

BETA VULGARIS MEDIATED BIO-INSPIRED COPPER NANOPARTICLE AND ITS APPLICATION AS A ANTIBACTERIAL AGENT

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ABSTRACT

Metal nanoparticles have witnessed incredible growth due to their wide applications in a variety of areas. The present paper describes the biological synthesis of Cu NPs by aqueous extract of Beta Vulgaris, commonly called beetroot. Betalains present in the Beta Vulgaris have the capability of reducing and capping Cu NPs preventing the agglomeration and oxidation of Cu NPs. The formation of Cu NPs was confirmed by various spectroscopic techniques such as UV-Visible spectrophotometer, FT-IR, SEM and EDAX analysis. After confirmation, antibacterial properties were analyzed against three bacterial strains E. coli, Promicromonospora and Bacillus subtilis. They were found to show good antibacterial properties.

Keywords: Beta Vulgaris, Green Method, Copper NPs, Antibacterial Properties.

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INTRODUCTION

Due to their ideal size and exclusive properties, nanoparticles have been the area of attention to be employed in several fields such as electronics, food industries, drug delivery, cancer treatment, catalysis, treatment of wastewater, antimicrobial agents, etc.¹⁻² Several efforts have been successfully made in synthesizing NPs with small size and excellent shape.³⁻⁴ But they are mainly limited to chemical and physical techniques.⁵⁻⁶ These approaches employ toxic chemicals, high-temperature conditions and radiations, involve the generation of hazardous by-products, need specialized apparatus and consume energy that limits their applications for further use.⁷⁻⁸ Bio-nanotechnology is the fast-growing area in which natural organisms/molecules are used to synthesize nano-size materials and in the future will be a crucial part of green chemistry.⁹ Biosynthesis of silver and gold nanoparticles as antibacterial agents have been reported in the literature.¹⁰⁻¹¹ Bactericidal effect of metal NPs is mainly the result of their very small size and high surface-to-volume ratio. As a result, there is a close interaction with microbial membranes.¹² Both silver and gold are expensive metals and also, they show low resistivity towards the migration of ions. Nanomaterials prepared from economical metals have engrossed considerable attention as they could be promising alternatives to expensive noble metals.¹³ Cu NPs in particular, are an area of attraction as copper is highly abundant in nature, has a high melting point and is cheaper, has low electrochemical migration behavior, chemical stability and possesses catalytic as well as antimicrobial properties.¹⁴ Copper is an important metal needed for various metabolic activities in human beings as it acts as a cofactor for various enzymes, cell signaling and defense mechanism.¹⁵⁻¹⁶ It is one of the important elements for plant growth.¹⁷ Cu nanoparticles can be a substitute for noble metals in the field such as catalysis, organic reaction, inkjet printing etc.¹⁸⁻¹⁹ Although Cu nanoparticles have been synthesized by various chemical, physical and biological methods.²⁰ Plants parts have extensively been used for the biosynthesis of Cu NPs due to abundant secondary metabolites present in the plants.²¹⁻²² Plant-based synthesis is simple, biocompatible, and involves a one-pot reaction. Aqueous extract of plant materials can reduce and stabilize nanoparticles during synthesis. It is amenable to scale up.²³ Moreover, the process is cost-effective. To design an experiment that involves the biosynthesis of Cu NPs, the choice of solvent, environment-friendly reducing agent and stabilizing agents are essential considerations. In the present paper, the authors have adopted green technology for the biosynthesis of Cu nanoparticles using an aqueous extract of Beta Vulgaris and

explored its antibacterial properties against three bacterial strains. Beta vulgaris is a rich source of flavonoids, carotenoids, limonene, sugars etc. that has the capability to reduce and stabilize the metallic Cu to its NPs.

EXPERIMENTAL

Materials and reagents

Analytical grade chemicals [$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, ethanol, peptone, yeast powder, Muller agar and NaCl] were procured from Merck, India and were used as received. Beta Vulgaris was procured from the local market.

