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Low-Cost and Eco-Friendly Green Synthesis of Antibacterial Copper Oxide Nanoparticles

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The present study reports a simple and eco-friendly synthesis of copper oxide nanoparticles (CuO NPs) using green synthetic route. The synthesis of copper oxide nanoparticles is explored using reducing as well as stabilizing properties of *Elettaria cardamomum* (elaichi). The copper oxide nanoparticles are duly characterized using ultraviolet–visible (UV–Vis) spectrometer, dynamic light scattering (DLS) and zeta-potential methods. The UV–Vis spectra confirm the formation of dark brown coloured CuO nanoparticles with a characteristic peak at the λ_{max} value of 220 nm. Due to capping of stabilizing agents around both copper oxide nanoparticles, the hydrodynamic particle size as revealed by DLS experiments is found to be of 175 nm. The stability and overall charge on the nanoparticles are found by its zeta potential, which is found to be of −16.2 mV. Antibacterial activities of copper oxide nanoparticles are tested against both Gram-positive and Gram-negative bacteria. Thus, the present method proves to be the most appropriate for large-scale green synthesis of copper oxide nanoparticles.

Дане дослідження повідомляє про просту й екологічно чисту синтезу наночастинок оксиду Купруму з використанням зеленого синтетичного підходу. Синтеза наночастинок оксиду Купруму досліджується за допомогою відновлювальних, а також стабілізувальних властивостей Елетарія кардамону. Наночастинки оксиду Купруму належним чином характеризуються за допомогою ультрафіолетово-видимого (УФ–вид) спектрометра, динамічного розсіяння світла (ДРС) і методів дзета-потенціялу. УФ–вид-спектри підтверджують утворення темно-коричневих кольорових наночастинок CuO з характерним піком при максимальному значенні λ у 220 нм. Через обмеження стабілізувальних аґентів навколо обох наночастинок оксиду Купруму, гідродинамічний розмір частинок, як показали експерименти ДРС, становить 175 нм. Стабільність і загальний заряд на наночастинках знаходяться за його дзета-потенціялом, який, як виявилося, становить −16,2 мВ. Антибактеріяльна активність наночастинок оксиду Купруму перевіряється як на грампозитивних, так і на грамнеґативних бактеріях. Таким чином, даний метод виявляється найбільш підходящим для великомасштабної зеленої синтези наночастинок оксиду Купруму.

Key words: *Elettaria cardamomum*, copper oxide nanoparticles, antibacterial activities.

Ключові слова: кардамон Елетарія, наночастинки оксиду Купруму, антибактеріяльна активність.

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1. INTRODUCTION

The rapid development of nanomaterials with new capabilities has maximized many desirable impacts on health and environment [1]. Decreasing bulk counterparts into smaller dimensions up to nanoscale influences the physicochemical properties, which depend on the involved practical route [2]. There are two main synthetic approaches of synthesizing nanoparticles, *i.e.*, top-down and bottom-up approach [3]. Bottom-up approach is the most suitable method for biogenic synthesis of metal nanoparticles, which uses synthetic chemistry and bioengineering tools [4]. The chemical reduction of metallic salt precursors using plant-based natural products as both reducing as well as stabilizing agents forms the basis of an eco-friendly synthesis of metal nanoparticles [5]. These bio-resources are not only easily available but also diminish the presence of toxic chemical contaminants in the environment [6]. The metal nanoparticles produced by plants and plant-derived materials includes several advantages such as good stability, use of non-toxic solvents, lower cost, fast rate of synthesis and possibility of its commercial production [7]. In recent years, there has been a lot of research in the synthesis of metal oxide nanoparticles, especially copper oxide and zinc oxide nanoparticles due to their unique physical and biological properties [8, 9]. The most appropriate method used for the low-cost and durable application of these nanoparticles is by green-synthetic route involving plants and plant spices [5, 10].

In literature, there are several reports of multistep synthesis of copper oxide nanoparticles using expensive and toxic reagents such as sodium borohydride and require external additives to complete the reaction [11, 12]. Therefore, there is need to study copper oxide nanoparticles and establish its synthesis using convenient and economical method. In this present study, copper oxide nanoparticles have been synthesized through a simple and eco-friendly route using *Elettaria cardamomum* (elaichi) as reductant and stabilizer. There are reports for the synthesis of gold and silver nanoparticles using the reducing properties of *Elettaria cardamomum* aqueous extract from seedpod [13, 14]. Copper oxide nanoparticles are of a particular interest because it is incorporated into a wide range of biological and industrial applications. To the best of our knowledge, we have not found green synthesis of copper oxide nanoparticles (CuO NPs) using *Elettaria cardamomum*. Thus, we develop green method to synthesis CuO NPs. A general representation for the preparation of copper oxide nanoparticles has also been discussed. The nanoparticles were characterized by UV–Vis spectrophotometer, zeta potential and DLS. The present study also examines the antibacterial properties of the green synthesized CuO NPs.

