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Synthesis and characterization of *Eichhornia*-mediated copper oxide nanoparticles and assessing their antifungal activity against plant pathogens

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Abstract. **In this paper, we report the biosynthesis and characterization of copper oxide nanoparticles from an aquatic noxious weed, *Eichhornia crassipes* by green chemistry approach. The aim of this work is to synthesize copper oxide nanoparticles by simple, cost-effective and ecofriendly method as an alternative to other available techniques. The synthesized copper oxide nanoparticles were characterized by UV–visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), Field emission scanning electron microscopy (FESEM) and Energy dispersive X-ray spectroscopy (EDX) analyses. The synthesized particles were highly stable, spherical in shape with an average diameter of 28 ± 4 nm. The synthesized nanoparticles were then explored to antifungal activity against plant pathogens. Highest zone of inhibition were observed in 100 μg ml^{-1} of *Eichhornia*-mediated copper oxide nanoparticle against *Fusarium culmorum* and *Aspergillus niger.* This *Eichhornia*-mediated copper oxide nanoparticles were proved to be good antifungal agents against plant fungal pathogens.**

Keywords. **Antifungal activity; copper oxide nanoparticles; *Eichhornia*; EDX; FESEM; plant pathogens.**

1. Introduction

In recent years, nanotechnology is emerging as advanced technology, interdisciplinary with physics, chemistry, biology, material science and medicine. Green chemistry method highlights the development of an ecofriendly approach for the synthesis of metal oxide nanoparticles [1,2]. It emphasizes the usage of natural organisms like plant leaf extract, bacteria, fungi and enzymes for the synthesis of metal oxide nanoparticles [3,4]. In this method, it was found that the extracts of living organisms act both as reducing and capping agents [5]. Recently, copper oxide nanoparticles have achieved significant importance due to their distinctive properties [6]. Copper oxide nanoparticles are used as gas sensors [7–10], batteries [11], catalysis [12], solar energy exchange tools [13], etc. Copper oxide nanoparticles are synthesized through diverse methods such as sol–gel [14], alkoxide-based method [15], thermal decomposition of precursor [16], one step solid-state reaction method [17], precipitation–pyrolysis [18], etc. Chemical synthesis methods lead to absorption of several toxic chemicals on the surface that may have undesirable effects in the medical applications [19]. To reduce the toxicity, we have focussed our attention on green synthesis of nanoparticles. Currently, zinc oxide, gold, silver and copper oxide nanoparticles are synthesized by green chemistry method using plants [19–21]. *Aloe vera*-mediated copper oxide nanoparticles were biosynthesized and the average particle size was between 15 and 30 nm [19]. *Tabernaemontana*-mediated copper oxide nanoparticles were reported to be monodispersed with average size of 48 ± 4 nm [22].

Plants are frequently affected by plant pathogens such as bacteria, fungi and viruses, which result in loss of agricultural productivity [23]. Several methods such as fungicides, pesticides have been used for the controlling these plant pathogens and each method has some drawbacks. Hence, nanoparticles are considered to be an alternative ecofriendly method for controlling these pathogens. Green-synthesized nanoparticles have an important role in controlling the plant infections as compared to man-made fungicides [24]. *Tabernaemontana*-mediated copper oxide nanoparticles have good antibacterial activity against UTI pathogens at 50 μg ml^{-1} concentration [22]. *Parthenium*-mediated zinc oxide nanoparticles have highest zone of inhibition at 25 μg ml^{-1} concentration against plant pathogens such as *Aspergillus flavus* and *A. niger* [25].

Eichhornia crassipes (Family: Pontederiaceae) is one of the worst aquatic weeds of the world. It is effectively resistant to all challenges of eradication methods (mechanical, chemical, biological or hybrid means). Therefore, nanobiotechnology approaches have been used to determine the problem of aquatic weed removal and management [26].

In this paper, we have reported the green synthesis of copper oxide nanoparticles using aqueous extract of *E. crassipes* leaf extract and shape and size of particles were detected

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by using standard techniques and the antifungal activity was performed using well-diffusion method against plant fungal pathogens.

2. Materials and methods

2.1 *Materials*

E. crassipes plants were collected from Ukkadam Lake, Coimbatore, Tamil Nadu, India (11°31′N; 76°39′E). All the chemicals used in this experiment were purchased from Sigma-Aldrich Chemicals, India. Laboratory glass wares were immersed overnight in acid cleaning solution and rinsed in tap water and distilled water. Milli-Q water has been used for synthesis of nanoparticles. *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Fusarium oxysporium* and *F. culmorum* were acquired from the Department of Microbiology, School of Life Sciences, Karpagam Academy of Higher Education, Coimbatore, India. The obtained cultures were maintained in potato dextrose broth at 200 rpm for 3 days in shaker at room temperature.

