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Anti-bacterial, Anti-inflammatory and Anti-cancer activity of green Synthesized Copper Metal Nanoparticles

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ABSTRACT
Copper (Cu) nanoparticles are one of the most important transition metal oxides in the burgeoning field of nanotechnology due to their intriguing features. Because of its simplicity, eco-friendliness, and cost-effectiveness, its synthesis using green chemistry principles is gaining traction as a next-generation antibiotic. Cu nano particles (CuNps) were generated from Cassia absus aqueous extract and analysed utilising several analytical techniques in this study. Antibacterial, anti-inflammatory, and anti-cancer properties were also assessed. All of the results indicate a high level of biological activity. Based on the current findings, it is possible that green produced Cu Nps will find potential applications in nanomedicine.
Keywords: *Cu Nps, Cassia absus, anti-bacterial, Anti-inflammatory and Anti-cancer activity.*

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INTRODUCTION

Nanoparticles' unusual features have sparked a surge in study into nanoparticle synthesis and uses [1-4]. Copper nanoparticles (Cu-NPLs) are useful in a variety of sectors, including catalysis [5-7], water treatment [8,] information storage, and solar cells [9]. Cu-NPLs are acceptable materials for use in printed electronics and good substitutes for conductive ink in ink-jet printing to make low-cost electronic components [10, 11]. Copper also plays an important role in sophisticated electronic circuits because of its excellent electrical conductivity and inexpensive cost. Because of their antibacterial qualities, Cu-NPLs have also been employed as disinfectants [12]. Cu-NPLs could also be used in the pharmaceutical, health-care, and environmental health fields.

Laser ablation [13], microwave-assisted process, sol-gel, co-precipitation, pulsed wire discharge, vacuum vapour deposition, high-energy irradiation, lithography, mechanical milling, photochemical reduction, electrochemistry, electrospray synthesis, hydrothermal reaction, microemulsion, and chemical reduction are just a few of the physical and chemical methods used to make nanoparticles. Physical and chemical procedures produce well-defined and pure nanoparticles, but they are neither cost-effective nor environmentally friendly because harmful substances are used. The creation of eco-friendly, nontoxic, and clean green chemical techniques is one of the most essential criteria in nanotechnology. As a result, the production of nanoparticles involves a green chemistry-based process that includes plants, actinomycetes, fungi, bacteria, yeast, and viruses. The use of biological entities to synthesis NPs with a wide range of size, physicochemical properties, morphologies, and compositions is nontoxic, clean, and environmentally benign [14-17].

In this chapter,Copper nanoparticles (CuNPs) are synthesized by biosynthesis method using *Cassia absus* seed act as a reducing agent and antibacterial, anti-inflammatory, and anti-cancer properties were also assessed.

MATERIALS AND METHODS

Biosynthesis of Nanoparticles

In an Erlenmeyer flask, 10 ml of aqueous *Cassia absus* seed extract was added to 90 ml of (1mM) copper nitrate solution. At room temperature, the reaction mixture was thoroughly agitated, and the development of a brown/green colour indicated the creation of CuNPs and CuNPs. To separate the colloidal suspension of metal nanoparticles, the solution was centrifuged at 2500 rpm for 15 minutes and rinsed twice with deionized water to eliminate contaminants.

Characterization of Nanoparticles

The biosynthesised metal nanoparticles were spectroscopically characterized using UV-Visible Spectroscopy (Jasco V670 Spectrophotometer), Fourier Transformed Infrared Spectroscopy (FTIR SHIMADZU). The morphological characteristics of the CuNPs were analyzed using microscopic techniques such as Scanning electron microscopy (SEM ZEISS EVO18).

Biological potential of the Nanoparticles

Anti-inflammatory activity

In 100 ml of distilled water, 2 g of dextrose, 0.8 g of sodium citrate, 0.05 g of citric acid, and 0.42 g of NaCl were combined to make Alsever solution. The prepared Alsever solution and fresh human blood were then combined and centrifuged for 15 minutes at 3000 rpm. The coagulated blood cell was collected and cleaned three times with isosaline after centrifugation (0.85 percent pH 7.2). Induced human erythrocyte haemolysis was investigated using these blood cells and CuNPs.Different concentrations (0-50 µg/ml) of NPs were combined with 5 ml of hypotonic (10 mM sodium phosphate 50 mM NaCl) buffered solution with RBC erythrocyte blood cells (100 l). At 28o C, blood cells containing NPs were treated for 10 minutes with the hypotonic solution (room temperature). The aforementioned solution was centrifuged for 10 minutes at 3000 rpm after incubation. A UV spectrophotometer was used to assess the absorbance of the supernatant solution at 560 nm. The proportion of activity was calculated using the algorithm below.