2. EXPERIMENTAL SECTION

2.1. Materials and Methods

Copper sulphate $CuSO_4 \cdot 5H_2O$ were purchased from Sigma-Aldrich (Mumbai, India) and used as received. All aqueous solutions were prepared from quartz distilled deionized water, which was further purified by a Millipore Milli-Q water purification. The colloidal solutions were centrifuged in REMI, Model No. C-24BL laboratory centrifuge. Absorption spectra were recorded on a Jasco V-570 UV–Vis spectrophotometer. Dynamic light scattering (DLS) and zeta potential measurements were operated with a Zetasizer NanoZS (Malvern Instruments).

2.2. Preparation of Extract

The extract was prepared by using fresh *Elettaria cardamomum* (elaichi) purchased from local market, and it was thoroughly washed with distilled water and dried at 37°C. The seeds were grinded to make fine powder using mortar and pestle. The dry seed powder (5 g) was suspended in 100 mL of distilled water and heated at 70°C temperature for 30 min. After filtration, the solution was taken as stock solution and used for further experimental use.

2.3. Green Synthesis of CuO NPs

In this procedure (Fig. 1), 10 mM solution of copper sulphate was

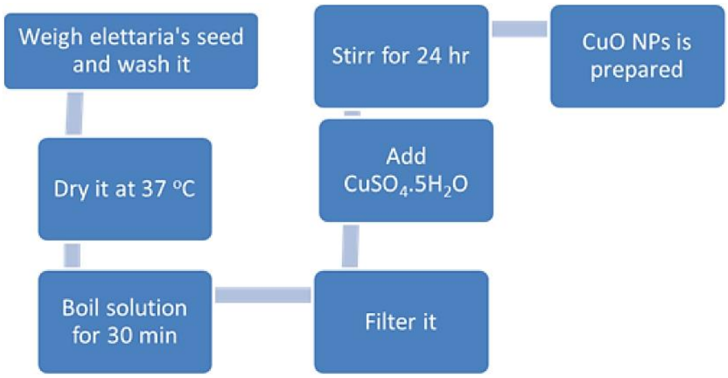

Fig. 1. General representation for the preparation of CuO NPs using *Elettaria cardamomum* (elaichi).

prepared in aqueous media and made up to 100 ml using standard flask. In a round bottom flask, 10 mL Elettaria extract was heated at 70°C temperature and the prepared solution of copper sulphate was added into it. The reaction mixture was then kept overnight at room temperature so that the bio reduction reaction may take place. The formation of dark brown colour indicated the formation of CuO NPs, which were centrifuged at 8000 rpm and washed several times with distilled water.

2.4. Antibacterial Studies

The antibacterial activity of green synthesized CuO NPs was evaluated using the disc diffusion and Kirby–Bauer method [15]. The antibacterial assays were done on bacterial organisms like *E. Coli, B. Megaterium, S. Aureus, B. Subtilis*. The plates were incubated for 24 h at 37°C overnight, and results were recorded as the inhibition zone diameter size (in mm).

3. RESULTS AND DISCUSSION

3.1 Characterization of Green Synthesized CuO NPs

Figure 2 depicts the UV–Vis spectrum of green synthesized CuO nanoparticles using *Elettaria cardamomum* (elaichi) extract. The nanoparticles were sonicated for uniform dispersion and then subjected for the absorbance measurements. The CuO NPs exhibited a peak at wavelength of 220 nm. The results are consistent with previously reported literature [16, 17].

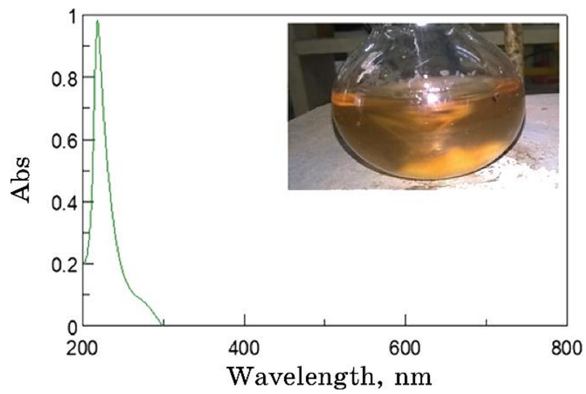

Fig. 2. UV–Vis spectrum of CuO NPs.

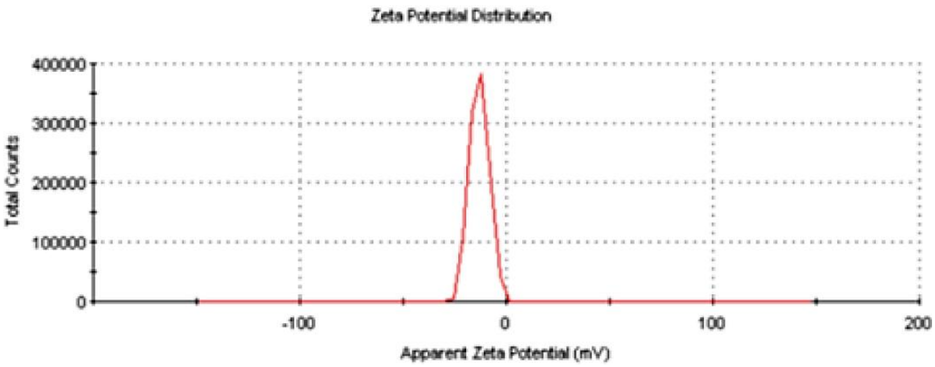

Fig. 3. Zeta potential of CuO NPs.

