

Synthesis Characterization and Antimicrobial Activity of Copper Nanoparticles using *Murdannia Spirata* Leaves Extract

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Abstract—Among the various synthesis methods the copper nanoparticles (CuNPs) can be synthesized by green methods using plant extracts. These methods are more environmentally friendly and offer improved properties of the synthesized NPs in terms of biocompatibility and functional capabilities. In the present study CuNPs were synthesized using *Murdannia Spirata* Leaves Extract and were characterized by FTIR, XRD and SEM analysis. The particle size of nanoparticles was found in the range of 12–20 nm. The CuNPs shows antifungal potential against *Aspergillus Flavus* and the zone of inhibition was recorded at 20 mm.

Key words—CuNPs, Green synthesis, Plant Extract, Antifungal.

I. INTRODUCTION

Copper is an essential trace mineral naturally present in ores and minerals. In biology in various metabolic pathways, it functions as a cofactor for numerous enzymes involved in energy production, and biosynthesis of connective tissue, neurotransmitters, collagen, and red blood cells [1]. Copper also plays a significant role in supporting normal development, immune functions, dismutation of superoxide radicals, skin regeneration, angiogenesis, and healing processes [2]. However, both copper deficiency and excess in body can cause illnesses in humans.[3] Moreover, the antibiotic properties of copper have been documented extensively throughout history, with records as early as 3000 BC. Copper based NPs have been proven to be effective against a broad range of clinically relevant pathogenic microorganisms and fungi [4]. Despite the widespread use of NPs, there are still concerns regarding their potential risks to both the ecosystem and human health [1]. Although the toxicity of copper metal is considered low for humans, there is limited research available on the toxicity of CuNPs. Recent studies have shown the synthetic copper nanoparticle-induced toxicity in the kidney, liver, and spleen, and the reproductive system in mice. Moreover, CuNPs caused apoptosis and suppressed cellular proliferation in human extravillous trophoblast cells and SW480 cells. [5] It is important to note that the characteristics and potential toxicity of CuNPs are influenced by several factors, including method of synthesis, the composition of the biological agent, the precursor salt used, the synthesis conditions, and the resulting particle size [6].

Classical NP synthesis process involves the use of hazardous materials, reducing agents, including toxic, corrosive, and explosive substances, and utilizes both chemical and physical methodologies. Furthermore, the proper disposal of potentially hazardous by-products and solvent waste becomes imperative to mitigate the already significant environmental impact of the process. [6] These risks primarily depend on the selected precursor material, for example, some copper salts are more dangerous than others. Moreover, classical methods require sophisticated equipment with high energy costs, which presents significant challenges in establishing and maintaining optimal operation conditions [8]. The need for more cost-effective, eco-friendly and sustainable alternatives for NP synthesis has drawn interest in biological methods. Among these, green synthesis using plant extract has emerged as a straightforward, rapid, and sustainable approach. [9]

Notably, studies involving plant extracts do not selectively pinpoint the specific biomolecule responsible for NP synthesis and stabilization due to the process's inherent complexity. However, several investigations have mentioned molecules such as ascorbic acid, which display dual roles as reducing and stabilizing agents in the synthesis of gold and silver NPs [10]. Recently, a biological method is also followed for the green chemistry synthesis of copper nanoparticles due to it being free from hazardous chemicals. The copper nanoparticle synthesis method from various plant extracts has more advantages over the microbial synthesis method because the microbial process requires high cost for microorganism isolation and their culture maintenance. *Murdannia* Royle is one of the dominant genera in the family of Commelinaceae. It is represented by c. 55 species and distributed in the tropical and subtropical regions of Asia and Africa [11]. The western region of India consists of 22 species. This western region of India is a major centre of *Murdannia* sp. diversification. Some reports showed that there are 50 species in tropical and subtropical regions and 20 endemic species in China. Also, the general characteristic of *Murdannia* sp. has been detailed studied [12]. In this study we have used *Murdannia* sp. (Fig. 1) for the synthesis of copper nanoparticles. The purpose of this study is to use leaves extract for the synthesis of CuNPs.



Fig. 1 Leaves of *Murdannia spirata*

II. EXPERIMENTAL PROCEDURE

Materials

Leaves of *Murdannia spirata* (collected from farm nearby Baramati area) (Fig.1), copper acetate, Soxhlet apparatus, distilled water used for the preparation of the aqueous extract



Fig. 2 Dried leaves of *Murdannia spirata*

Preparation of *Murdannia spirata* leaves extract

Fresh leaves of *Murdannia spirata* were collected and washed with distilled water to remove dust and then sun dried for 3-4 days (Fig. 2). Dried leaves were grinded to fine powder. The aqueous extract was prepared by refluxing 5 gm of leaves powder and 150 ml of distilled water by using Soxhlet apparatus at 100° C for 90 minutes. Then extract were filtered through Whatman filter paper no.42 and the extracts were collected in an airtight bottle.

