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A Green Strategy to Synthesize and Characterization of CuO Nanoparticles for Antimicrobial Applications

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Abstract: Green synthesis of nanoparticles is the recent and advanced technology in the fields of nanoscience and biotechnology which produces the eco-friendly and more efficient antimicrobial agents. Therefore, present study reported a simple, suitable and cost effective method of preparation of bioactive antimicrobial agents by the green strategy of copper oxide nanoparticles with Moringa oleifera through a green route method. In this green process the vital and varsatile medicinal quality plant i.e., Moringa oleifera leaves extract as a reducing agent as well as stabilizing agent were used to prepare the copper oxide nanoparticles. The synthesized copper oxide nanoparticles were characterized by UV- Vis, SEM, and TEM techniques. The antibacterial activity of the samples were tested by disc diffusion method against gram positive bacteria (S.aureus), gram negative bacteria (E.coli). The size of synthesized copper oxide nanoparticles was in the ranges around 60-100 nm with dot and spherical shaped morphology. The antimicrobial activities of biofunctionalized copper oxide nanoparticles were observed significant inhibition activity. From this analysis, the green synthesized biofunctionalization method what we have suggested is exposed assure results in the antibacterial activity tests. Moreover, biofunctionalized copper oxide nanoparticles are shown superior performance in the opposed to of microbes.
Keywords: Green synthesis, Copper Oxide nanoparticles, Antimicrobial Activity, Moringa oleifera.

I. INTRODUCTION

Nanotechnology is a fast-growing area in the field of science which is an interdisciplinary field of both science and technology that increases the scope of investing and regulating at cell level between synthetic material and biological system [1, 2].

Nanosize materials are quite unique in nature because nano size enhance surface to volume ratio and also its physical, chemical, and biological properties are different from bulk material. Thus in recent years, much research is going on metallic nanoparticle properties like a catalyst, optics, antibacterial activity, sensor, medicine and data storage capacity [3]. Nanomaterials are the chief in the different fields of science, however there is growing need to develop environmentally benign nanoparticles synthesis routes. Biosynthesis of nanoparticles researchers to develop synthetic strategies using biological entities like enzymes [4], microorganisms [5] and plant extracts [6-8] play a major role in the formation of nanoparticles, instead of using toxic chemicals. Metal and metal oxide nanoparticles containing magnesium oxide, silver, iron, zinc and nickel oxides are of enormous interest due to its well exhibited antimicrobial behavior. Copper nanoparticles have concerned considerable attention because they are very reactive and their high surface-to-volume ratio helps to interact with bacterial surfaces effectively [9, 10]. Moreover, it's cheap, high yields and short reaction times under normal reaction conditions are the advantages of green-nano preparation. The copper nanoparticles were prepared through different green methodologies and proved their higher antimicrobial activity against various bacterial strains from many types of researches [11-13]. Green biosynthesis of copper nanoparticles is of huge interest because of many advantages: copper is highly conductive and also cheaper than silver and gold [14] Copper oxide (CuO) nanoparticles are significant due to their applications as antimicrobials. *M. oleifera* has enormous medicinal potential which has been long recognized in the Ayurvedic and Unani system [15]. It has a high nutrient value and contains carbohydrates, fat, and protein. *Moringa oleifera* leaves are known for their antimicrobial activity against many organisms. Extracts of *Moringa oleifera* leaves contain superior amounts of alkaloids, anthocyanins, proanthocyanidins, flavonoids, and cinnamates [16, 17].

The present study reports for the synthesis and characterization of copper oxide (CuO NPs) using *Moringa oleifera* (Drumstick) leaves extract and copper sulphate [$Cu(SO_4)_2.5H_2O$]. Also, the antibacterial characteristics of the synthesized CuO nanoparticles were evaluated in this study.