Zeta-potential value is a very important parameter for colloids or nanoparticles in suspension. Its value is closely related to suspension stability and particle surface morphology [18]. To describe the stability of CuO NPs, the zeta-potential values were measured. The average zeta-potential value was found to be of −16.2 mV (Fig. 3). This value accounts for good to better stability of the CuO NPs that may be attributed to the presence of stabilizing agents present in the *Elettaria cardamomum* extract.

Nanoparticles synthesized using green synthesis approach and bottom-up reduction method often results with large size units [19]. Dynamic light scattering (DLS) technique was used to analyse the hydrodynamic size range of the nanoparticles colloidal dispersions. The size distribution of CuO NPs is shown in Fig. 4 with an average particle diameter of 175 nm. The variation in the particle size is attributed to the polydispersity index value (PdI) which was found to be 0.410. This describes the degree of 'non-uniformity' of a distribution and in the case of nanoparticle suspensions, which in turn causing variability in calculated particle size in comparison with the actual size of the particles [20].

3.2. Antimicrobial Studies

The synthesized copper oxide nanoparticles were studied for evaluation of their antibacterial action against bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus megaterium* and *Bacillus subtilis*, which were compared with standard antibiotic such as chloramphenicol. Zone of inhibition a qualitative method to measure and compare levels of inhibitory action of certain bacteria [21]. It has been reported that *Elettaria cardamomum* can potentially be used in the treatment of various infectious diseases caused by microorganisms that are showing resistance to currently available antibiotics [22]. On this basis, the antibacterial activity of copper oxide nanoparticles was examined which was found to be comparative

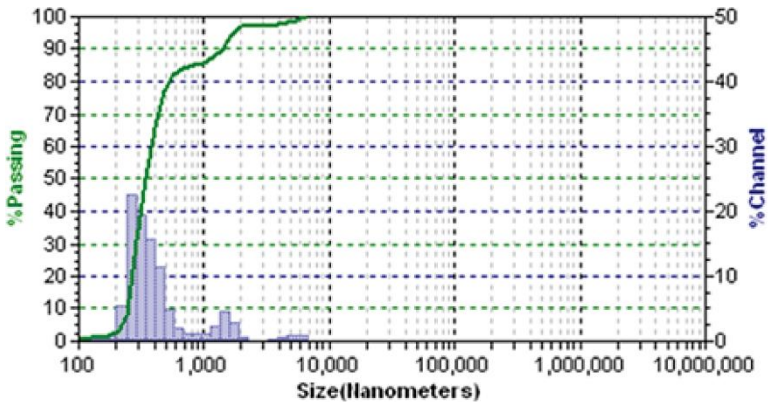

Fig. 4. Dynamic light scattering (DLS) of CuO NPs.

TABLE. Antibacterial study of CuO NPs.

Bacteria	Diameter of zone of inhibition, mm		
	CuO NPs	*Elettaria cardamomum* extract	Chloramphenicol (antibiotic control)
E.coli	15 ± 0.82	11 ± 0.12	9 ± 0.21
B.subtilis	12 ± 0.75	10 ± 0.35	10
S.aureus	15 ± 0.11	12 ± 0.81	10 ± 0.15
B.megaterium	10 ± 0.92	11 ± 0.45	11

with that of the *Elettaria cardamomum* (elaichi) extract. This led us to conclude the copper oxide nanoparticles can be considered as a good antibacterial agent. The exact mechanism of the antibacterial effect of copper oxide nanoparticles is partially understood; still some mechanistic pathways have been reported [23]. Table shows the values of the observed diameter zone of inhibition (mm) after 24 h incubation time. The different bacterial species were subjected to different extracts in agar well diffusion assay and in each well, the sample used was 100 μL.

4. CONCLUSION

Colloidal stable CuO NPs with a good stability and potent antibacterial activity been successfully synthesized through green synthesis in the presence of *Elettaria cardamomum* (elaichi) extract. The synthesized NPs were characterized by UV–Vis, DLS and zeta potential analyses. The main role attributed to the *Elettaria cardamomum* is related to its reducing capabilities. The simple single step synthetic method provides convenient, eco-friendly and cost-effective substitute to other synthetic chemical methods. The potential of CuO NPs for combating bacteria makes it suitable for formulation of pharmaceuticals and medicinal applications. However, *in vivo* studies may determine the toxicity of these nanoparticles, which are necessary.

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