Preparation of Beta Vulgaris Extract

A weighed amount of dried Beta Vulgaris was powdered in a mortar and pestle. To the groundmass, about 70 ml of double distilled water was added and stirred at 40°C on a magnetic stirrer for about 10-15 min. Then, it was filtered and centrifuged for about 15 min. The obtained supernatant was used as plant extract throughout the experiment.

Green Synthesis of Cu NPs

20 ml of copper salt solution (1 mM) was mixed with 5 ml of Beta Vulgaris extract. It was stirred on a magnetic stirrer for 1 h. A change in color of the reaction mixture is the primary confirmation for the biosynthesis of nanoparticles. The formation of Cu nanoparticles was indicated by the color change of Beta Vulgaris extract from blood red to deep brown (Fig.-1). The spectroscopic studies were made in the UV-Vis Spectrophotometer.



Fig.-1: Color Change Showing the Visual Formation of Cu NPs

Antibacterial Activity

The disk diffusion method was adopted to assess the antibacterial efficacy of biosynthesized copper nanoparticles from Beta Vulgaris. The nutrient media Luria Bertani (LB) was prepared by mixing peptone, agar, yeast extract powder and NaCl in double distilled water. Approximately 20 ml of sterile molten and cooled media was poured into pre-sterilized Petri dishes. The plates were left overnight at 35°C to check for any contamination to appear. After inoculation of different target bacteria on the nutrient media, sterile paper discs of 3 mm diameter were impregnated with 50 μl copper nanoparticles placed on each plate. Chloramphenicol and tetracycline were used as the standard for comparing antibacterial activity. The experimental plates were then incubated at 35°C for twenty-four hours and forty-eight hours along with the control.

RESULTS AND DISCUSSION

The potential ability of Beta Vulgaris for the reduction of an aqueous solution of copper salt to copper nanoparticles was investigated. The results were analyzed using spectroscopic techniques.

UV-Vis Spectral Analysis

The preliminary detection of copper NPs was studied with the aid of a UV-Visible spectrophotometer. It is an indirect method to study the bioreduction of an aqueous solution of copper salt to copper NPs. The UV-Visible spectrum showed an absorption maximum band (SPR, λ_{spr}) in the range of 550-575 nm (Fig.-2) which appears as a result of collective electron oscillation around the surface mode of the particles and confirmed the formation of Cu nanoparticles (Cu NPs).

Scanning Electron Microscopy (SEM)

The morphology and size of biologically synthesized copper NPs using Beta Vulgaris extract were studied by Scanning Electron Microscopy. The SEM micrograph showed the monodispersed nature of Cu NPs mostly having a cubical shape and few are spherical with the size ranging from 35 to 60 nm (Fig.-3).

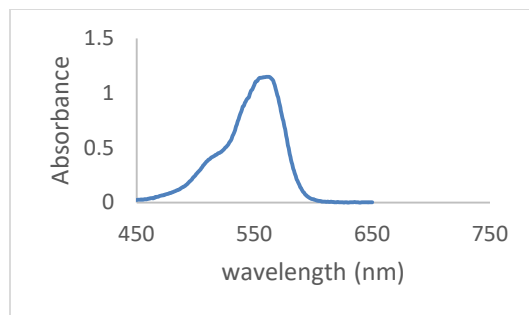


Fig.-2: UV-Visible Spectrum of Biologically Synthesized Cu-NPs

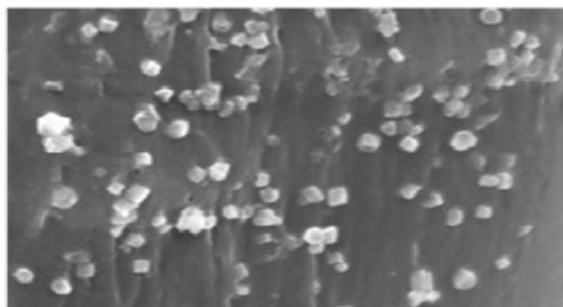


Fig.-3: SEM Image of Biologically Synthesized Copper Nanoparticles

EDAX

The elemental analysis of the synthesized sample was done using electron dispersive X-ray spectroscopy. EDAX image (Fig.-4) showed the characteristic peaks of metallic copper along with peaks of C and O which might be due to the presence of biomolecules (betalains) that are involved in the stabilization of Cu NPs. It suggests that nanoparticles were synthesized successfully from an aqueous solution of Beta Vulgaris.