Synthesis of CuNPs using *Murdannia spirata* leaves extract

For the synthesis of CuNPs, The leaf extract was diluted 10 times in distilled water. 0.002 M moles of copper solution was added in 20 ml diluted extract in beaker and the mixture was placed at room temperature for half an hour. After 30 minutes change in the solution colour was observed, indicating the preliminary stage of the synthesis of copper nanoparticles. Precipitate formed at the bottom of the beaker. Precipitate was collected the in petridish and dried at 60° C, The dry powder was obtained.

Preparation of Bacterial Culture

Selection of Bacterial Strain: bacterial strain was chosen for testing, including Gram-negative bacteria (*Pseudomonas aeruginosa*). Inoculum Preparation: Grow bacterial culture overnight in appropriate growth media (Mueller-Hinton broth) at 37° C. Some phytoconstituents of plant *Murdannia spirata* ability to inhibit to *Pseudomonas aeruginosa* zone of inhibition observed in well plate assay.

Antifungal activity

Agar well diffusion method was employed to evaluate the anti-fungal activity against two pathogenic fungal strains i.e., *Aspergillus Flavus*. Four wells were made in agar medium using gel puncture under aseptic conditions. Then 10 µl of CuNPs solution was added in the well 1. Distilled water and streptomycin were used as control in well 2 and 3, respectively. The plates were then incubated for 24 h at 35°C. The zone of inhibition was observed after incubation

III. RESULT AND DISCUSSION

Characterization of CuNPs

1. Characterization of synthesized CuNPs by UV-Vis spectroscopy:

The purified CuNPs extract was dispersed in distilled water and subjected to double-beam BMS-2800 UV-spectrophotometry within 200–800 nm. Figure 3 shows a distinct characteristic peak for CuNP due to the nanoparticles CuNP are formed when the color changes from blue (aqueous salt solution) to dark brown. Apart from the color change, the development of a specific absorption peak associated with CuNP at 392–400 nm verifies the green method of nanoparticle synthesis (as seen in Fig 3). [13] The characteristic peak in the prescribed reporting range for CuNP was seen.

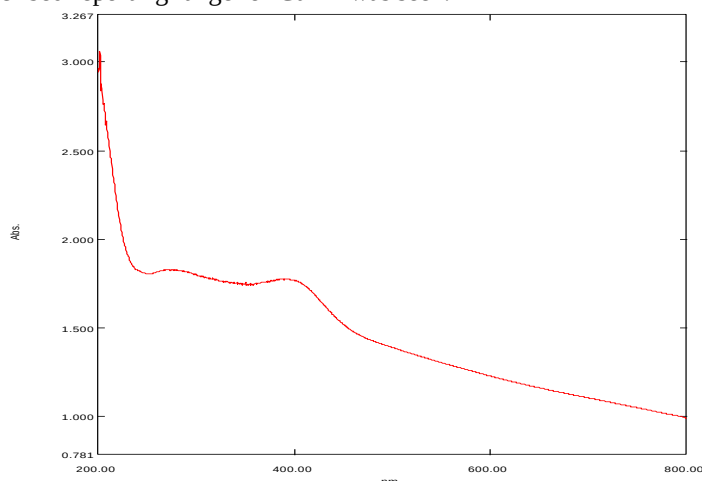


Fig. 3 UV–Vis spectrum for CuNPs prepared by using *Murdannia spirata*.

2. Characterization of synthesized CuNPs by FTIR Spectrophotometer

The FTIR spectrum revealed the various functional groups in the Cu-NPs. It revealed various interactions as well as compositions of the leave extract (Fig. 4). The presence of broad peak at 3325.28 cm^{-1} highlighted the stretching due O–H from the surface water present in the CuNPs. while the peak at 1085.92 cm^{-1} indicated the stretching due to C–O band of the primary alcohols. Peak at 885.62 cm^{-1} shows C–H bending in the molecules, while the peak at 601.79 cm^{-1} was a result of C–Cu vibration of the halo species. The occurring of peak around 482.20 cm^{-1} corresponding on the of Cu ion species.