2.2 *Synthesis of copper oxide nanoparticles using Eichhornia leaf extract*

Five grams of (fresh) *E. crassipes* leaves were weighed and washed with tap water and distilled water. Samples were ground well by mortar and pestle using deionized water and boiled with 100 ml of deionized water for 15 min. After cooling, the leaf extract was filtered using filter paper (Whatman No. 42, Maidstone, England) and stored in refrigerator for further investigation. The schematic diagram for green synthesis of *Eichhornia*-mediated copper oxide nanoparticles synthesis is shown in figure 1.

A 50% of the leaf extract was made up to 250 ml with deionized water. Copper sulphate was used as a precursor and the copper sulphate solution was prepared using deionized water and was mixed with 50% leaf extract under continuous stirring with magnetic stirrer. The mixture of this solution was kept under vigorous and continuous stirring at 100°C for 7–8 h and allowed to cool at room temperature and the supernatant was discarded. After this process, a brownish black colour solid product was obtained which was washed twice with deionized water and dried at 80°C for 8 h. Finally, the resulting dried powder was stored in properly labelled air tight containers and used for further studies.

2.3 *Characterization of copper oxide nanoparticles*

The optical property of copper oxide nanoparticles was analysed by UV-absorption spectra (Shimadzu). The functional groups in the copper oxide nanoparticles were analysed by Fourier transform infrared (FTIR) spectrometer (Perkin–Elmer 1725x). Elemental analysis of copper oxide nanoparticles was examined using energy dispersive X-ray spectrometer (RONTEC's EDX system, Model QuanTax 200, Germany). Field emission scanning electron microscopy (FESEM) (Model JSM6390LV, JOEL, USA) was used to study the morphology and size of the copper oxide nanoparticles.

2.4 *Determination of antifungal activity of Eichhornia-mediated copper oxide nanoparticles against plant pathogens by well-diffusion method*

Antifungal activity of green synthesized copper oxide nanoparticles were determined using plant fungal pathogens by well-diffusion method [27]. The fungal cultures were maintained in potato dextrose broth at 200 rpm in shaker for

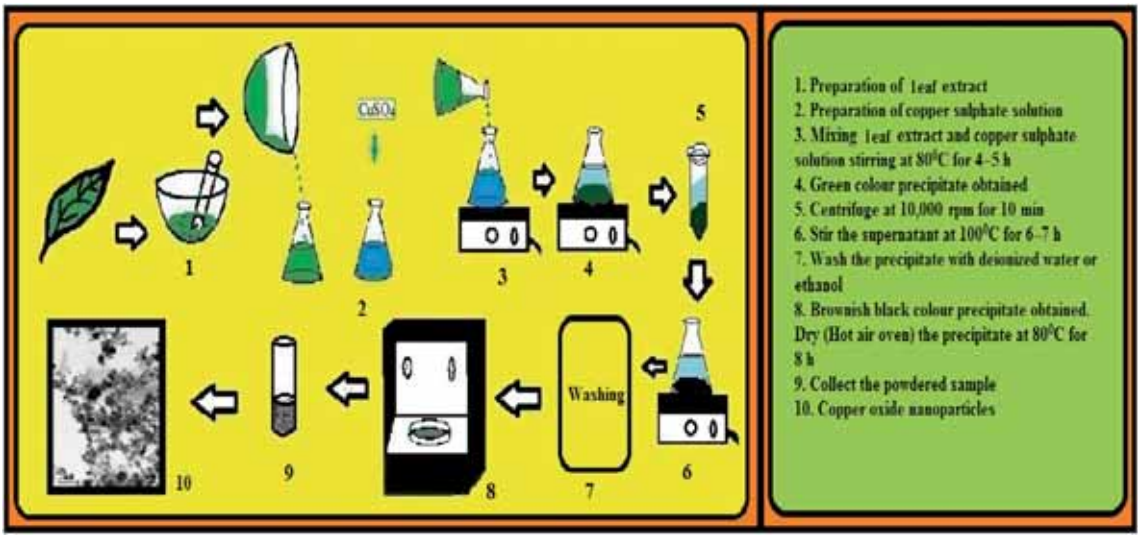

Figure 1. Schematic diagram of biosynthesis of copper oxide nanoparticles from *E. crassipes* leaf extract.