II. MATERIALS AND METHODS

A. Materials and Chemicals Used

Copper sulfate pentahydrate ($CuSO_4$ $5H_2O$), Ethanol (EtOH), and double-distilled water were purchased from Himedia Ltd. Mumbai. The all organic solvents, reagents, and chemicals used in the study were of analytical reagent (AR) quality. Double distilled water was used throughout the experiment.

B. Collection of Plants

The plant of *Moringa oleifera* (Drumstick) was collected from the campus of Siddhartha College, Jafrabad, Jalna District, Maharastra, India. The plant was identified and authenticated by Department of Botany, Siddhartha College Jafrabad. The collected plant materials were thoroughly washed numerous times using normal tap water and then followed by distilled water to remove impurities.

C. Biosynthesis of CuO Nanoparticles

24.9 gm of copper sulfate [$Cu(SO_4)_2.5H_2O$] obtained from Himedia Pvt. Ltd. chemicals were dissolved in 1000 mL of double distilled water to obtain 0.1M of copper sulfate precursor solution. For the synthesis of copper oxide nanoparticles, 0.1M of copper sulfate [$Cu(SO_4)_2.5H_2O$] were taken in 50 mL, and then 10 mL of *Moringa oleifera*, leaf extract was added slowly into the solution under magnetic stirring at 70°C for about 2 hours to form a light green copper hydroxide. The precipitate formed after an adequate time of stirring was collected by centrifugation at 10000 rpm for 10 minutes. Then the centrifuged particles were washed with distilled water and subjected to further centrifugation at 5000 rpm for 10 minutes. The separated light green precipitate was dried in an oven at 70°C for 2 hours and ground with help of a mortar. The powdered sample was calcined in a muffle furnace at 350-450°C to get copper oxide nanoparticles.

D. Characterization of Copper Oxide Nanoparticles

Copper oxide nanoparticles (CuO NPs) synthesized by this green method were characterized by UV-Vis spectrophotometer (Equip-Tronics) in the 200 to 600 nm range. Detailed analysis of the morphology, size, and distribution of the nanoparticles was documented by Scanning Electron Microscopy (SEM) using CARL ZEISS, EVO 18 model instrument. TEM analysis was performed to confirm the size distribution, morphological structure, crystalline nature of nanoparticles, by using the JOEL instrument.

E. Antimicrobial Assay

The antibacterial activity of the synthesized CuO NPs were tested by disc diffusion method [18, 19] against gram positive bacteria (Staphylococcus aureus, NCIM 2079), and gram negative bacteria (Escherichia coli, NCIM 2109).
For disc diffusion method, preparation of microbiological media Nutrient Agar (NA-Himedia) Media for Bacteria (*S. aureus* and *E. coli)* Composition of media Sodium chloride: 5.00 g Beef extract: 10.0 g Peptones: 10.0 g (pH = 7.2).
Preparation of medium: Suspend 28.0 grams in 1000 mL distilled water. Dissolve the medium completely and heat to boiling. Sterilize by autoclaving at 15 Ibs pressure (121ºC) for 15-20 minutes. Mix well properly and pour into sterile Petri plates.The sample was loaded placed on the surface of the cultured agar plates and incubated at 37°C for 24 hours. The inhibition zones observed around the disc were measured and the results were compared with standard antibiotic, Gentamicin.

III. RESULT AND DISSCUSSION

A. UV-Vis spectroscopy of CuO NPs

The confirmation of the synthesized CuO NPs was done by recording UV-Vis spectrum; this was the preliminary step to confirm the result. In the present works, the biosynthesis of CuO NPs is formed after the addition of *Moringa oleifera* leaves extract. The UV – Visible spectrum was recorded for aqueous leaves extracts of *Moringa oleifera* with CuO NPs. Fig. 1. Showed the copper nanoparticles prepared have displayed an absorption peak at between 290 and 300 nm [20] which is assigned to the absorption of CuO NPs. This spectrum confirms the presence of CuO only, as there is no other measurable peak observed**.**

B. SEM Studies

The morphological studies of biosynthesized copper oxide nanoparticles were studied by using the scanning electron microscopy (SEM) method and magnification of SEM micrograph carried out at 67.55 KX. It was observed that the particles were aggregated with small grains. It is evident from the SEM image that the morphology of particles is uniformly distributed, well dispersed, and equally distributed in all directions most of the CuO nanoparticles are less agglomerated with spherical shapes and clear dot-shaped morphology. The experimental results show that the prepared copper nanoparticles using the plant extract of moringa oleifera are in the range 30-50 nm.

