311001, India
*Corresponding author E-mail: anjali.22289@gmail.com
(Date of Receiving : 27-04-2025; Date of Acceptance : 07-07-2025)

ABSTRACT

Creating an environmentally friendly process to synthesize copper nanoparticles (CuNPs) is a critical field of nanotechnology research. A ground-breaking method for producing various nanoparticles has emerged in recent years. The production of copper nanoparticles (CuNPs) using an aqueous extract of *Nyctanthes arbor–tristis* leaves is described in this paper as a straight forward and economical process. The characterization of these CuNPs was tracked using XRD and SEM. The plant biomolecules promote the reduction of Cu^{2+} ions to CuNPs in addition to capping and stabilizing the CuNPs. This study describes cytotoxic activity of green synthesized copper nanoparticles. Cytotoxic activity was studied on THP1 cell line was determined by MTT assay. Both plant extract and copper nanoparticles of *Nyctanthes arbor-tristis* are not effective for cytotoxicity.
Keywords: Green synthesis, XRD, SEM, *Nyctanthes arbor-tristis*, plant biomolecules, characterization, Cytotoxic.

Introduction

The discovery of nanomaterials is regarded as one of the century's scientific revolutions. All nanomaterials, including nanoparticles, have garnered particular interest because of their numerous uses in antimicrobial, agricultural, medicinal, antifungal, anticancer, and cosmetic applications.

For the creation of nanoparticles, a number of techniques have been devised, including microwave, chemical reduction, thermal deposition, and so nonchemical reduction. The substances used in all of these techniques are harmful to the ecosystem. Consequently, eco-friendly green nanoparticle production has been the focus of research.

We present an environmentally acceptable, low-cost, and nontoxic technique for synthesizing copper nanoparticles using *Nyctanthes arbor-tristis*.

CuNPs have been synthesized using a variety of techniques, including physical, chemical, and biological methods. Extracts from a variety of plant parts, such as the peel of *Punica granatum* (Kaur *et al*., 2016), the stem of *Zingiber officinale* (Delma *et al*., 2016), the juice of *Citrus medica* (Idilimbu) (Shende *et al*., 2015), the wild fruit of *Ziziphus spina-christi* (L.) (Khani *et al*., 2018), the root and leaf of *Asparagus adscendens Roxb.* (Thakur *et al*., 2018), the leaves of *Eclipta prostrata* (Chung *et al*., 2017), the leaves of *Ginkgo biloba* (Nasrollahzadeh *et al*., 2015), *Plantago asiatica* (Nasrollahzadeh *et al*., 2017), *Thymus vulgaris* (Issaabadi *et al*., 2017), the black tea leaf (Asghar *et al*., 2018), the *Terminali acatappa* leaf (Muthulakshmi *et al*.,2017), and the *Azadirachta indica* leaf (Ahmed *et al*.,2019) have all been used successfully in the synthesis of CuNPs.