3 days. The fungal cultures of 100 µl were spread on potato dextrose agar plates using sterile L-rod. The spread plates were allowed to stand for 10 min for absorption of culture in the agar plate. The wells (5 mm) were punctured on plates using sterile gel puncture and different concentrations (25, 50, 75 and 100 µg ml^{-1}) of the biosynthesized copper oxide nanoparticles were added in the well. The positive control (10 µg ml^{-1}) (amphotricin B) was prepared and added into wells. The plates were incubated at room temperature for 48 h. After incubation, the zone of inhibition was measured in millimetre. Each screening test was performed with three replicates and the mean values were recorded.

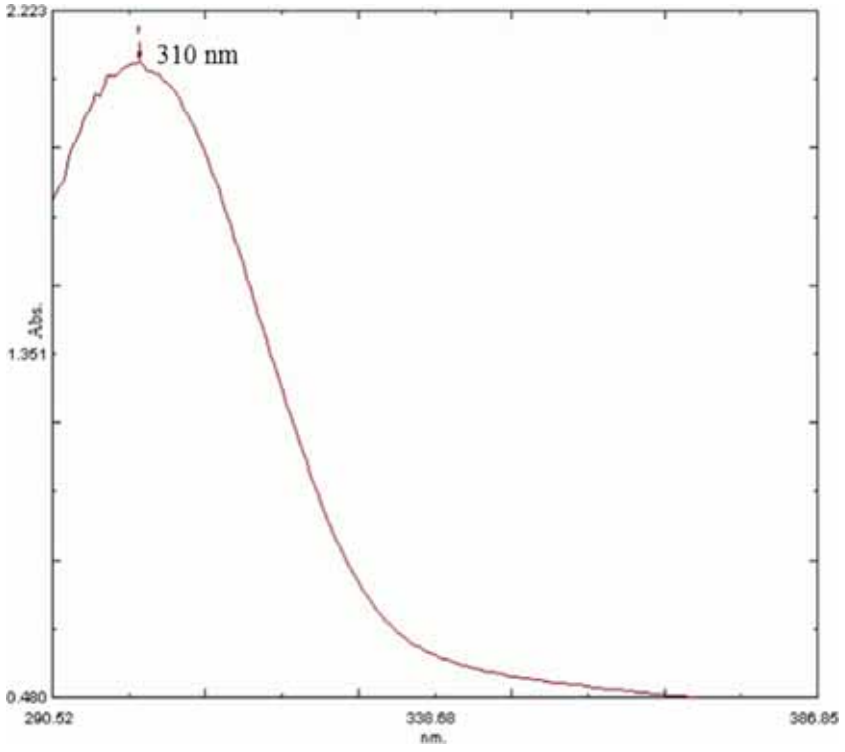

Figure 2. UV spectra of *Eichhornia*-mediated copper oxide nanoparticles.

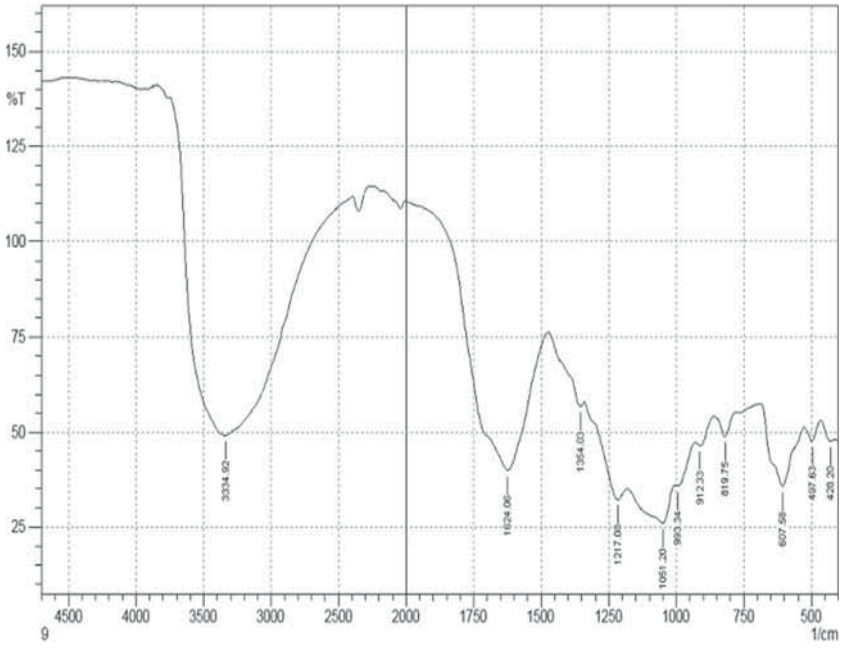

Figure 3. FTIR spectra of *Eichhornia* copper oxide nanoparticles.