$$\% \text{ activity} = \frac{\text{The optical density of the test sample}}{\text{The optical density of control}} \text{x} 100 \quad \text{-------} (1)$$

Anti-cancer activity

Cellular viability and proliferation assays, which include cytostatic and cytotoxic drugs, are widely employed to evaluate the efficacy of proposed anti-cancer treatments. The number of healthy cells in a population is referred to as cellular viability. The ability of healthy cells to divide and procreate is referred to as cellular proliferation. As a result, cell viability and cell proliferation assays are used to determine the number of healthy cells in a population and/or the rate of cell growth. This is done by assessing cell activity markers such metabolic activity, the number of cells present or divisions within the population, ATP production, or DNA synthesis. Colorimetric, binding, and staining tests are frequently used to quantify these properties. However, using these methods alone, it can be difficult to discern between cytotoxicity and cytostasis, resulting in unclear cell survival statistics. The adenosine triphosphate-based tumour chemosensitivity assay (ATP-TCA) or laser scanning cytometry are frequently used to distinguish these effects.

Anti-bacterial activity

Plant extracts were tested for antimicrobial activity using the agar well diffusion method in Mueller Hinton Agar (MHA) plates. *Klebsiella pneumoniae* and *S. aureus* were inoculated in Nutrient broth and incubated overnight at 37 C to adjust the turbidity to 0.5 McFarland standards, yielding a final inoculum of 1.5 108 CFU/ml. Using standardised microbial culture broth, the MHA plate was lawn cultivated. Dimethyl Sulfoxide was used to create plant extracts at a concentration of 50 mµg/ml (DMSO). Using a sterile cork-borer, six 6 mm wells were drilled in the inoculation material (6 mm).Positive control Ciprofloxacin was added to each well at 30 µg/ml extracts from various plants. It was allowed to diffuse for 30 minutes at room temperature before being incubated at 37 C for 18-24 hours. Plates were examined after incubation for the formation of a clear zone around the well, which correlates to the antibacterial activity of the substances studied. The zone of inhibition (ZOI) was measured in millimetres.

RESULT AND DISCUSSIONS

Characterization of metal nanoparticles

UV-Visible analysis

UV-Vis spectroscopic analysis showed the presence of absorption peak at 267 nm [Fig. 1] which confirmed the formation of the CuNPs. The spectrum depicted the *Cassia absus*seed extract mediated bio reduction of the $Cu^{(2+)}$ to $Cu^{(0)}$. The obtained result is in agreement with *Shewanella loihica* mediated biosynthesis of CuNPs.