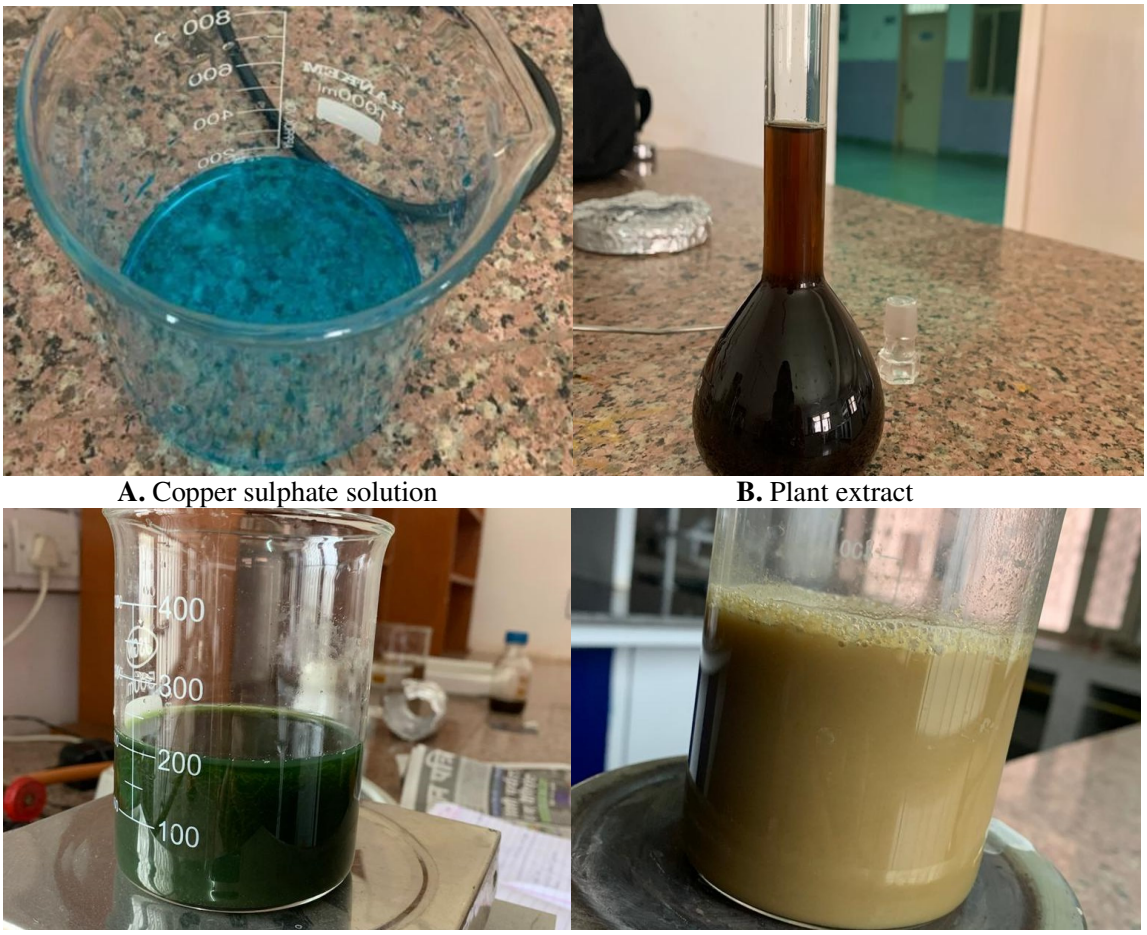
Although successful nanoparticle synthesis utilizing fungus, algae, and plants has been documented, plant-assisted synthesis is safer, more environmentally friendly, less expensive, simpler, and comparatively quicker than microbe-assisted synthesis.

Materials and Methods

Preparation of Plant extract

Nyctanthes arbor-tristis leaves were gathered from Bhilwara and identified by the MLV Govt. College Bhilwara, Rajasthan, botany department. First,

tap water was used to wash the leaves, and then distilled water. Following washing, the leaves were left to dry in the dark. Crushed dried leaves were used. The plant extract was made by boiling 100 g of crushed leaves in 700 ML of distilled water for an hour. The maximum temperature should be 60°C.350 ML of water was left, and it was then allowed to cool. After being filtered twice using What- man filter paper, we were given plant extract in a beaker (Fig. 1B).



A. Copper sulphate solution **B.** Plant extract

C. Before stirrer **D.** After stirrer

Fig. 1: (A-D): Different solutions and mixture

Preparation of copper nanoparticles using plant extract

Distilled water was incorporated into 12.484 g of $CUSO_4.5H_2O$ to formulate a 100 mL solution (Fig. 1A). This solution had a morality of 0.5 M. One hundred milliliters of newly prepared plant extract were included into this combination. The solution that was used exhibited a green hue (Fig. 1C). Drop by drop, add the plant extract. For an hour, the solution-filled beaker was placed on a magnetic stirrer set at 70 to 80°C.Precipitation was clearly visible in the beaker (Fig. 1D). The mixture was heated for 24 hours at 70 to 80°C.The solution's color changed from green to brick brown, confirming that Cu NPs had formed (Shinde *et al*., 2015; Chung *et al*., 2017).

Characterization of copper nanoparticles

Visual observation

Visual observation was used for the primary detection. The creation of CuNPs is indicated by the formation of a reddish, shiny brown precipitate on the inner surface of the vessel and the slow color change of the precursor solution from olive green to dark brown (Shinde *et al*., 2015)

X-ray diffraction (XRD)

X-ray diffraction (XRD) studies were carried out using an X-ray diffractometer. The XRD data was analyzed using the ISDD program JCPDS. The generated CuNPs powder was used for X-ray diffraction investigations, and the related XRD pattern is shown in Fig. 2(c).