C. TEM studies

TEM images of the phytochemical fabricated synthesized copper oxide nanoparticles at scale 50 nm and 100 nm magnifications. These images show that the nanoparticles formed are nearly spherical and elliptical uniformly morphology. It is observed that there is variation in particle size and the average size estimated was 35 nm.

D. Antibacterial study of CuO NPs

The antibacterial activities of CuO NPs against gram-positive (*Staphylococcus aureus*) strain reference NCIM 2079 and gram-negative bacteria (*Escherichia coli*) strain reference NCIM 2109 were performed using the disc diffusion method and their activity was compared to a well-known commercial antibiotic Gentamicin are shown in fig.4. The results are evaluated and listed in Table 1 and the graphical representation showed in fig.5. From the antibacterial inhibition results, CuO NPs observed bacterial activity against all bacterial strains. The *S.aureus* gram-positive found more zone of inhibition (9.85±0.2) as compared to *E.coli* (9.08±0.2) gram-negative. Especially, *S.aureus* gram-positive showed higher activity than standard drug Gentamicin (9.40±0.4). Therefore, we can conclude that the biosynthesized copper oxide nanoparticles were shown considerable antibacterial activity.

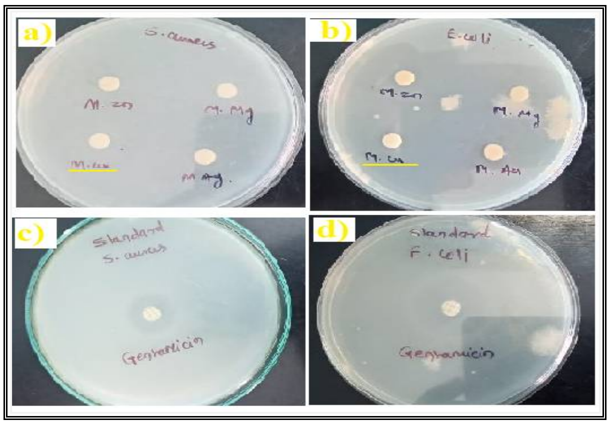

Figure.4.Plate having underlined disc showing antibacterial activity of CuO nanoparticles against (a) S.aureus (b) E.coli (c) positive control of S.aureus and (d) positive control of E.coli

Table .1..Zone of Inhibition (ZOI) of CuO NPs against S.aureus E.coli and positive (P) control

Pathogenic bacteria	Zone of Inhibition (mm)	
	CuO NPs	Positive control (Gentamicin)
S.aureus	9.85 ±0.2	9.40 ±0.4
E.coli	9.08 ±0.2	9.65 ±0.2

*Zone of inhibition values are expressed as the mean of triplicate measurements ± standard deviation

Figure.4.8 Antimicrobial activity of copper oxide NPs against bacterial pathogens

IV. CONCLUSION

In this paper, it was reported that, the use of *M.oleifera* extracts for the green route synthesis of CuO nanoparticles. This method has several advantages such as ease to scale up, eco-friendly, less toxic and less time consuming. The characteristics of the obtained biosynthesis CuO nanoparticles were studied using the UV-vis, SEM and TEM techniques. The use of environmentally benevolent materials like plant extracts, for the synthesis of nanoparticles offers several benefits of eco-friendliness and compatibility. The synthesized CuO NPs are highly stable and have noteworthy effect on both Gram-positive and Gram-negative bacteria. The green synthesis route provide a faster rate as compare to chemical route. CuO NPs potentially used in areas such as food, sensor and medical applications.