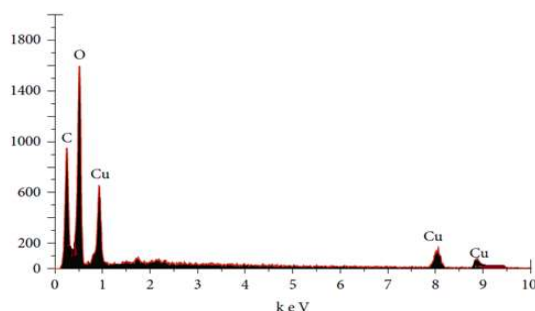


Fig.-4: EDAX of Copper Nanoparticle

Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR is an important technique to identify the biomolecules that are involved in the reduction and stabilization of the synthesized Cu NPs. It was recorded in the range from 4,000 to 400 cm^{-1} . Broad band observed at 3400 cm^{-1} was assigned to the O-H stretching vibration that represents the high concentration of alcoholic groups present in Beta Vulgaris. The hydroxyl groups present in the biomolecules have a stronger ability to bind with copper ions and are involved in the green synthesis of Cu NPs (Fig.-5). The peaks at 2900 cm^{-1} and 2976 cm^{-1} showed symmetric & asymmetric C-H stretching vibrations. The carbonyl group band appeared at 1654 cm^{-1} indicating the presence of aldehydes, ketones or carboxylic groups.

Antibacterial Activity

The biologically synthesized copper nanoparticles were tested against three bacterial strains; *Bacillus subtilis* (gram +ve), *Escherichia Coli* (gram -ve), and *Promicromonospora* (actinobacteria). Table-1 summarizes the zone of inhibition (ZOI), which appeared as a clear area around the discs. It was observed

that the zone of inhibition was maximum for E. Coli and minimum for Bacillus subtilis (Fig.-6). The exact mechanism of the antibacterial activity of Cu NPs is uncertain. It is believed that the Cu NPs get attached to Sulphur and -COOH group of amino acids that lead to the deactivation of the enzymes essential for DNA biosynthesis.²⁴⁻²⁷

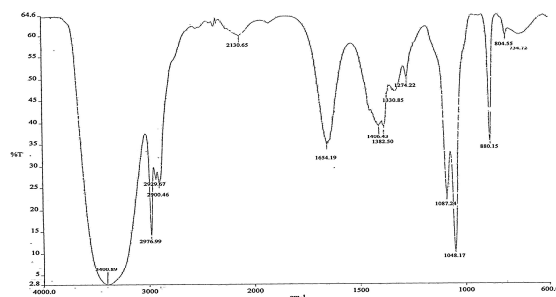


Fig.-5: FT-IR Spectrum of Copper NPs

Table-1: Antibacterial Activity of Copper nanoparticles

No	Bacteria	Inhibition zone diameter (in mm)
1	Promicrospora	18±2
2	Escherichia coli	20±2
3	Bacillus subtilis	7±2



Fig.-6: Zone of Inhibition Diameter Against (a) Promicrospora (b) E. coli (c) Bacillus subtilis

CONCLUSION

We proposed an environmentally benign method to synthesize Cu NPs using Beta Vulgaris aqueous extract and without using any external stabilizer. Copper is prone to oxidation and therefore, the synthesis of copper NPs is generally carried out in a non-aqueous medium. Plenty of betalains in Beta Vulgaris might be imparting additional stability to the synthesized Cu NPs. They were confirmed by FT-IR, UV-Visible spectrophotometer, SEM and EDAX spectroscopic techniques. The particles of the size range 35-60 nm were obtained in the present study and could be further controlled by adopting suitable reaction conditions. The growth of E. coli was suppressed significantly with copper NPs. Biologically synthesized copper NPs showed good antibacterial activity.

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