Scanning Electron Microscopy (SEM) analysis

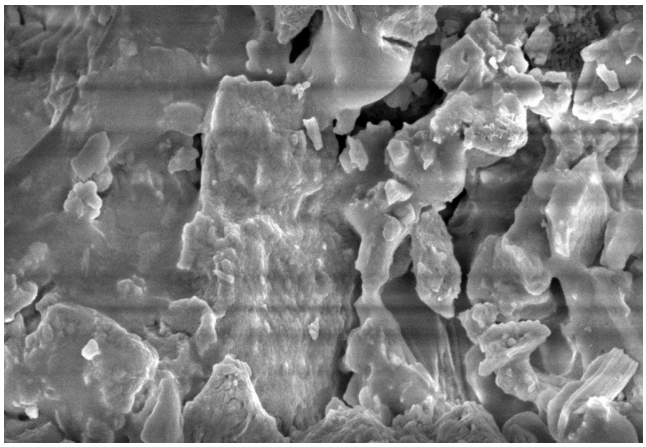
Using scanning electron microscopy (SEM), the surface morphology of the nanoparticles was investigated. This microscopy further reveals the morphological features of the green generated copper nanoparticles. The generated copper nanoparticles are asymmetrically scattered and occasionally come together to form free crystal forms, as shown by the SEM image. Figure 2(a–b)

Cytotoxicity Evaluation of CuNP

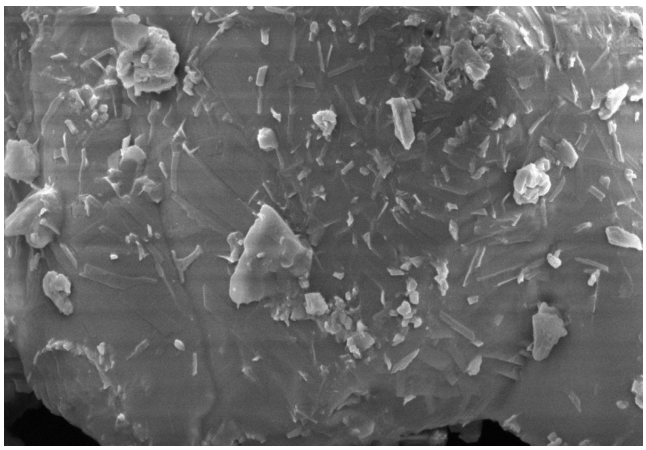
Cytotoxicity of the provided samples on THP-1 (Procured from NCCS Pune- Human leukemia monocytic cell) cell line was determined by MTT Assay. (Morgan DML. Methods Mol Bio1 1998) The cells (10000 cells/well) were cultured in 96 well plate for 24 h in DMEM medium (Dulbecco's Modified Eagle Medium-AT149-1L) supplemented with 10% FBS (Fetal Bovine Serum - HIMEDIA-RM 10432) and 1% antibiotic solution at 37°C with 5% CO2. Next day cells were treated from different concentrations. After incubation for 24 hours, MTT Solution was added to cell culture and further incubated for 2 h. At the end of the experiment, culture supernatant was removed and cell layer matrix was dissolved in 100 µl Dimethyl Sulfoxide (DMSO –SRL- Cat no.- 67685) and read in an Elisa plate reader (iMark, Biorad, USA) at 540 nm and 660 nm. IC50 Was calculated by using software Graph Pad Prism-6. (Fig. 3 a-b) and (Table 1)

Result and Discussion

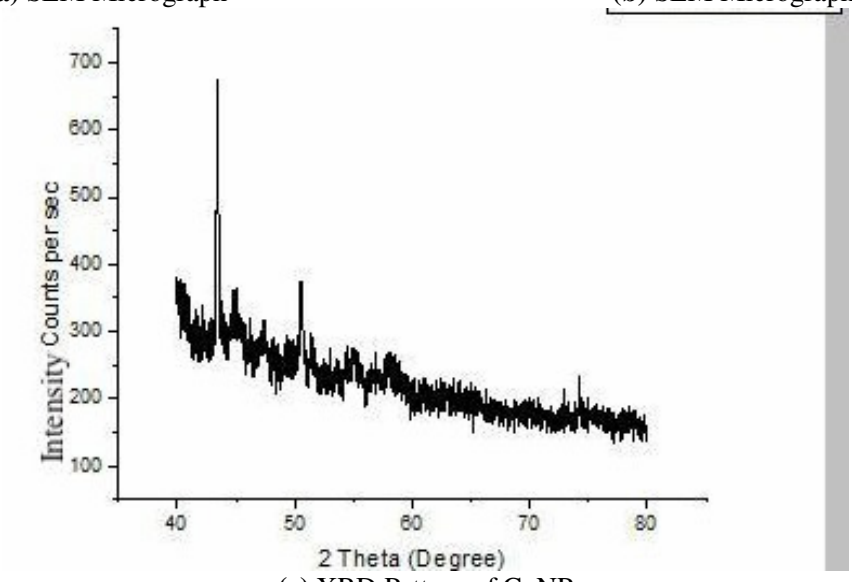
When leaf extract is added, the blue hue of the copper sulphate (0.5M) solution turns green. When this mixture is heated with a stirrer, a precipitate occurs, which indicates the creation of copper