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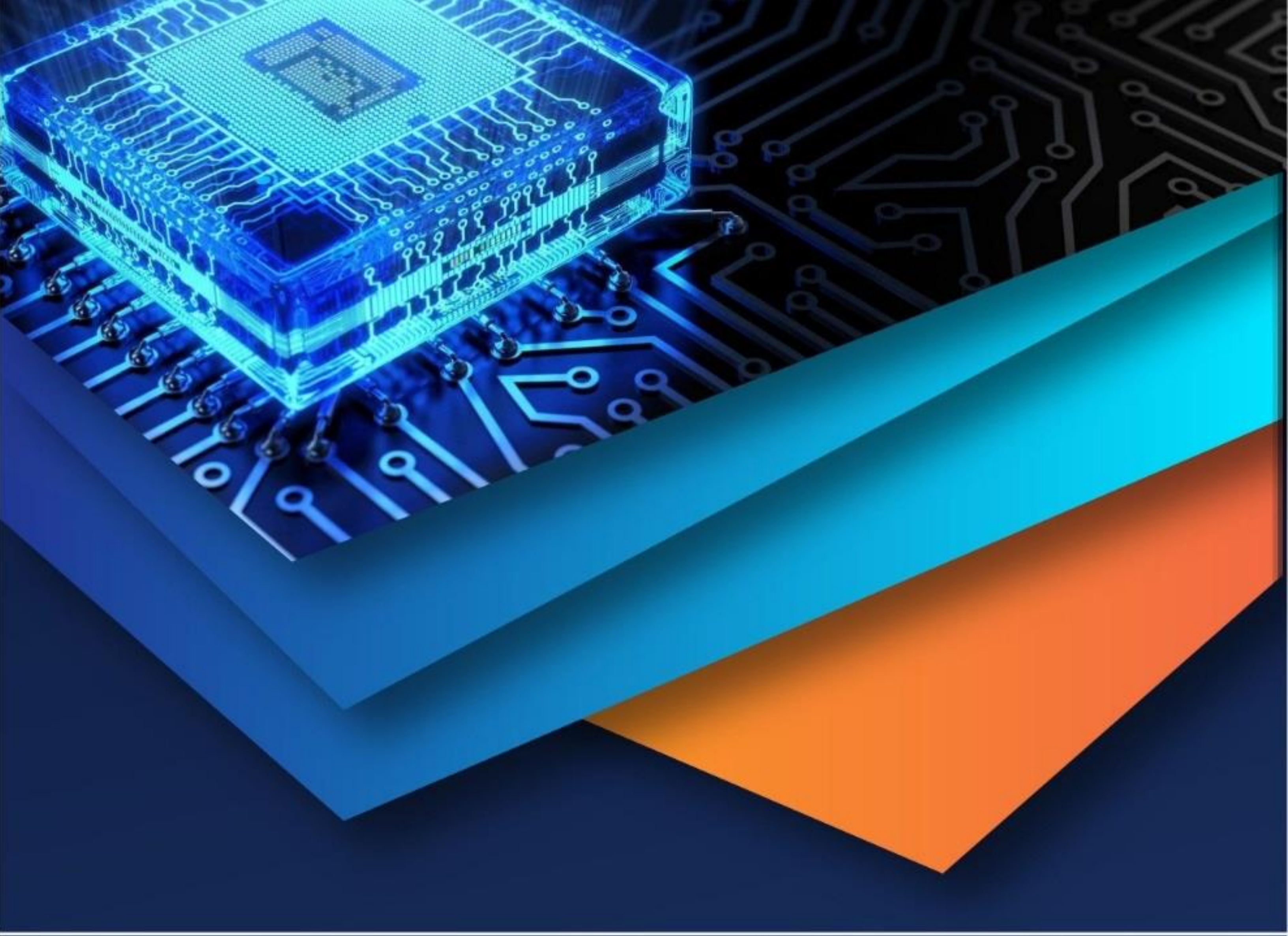

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