3. Results and discussion

3.1 *Characterization of copper oxide nanoparticles*

UV–Vis absorption spectrum of copper oxide nanoparticles is shown in figure 2. UV–Vis absorption spectra reveal that green synthesized copper oxide nanoparticles are monodispersed and show a broad absorption peak at 310 nm. The band gap of copper oxide nanoparticles was calculated by using formula $E = hc/\lambda$, where h is the Plank's constant, c the velocity of light and λ the wavelength. The band gap of zinc oxide was found to be 3.32 eV, as reported earlier [25,28].

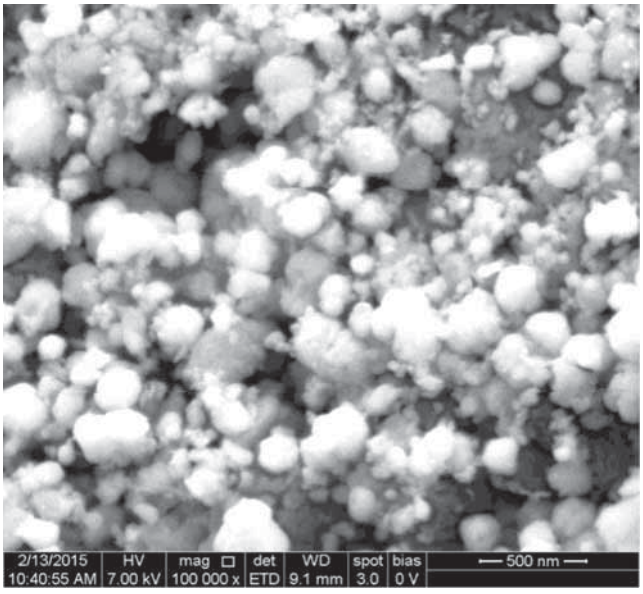

Figure 4. FESEM image of *Eichhornia*-mediated copper oxide nanoparticles.

FTIR spectra helped to find the biomolecules that are attached to surface of green synthesized copper oxide nanoparticles and are shown in figure 3. The spectrum showed bands at 607, 428 and 497 cm^{-1} corresponding to metal–oxygen (M–O). The bands at 819 and 912 cm^{-1} show the presence of C–C stretching of alkanes [29,30]. The bands at 1354 and 1624 cm^{-1} refer to N–H bending mode. The synthesized copper oxide nanoparticles show peak at 3314 cm^{-1}, which corresponds to phosphorous compounds. The intense bands observed at 1217 cm^{-1} corresponding to C–O–C stretch was also observed [29].

Field emission scanning electron microscopy (FESEM) confirmed that the copper oxide nanoparticles had well-defined morphology (figure 4) and the nanoparticles were found to be spherical in shape. Similar results were seen in previous literature [31]. No diffraction peaks arising from any impurity has been detected in the pattern, which confirms that the grown products are pure. It is confirmed from the EDX analysis that the synthesized nanoparticles were composed of copper and oxygen (figure 5). Vanathi *et al* [23] and Rajiv *et al* [25] also confirmed the zinc oxide composition in green synthesized zinc oxide nanoparticles through EDAX. Gunalan *et al* [19] reported that *Aloe barbadensis*-mediated copper oxide nanoparticles were spherical in nature and sizes ranged from 15 to 30 nm.

3.2 *Antifungal activity of Eichhornia-mediated copper oxide nanoparticle against plant fungal pathogens*

Figure 6 shows the results of the antifungal activity of *Eichhornia*-mediated copper oxide nanoparticles against plant fungal pathogens. Highest zone of inhibition was observed in *F. culmorum* (21.26 ± 1 mm) and *A. niger* (18.33 ± 1 mm) at a concentration of 100 μg ml^{-1}, which is more than that