(a) SEM Micrograph



(b) SEM Micrograph



(c) XRD Pattern of CuNP

Fig. 2(a-c): (a-b) SEM Micrograph and (c) XRD Pattern nanoparticles. The inside sides of the beaker were scraped or brushed in order to collect the synthesized CuNPs.

Fig. 2(c) displays the XRD patterns for the CuNPs that were synthesized using *Nyctanthes arbor- tristis* leaves. Acrystalline copper FCC phase was indicated by the existence of a strong peak that corresponded to 111, 200, and 220 (Shinde *et al*., 2015). Using Debye-Scherrer's equation (D = 0.9k/b cos h), the average size is determined to be 47 nm for CuNP. Here, D stands for the average grain size of the crystallite, k for the incident wave length, h for the Bragg angle, and b for the diffracted full width at half maximum (in radians), respectively (Shinde *et al*., 2015). The crystal line nature of the nanoparticles is confirmed by the appearance of diffraction peaks at 43.41o, 50.52o, and 74.34o, which are indexed as the (111), (200), and (220) planes of Cu NPs, respectively (JCPDS # 04-014-0265).

Synthesized copper nanoparticles were revealed to be free crystal formations via SEM pictures. It was observed that the samples NP, and Plant extract have no cytotoxic activity up to highest dose (1000 µg/ml) against the THP-1 cell line.Fig. 3 (a) showed cytotoxic activity of nanoparticles against THP1 cell line that there is no cytotoxic activity up to highest dose (IC_{50} value 1000 µg/ ml). Fig. 3 (b) represents cytotoxic activity of plant extract against THP1 cell line that at 1000 µg/ml concentration cells are viable it means plant extracts have no anti-cancerous activity. When we compare Fig. 3 a and b it shows that plant extract has more cytotoxic activity as compare to copper nanoparticles of *Nyctanthes arbor-tristis*