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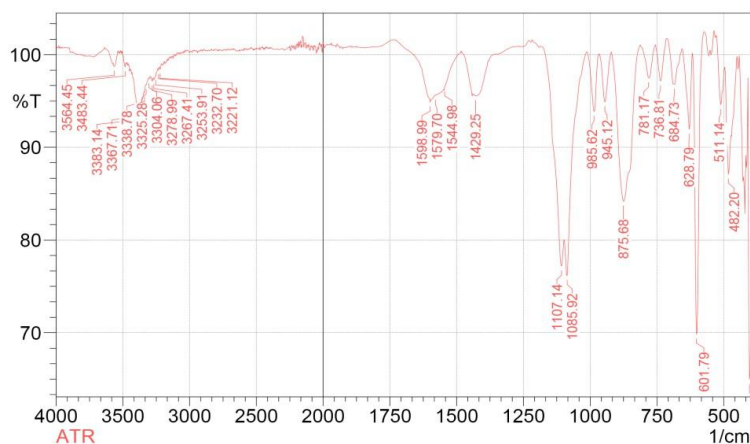


Fig. 4 FTIR spectrum of Cu-NPs

3 Scanning electron microscopy (SEM Analysis)

External morphology and surface features of CuNPs was analyzed using a scanning electron microscope. The CuNP was rinsed with distilled water. Thin films of the sample were prepared on a silicon plate. The diverse shapes of the surface particles of the synthesized CuNPs, as observed in the SEM images in Fig. 5, suggest that the nanoparticles have a complex and heterogeneous structure. The presence of triangular, cylindrical, polygonal, and nearly spherical shapes indicates that the CuNPs have different facets and orientations, which may influence their physical and chemical properties. The irregular shapes of the CuNPs could be attributed to the synthesis method used, as well as phytochemicals. SEM images of the synthesized Cu-NPs contained irregular particles arranged in clusters comprising of different structures from polygons to spherical shapes [14]

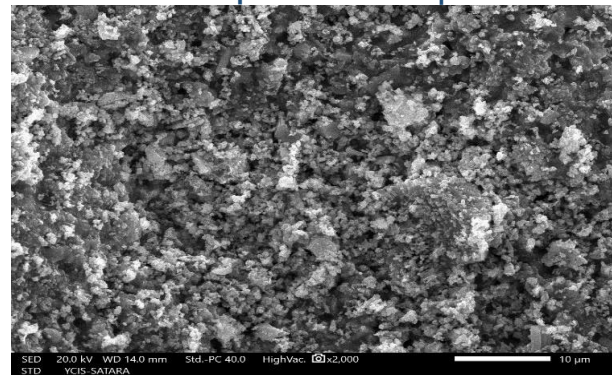
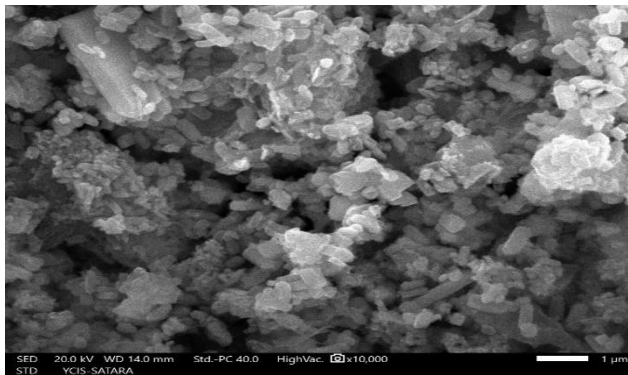


Fig. 5 SEM micrographs of CuNPs

4 Energy Dispersive X-ray Analysis

The elemental composition revealed by the EDX analysis provides valuable information about the chemical composition of the CuNPs and can help in understanding their properties and potential applications. The mapping result highlighted the major elements present (Fig. 6). The presence of a prominent Cu peak in the elemental analysis indicates that the CuNPs synthesized have a high purity level of copper ions, with a relative abundance of 64.32%. This suggests that the majority of the elemental composition of the nanoparticles is copper, which is desirable for applications where high purity is important. (Table 1) It is then followed by the O with the relative abundance of 35.68%.

Table 1 Atom % by EDX Analysis

Element	Mass%	Atom%
O	35.68±1.46	68.78±2.82
Cu	64.32±3.67	31.22±1.78
Total	100.00	100.00