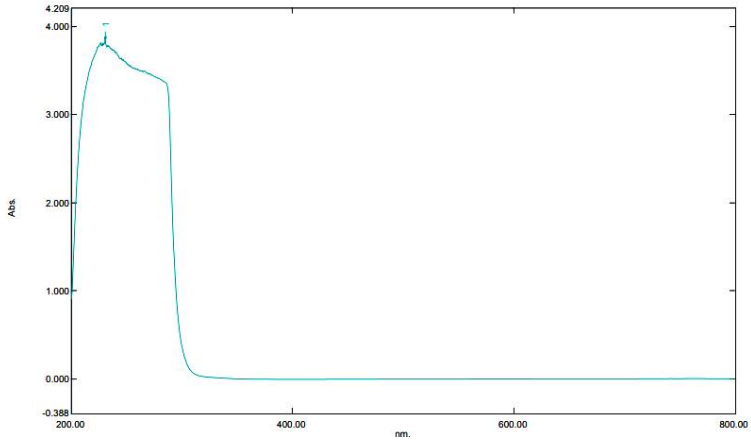

Fig.1. UV-Visible analysis of *Cassia absus* seed mediated synthesis CuNPs

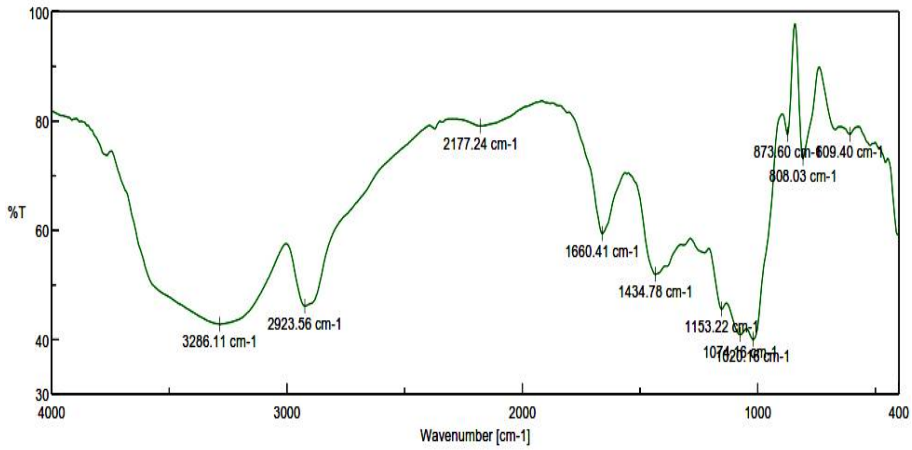

Fig.2 FTIR analysis of *Cassia absus* seed mediated a) CuNPs b)CuNPs

The IR spectra investigated [Fig 2] for the mushroom extract and CuNPs revealed the absorption peaks at (i) 3286 cm^{-1}, 2923 cm^{-1}, 2777 cm^{-1}, 1660 cm^{-1}, 1434 cm^{-1}, 1153 cm^{-1}, confirm the OH group of alcohols and phenols ,C-O group of carboxylic acid group); C=O stretching of carboxylic acid group, C-OH vibrations of the protein/polysaccharide. The peak intensities of the IR spectra of the aqueous extract of *Cassia absus* seed and the biofabricated CuNPs were found to be significantly different. There were significant differences in the signal strength of these peaks between the *Cassia absus* seed extract and the CuNPs, implying that bio molecules like phenols, carboxylic acids, and polysaccharides present in the *Cassia absus* seed aqueous extract played an important role in the reducing agent for the CuNPs synthesis.

SEM-analysis

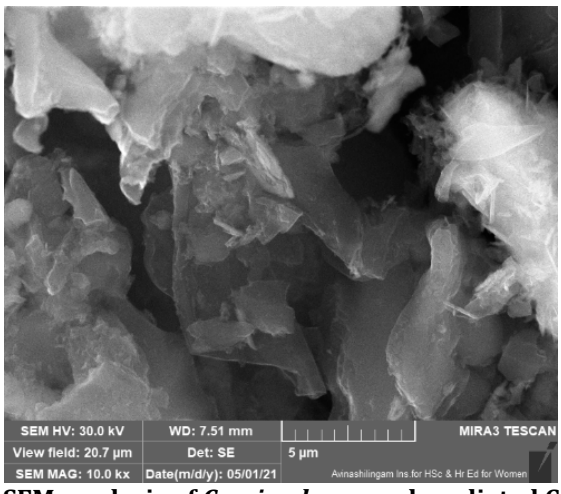

Fig.3. SEM, analysis of *Cassia absus* seed mediated CuNPs

Figure 3 shows a SEM image of *Cassia absus* seed mediated copper nanoparticles. CuNPs were seen as an agglomerated structure. Agglomeration could be caused by the hydroxyl groups found in *Cassia absus* seed. The elemental composition was investigated using the energy dispersive spectrum (EDAX). The presence of Copper is confirmed by the peak in the EDAX spectrum.

Biological Applications:

Anti-inflammatory activity

CuNPs mediated by mushrooms were studied for their anti-inflammatory properties. Figure 4 shows the percentage of RBC stabilisation by biosynthesized CuNPs. The oxidation of haemoglobin and the rupturing of the human RBC membrane occurred when RBC cells were placed in a hypotonic solution. Lipid peroxidation then causes free radical generation in burst cells. *Cassia absus* seed extract mediated CuNPs were thought to have anti-inflammatory properties through stabilising the RBC membrane.The RBC membrane stabilisation data revealed a concentration range of 10–100 µg/ml. CuNPs had the highest inhibition of 98 percent at 100 µg/ml. Finally, even at a concentration of 10 g, the biosynthesized CuNPs showed efficient RBC membrane stability. CuNPs contain bioactive components such as flavonoids and phenolic compounds, which have anti-inflammatory properties.