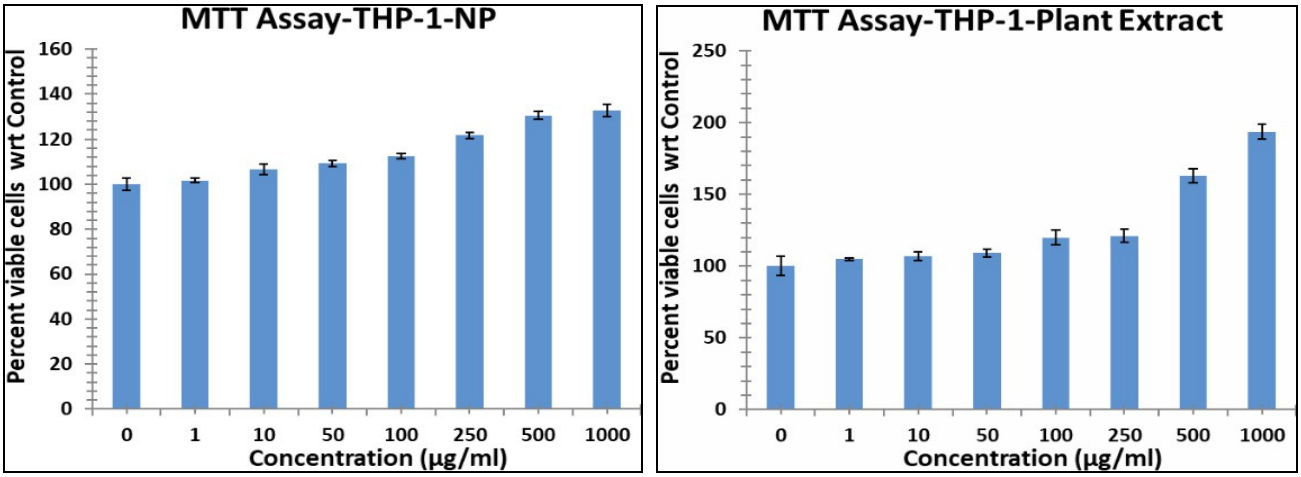


Fig. 3(a-b): A plot of cell viability versus sample concentration

Table 1: IC_{50} Value

Sample	Ic_{50}value
Nanoparticles	Not active
Plant extract	Not active

Acknowledgement

The authors would like to acknowledge Malviya national Institute of technology, Jaipur for providing facilities to carry out XRD & SEM and Aakaar Biotechnology Lucknow to carry out cytotoxic activity.

References

Ahmed, S., Saifullah, M., Ahmad, B., Swami, L. and Ikram, S. (2019). Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract, *Journal of Radiation Research andApplied Science*. **9**(1), 1–7.

Asghar, M.A., Zahir, E., Shahid, S.M. *et al*. (2018). Iron, copper and silver nanoparticles: green synthesis using green and black tea leaves extracts and evaluation of antibacterial, antifungal and aflatoxin B1 adsorption activity, LWT. **90**, 98–107.

Chung, I., Abdul, R.A., Marimuthu, S. (2017). Green synthesis of copper nanoparticles using *Eclipta prostrata* leaves extract and their antioxidant and cytotoxic activities," *Experimental and Therapeutic Medicine*. **14**(1), 18–24.

Delma, M.T. and Jaya, R.M. (2016). Green synthesis of copper and lead nanoparticles using *Zingiber officinale* stem extract, *International Journal of Scientific and Research Publications*. **6**(11), 134–137.

Issaabadi, Z., Nasrollahzadeh, M. and Sajadi, S.M. (2017). Green synthesis of the copper nanoparticles supported on bentonite and investigation of its catalytic activity, *Journal of Cleaner Production*. **142**, 3584–3591.

Kaur, P., Thakur, R. and Chaudhury, A. (2016). Biogenesis of copper nanoparticles using peel extract of *Punica granatum* and their anti microbial activity against opportunistic pathogens, *Green Chemistry Letters and Reviews*. **9**(1), 33–38.

Khani, R., Roostaei, B., Bagherzade, G. and Moudi, M. (2018). Green synthesis of copper nanoparticles by fruit extract of *Ziziphus spina-christi* (L.)Willd.: application for adsorption of triphenylmethane dye and antibacterial assay, *Journal of Molecular Liquids*. **255**, 541–549.

Morgan, D.M.L. (1998). Tetrazolium (MTT) assay for cellular viability and activity. *Methods Mol Biol.* **79**: 179-84.

Muthulakshmi, L., Rajini, N., Nellaiah, H., Kathiresan, T., Jawaid, M. and Rajulu, V. (2017). Preparation and

properties of cellulose nanocomposite films with in situ generated copper nanoparticles using *Terminalia catappa* leaf extract, *International Journal of Biological Macromolecules*, **95**, 1064–1071.

Nasrollahzadeh, M. and Mohammad, S.S. (2015). Green synthesis of copper nanoparticles using *Ginkgo biloba* L. leaf extract and their catalytic activity for the Huisgen [3 + 2] cycloaddition of azides and alkynes at room temperature, *Journal of Colloid and Interface Science*, **457**, 141–147.

Nasrollahzadeh, M., Momeni, S.S. and Sajadi, S.M. (2017). Green synthesis of copper nanoparticles using *Plantago asiatica* leaf extract and their application for the cyanation of aldehydes using K4Fe(CN)6, *Journal of Colloid and Interface Science*, **506**, 471–477.

Shende, S., Ingle, A.P., Gade, A. and Rai, M. (2015). Green synthesis of copper nanoparticles by *Citrus medica* Linn. (Idilimbu) juice and its antimicrobial activity, *World Journal of Microbiology and Biotechnology*. **31**(6), 865–873.

Thakur, S., Sharma, S., Thakur, S., and Rai, R. (2018). Green synthesis of coppernano-particlesusing *Asparagus adscendens* Roxb. root and leaf extract and their antimicrobial activities, *International Journal of Current Microbiology and Applied Sciences*, 7(4), 683–694.