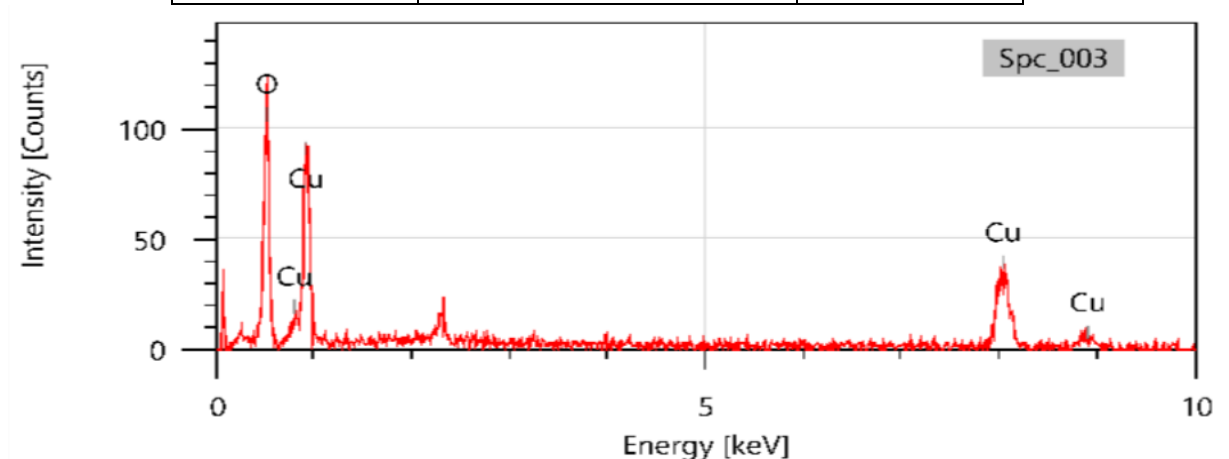


Fig 6. EDX mapping of the CuNPs.

5 X-ray Diffraction (XRD) Analysis

The spectrum obtained for XRD of biosynthesized CuNPs is represented in Fig. 7. Five characteristic peaks were observed at 2 theta angles of 16.04, 33.34, 36.73, 49.02 and 52.65, and these correspond to the h, k, l, values of the reflections from (110), (111), (220), (800) and (713), corroborating with the values of the JCPDS card (card no. 89- 5899)13 (Fig. 7). The Scherrer formula ($D = \frac{0.9 \lambda}{b \cos \theta}$, where λ wavelength and b full width at half maximum at the θ angle) was used to calculate the average crystalline size of the JC-CuNPs, showing 12 - 20 nm on the (111) plane. The peak values are also inconsistent with the joint committee of powder diffraction standards (file no. 89- 5899) which further support the formation of crystals of CuNPs. [15]

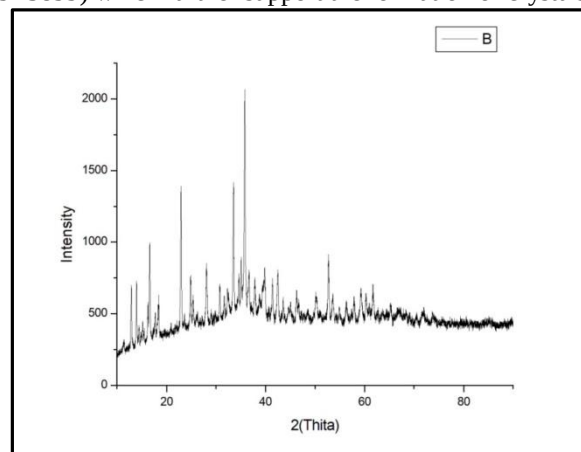


Fig. 7 XRD of the CuNPs

Applications

Antibacterial activity

Antimicrobial properties of the CuNPs was analyzed. The standard antibiotics used as a standard control in antibacterial activity test. The results from the assessment of the antibacterial properties Gram-negative bacteria (*Pseudomonas aeruginosa*) has been determined. It has shown the effects of extracts on the chosen bacterial pathogens under the zone of inhibitory study. Figure 8 have shown the antibacterial activities on the CuNPs the zone of inhibition was calculated as 14 mm (Fig. 8). The results revealed the excellent inhibition against the growth of all the bacteria tested at predetermined studied concentrations.

Antifungal activity

In the case of the fungal specie *Aspergillus Flavus* the zone of inhibition was calculated as 20 mm (Fig. 9). It is exhibited that CuNPs damage the membrane fungal cells which contain ergosterol that makes distinctive inclinations inside the cytoplasmic membrane. CuNPs damage membrane structure and cause the death of the cell.



Fig. 8 Antibacterial activity of CuNPs



Fig. 9 Antifungal activity of CuNPs

IV. CONCLUSION

The present research shows that a green synthesis of copper nanoparticles is indeed a very useful method because of its economic and environmental aspects. The synthesized CuNPs were characterized by FTIR, XRD and SEM and EDX. The particle size of nanoparticles was found in the range of 12–20 nm. The *Murdannia spirata* were used as a reducing agent for the synthesis CuNPs. The Copper nanoparticles exhibit significant antibacterial activity against *Pseudomonas aeruginosa*, their zone of inhibition was 14 nm. The antifungal potential was checked against *Aspergillus Flavus* and the zone of inhibition was recorded at 20 mm. The images according to the SEM analysis revealed the irregular shapes the particles have consisting of triangular, cylindrical, polygonal spherical shapes. The EDX spectrum indicated the formation of the Cu-NPs based on the sharp and strong peak of the Cu ions.

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