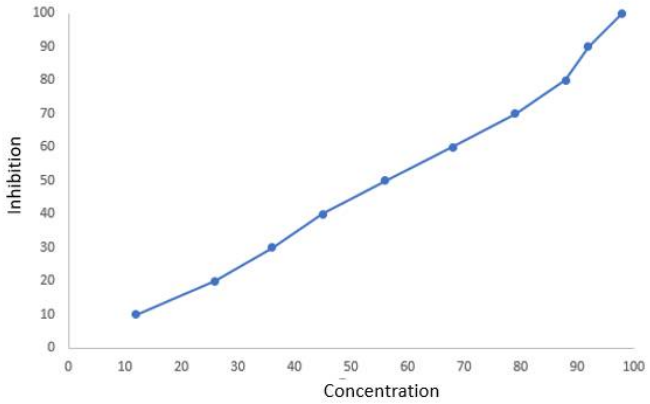

Fig. 4: Anti-inflammatory activity of CuNPs

Anticancer activity

When mushroom-mediated CuNPs were tested against SW620 colon cancer cell lines, they displayed dose-dependent cytotoxicity (20 to 100 µg/ml) (Fig.5b). At modest concentrations of CuNPs (25 µg/ml), maximum cell viability (98%) was found. At high concentrations (100 µg/ml), however, the viability of SW620 cells dropped by up to 82 percent. When copper nanoparticles were put into the MTT experiment, they caused morphological alterations in SW620 cancer cell lines, but no morphological changes were found in untreated SW620 cancer cells. At a maximal concentration of 100 µg/ml, copper nanoparticles were less harmful to SW-620 colon cancer cells. After 24 hours, cell mortality rates of 44.3 and 82.97 percent were observed at varied concentrations of nanoparticles, such as 25 and 50 µg/ml.

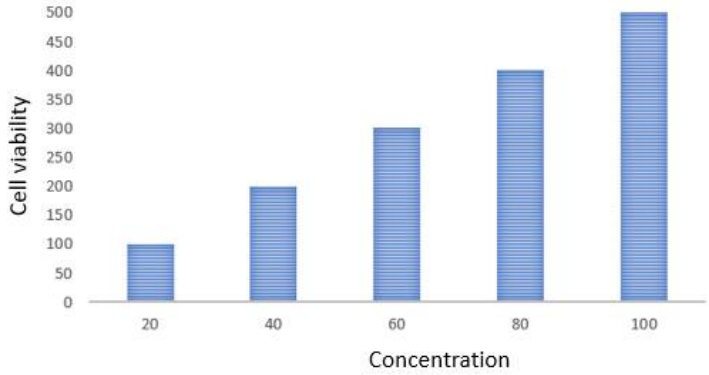

Fig.5. Anticancer activity of *Cassia absus* seed mediated CuNPs

Anti-bacterial activity

The antibacterial and antifungal curative efficacy of green produced CuNps was investigated using the agar well diffusion method, which was consistent with prior research. 20,41 CuNps' antibacterial activity was measured in zones of inhibition against *Klebsiella pneumoniae* and *Staphylococcus aureus* (ZoI).

CuNps efficacy was shown to be considerable for all strains when compared to the standard treatment (Fig 6), with p p0.0001 in most cases. With a 21.37 mm zone of inhibition, the most efficacy was reported against *Klebsiella pneumoniae* and *S. aureus*.In bacteria, the zone of inhibition has been observed. The cell wall of bacteria, on the other hand, is comprised of peptidoglycan (a polymer containing sugars and amino acids), which is less rigid and allows CuNps to pass through easily. Furthermore, CuNps' antibacterial activity was found to be more effective against *Klebsiella pneumoniae* and *S. aureus* than other bacterial strains. CuONps penetrate within the bacterial cell due to alterations in membrane shape, which dramatically increases cell permeability and affects transport over the plasma membrane, resulting in cell death, according to previous research.

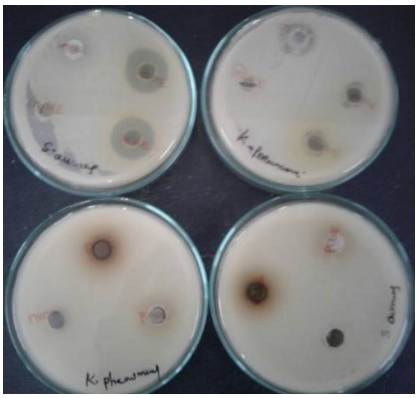

Figure 6. Anti bacterial activity of the Cu-Nano particles synthesis from *Cassia absus*seed.

CONCLUSION

Antibacterial, anti-inflammatory, and anti-cancer properties of green produced CuNps were investigated. All of the findings indicate that biological activity is high. Furthermore, the current research allows researchers to not only create pilot-scale procedures for sustainable nanoparticle synthesis from natural resources using a simple method, but also to test their antibacterial and antiviral properties. In comparison to other nanometals, there are very few research on microbial interactions with copper nanoparticles. They could open up new possibilities in the realm of nanomedicine by investigating the mechanisms of interaction of CuO Nps on long-term antibacterial efficacy.

CONFLICT OF INTEREST

The authors report no conflicts of interest in this work.

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