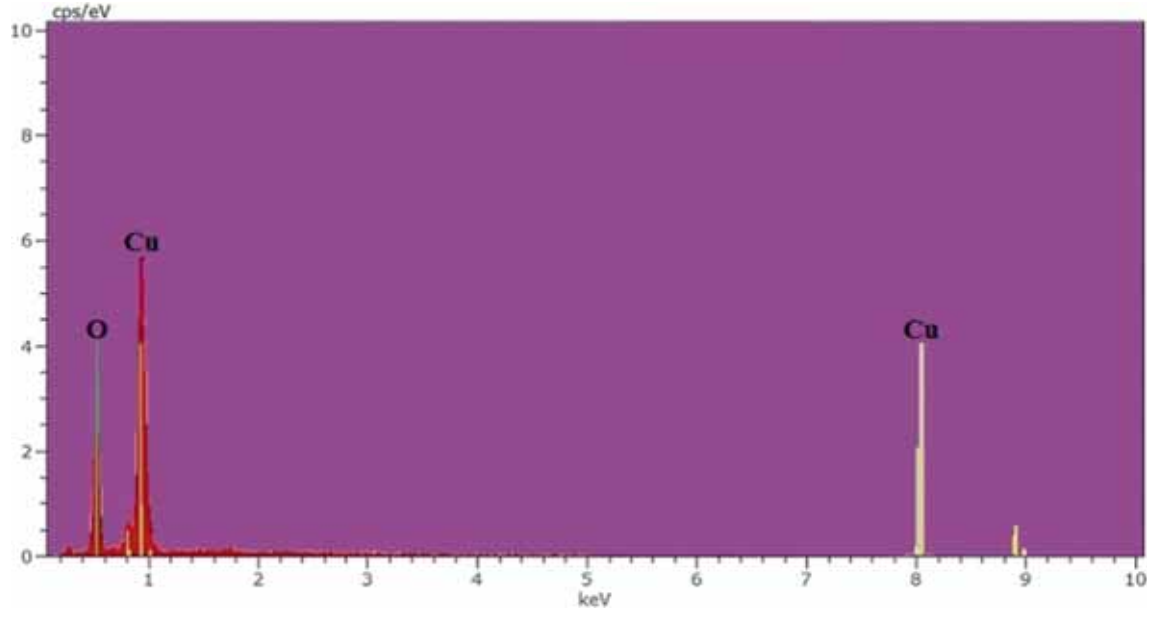

Figure 5. EDX pattern of *Eichhornia*-mediated copper oxide nanoparticles.

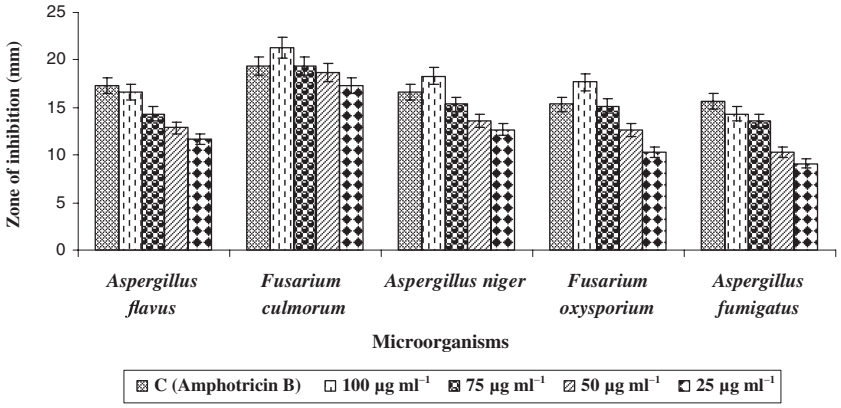

Figure 6. Antifungal activity of *Eichhornia*-mediated copper oxide nanoparticles against plant pathogens. Mean that were followed, differ significantly with $P < 0.05$ are established by ANOVA (Tukey's test).

of positive control (19.33 ± 1 mm and 16.66 ± 1 mm). Similarly, the lowest zone of inhibition was observed in *A. fumigatus* (9.10 ± 1 mm) at a concentration of 25 µg ml^{-1}, which is similar to the previous studies [24]. Copper nanoparticles have efficient bactericidal effect against *E. coli* and *P. aeruginosa* [32]. The inhibition in the growth of fungi may be due to the disruption and breakage of cell membrane and enzymes of fungi by copper oxide nanoparticles [33]. These results confirmed that the *Eichhornia*-mediated copper oxide nanoparticles have high antifungal activity against plant fungal pathogens under normal room conditions. The statistical analysis was done using SPSS 16.0. All the results were analysed by one-way Anova.

4. Conclusion

The present study reported that copper oxide nanoparticles can be synthesized in a simple and ecofriendly method using *E. crassipes* leaf extract. *E. crassipes* leaf extract acts as reducing- and capping-agents in the synthesized copper oxide nanoparticles because of the presence of flavonoids, proteins and other functional groups present in the *E. crassipes* leaf extract. Antifungal activity was performed on various plant pathogens which confirmed that the *Eichhornia*-mediated copper oxide nanoparticles have highest inhibitory effect against plant fungal pathogens compared to control. The investigated green synthetic method has efficient function in inhibiting the growth of plant fungus in crop plants in the agricultural fields.

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