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"Green Synthesis of Copper Nanoparticles Using Edible Mushroom Cantharellus Species and Its Antimicrobial Potential"

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Abstract

Antimicrobial agents are the mainstay of treatment in case of any bacterial and fungal infections in humans. But a tremendous challenge is being poised at the scientific community as more and more of the microbial pathogens are developing resistance to the currently used antibiotics and antifungals day by day. Finding novel antimicrobial agents is the need of the current era. Nanotechnology is a relatively juvenile branch in science with great future potentials. In this study we have biologically synthesised copper nanoparticles by a cost effective and robust method using an edible mushroom *Cantharellus Species* found in Madhya Pradesh, India. Copper Nanoparticles showed significant activity against various bacteria and fungus. Nanoparticles were also characterized using Fourier Transform Infrared Spectroscopy (FTIR), Transmission Electron Microscope (TEM) and Atomic Absorption spectroscope (AAS).

Keywords: Cantharellus, mushroom, copper nanoparticles, green synthesis, antimicrobial.

1. Introduction

The increasing incidences of microbial resistance to a wide range of antimicrobial agents are raising a great concern worldwide. The extensive use of antibiotics has contributed to escalate antimicrobial resistance incidences (**Lee *et al.*, 2013; El Zowalaty, 2012**). There is no single antimicrobial agent available against which resistance has not been developed by either human or animal pathogen. In this regard, development of novel antimicrobial compound is need of the hour.

Nanotechnology is the study of behaviour of materials at nano scale. At this scale, the particles show different behaviour from that of their bulk materials. Among the various metals, the use of Copper and copper-based materials play a significant role because of their easy availability and low cost.

During the last few years, the green technology to synthesize nanoparticles (NPs) has found great significance owing to their ecofriendly characteristics and cost effectiveness as

compared to chemical and physical methods which are expensive and toxic. Green method involves the use of bacteria, fungi, different parts of plants or any biological material to synthesize NPs.

Among the wild edible mushrooms, bamboo mushroom, found in the Balaghat district of Madhya Pradesh, India, acquires remarkable position. It is locally known as "Baans Pihri" (Figure 1). It grows on decayed bamboo stems and rhizomes during rainy season. Its protein rich nutritive profile makes it a better choice for food and medicinal purpose. This study was aimed to derive a method for green synthesis of Copper Nanoparticles (CuNPs) from *Cantharellus Species* and evaluating its antimicrobial properties.

Figure 1. *Cantharellus Sp*.

2.Methodology

2.1 Collection of Mushrooms Sample:

Cantharellus sp. was collected from the Balaghat district of Madhya Pradesh in monsoon season and analyzed carefully for macroscopic and microscopic characteristics. The identification was done with the help of authentic standard literatures available.

2.2 Preparation of Mushroom Extracts:

Potato Dextrose Broth was used as a growth medium for fungal mycelia. The flask was kept in a rotary shaker at 28^{o}C for 5 to 7 days at 120 rpm. The broth was filtered and the filtrate was stored at 4°C.

2.3 Aqueous $CuSO_4$ Solution Preparation:

5mM $CuSO_4$ solution was prepared for synthesis of CuNPs.

2.4: CuNPs Synthesis:

Mushroom extract was mixed with 5 mM aqueous $CuSO_4$ in 1:2 ratio and kept in rotary shaker for 72 hours at 120 rpm **(Nachiyar *et al.*, 2015).** Visual changes in solution indicated the synthesis of Cu Nanoparticles. Presence of CuNPs was confirmed by UV-visible spectrometer (make Systronics, 2203) in the range of 200-600 nm. Different NPs exhibit characteristic peaks in UV-Vis spectrum making it easier to identify them **(Borgohain *et al.*, 2002; Yin *et al.*, 2005)**

Remaining solution containing CuNPs was purified using centrifugation and repeated washing with deionised water.

2.5 Determining the Antibacterial Efficacy of CuNPs:

Antibacterial activity of CuNPs was estimated using Disc diffusion method **(Bauer *et al.*, 1996).** 10 samples including both Gram positive and gram negative bacteria were procured for this purpose from MTCC, Pune, India.

To determine the antibacterial property of these CuNPs, sterilized discs were dipped in previously prepared mushroom's mycelial extract, $CuSO_4$ solution, CuNPs, ampicilin and water. After drying, these were placed on Muller-Hinton Agar (MHA) plates. Plates were incubated at 35^0C for 24 hours. After 24 hours, zone of inhibition of each disc was measured. All experiments were conducted 3 times and the mean value was taken into consideration.

2.6 Antifungal Activity: Antifungal activity against 5 fungi was determined using the technique of **Grover and Moore (Poisoned food technique, 1962).**

2.7 Characterization of CuNPs: CuNPs were characterized for shape, size and presence of capping agents using various techniques.

3. Results and Discussion

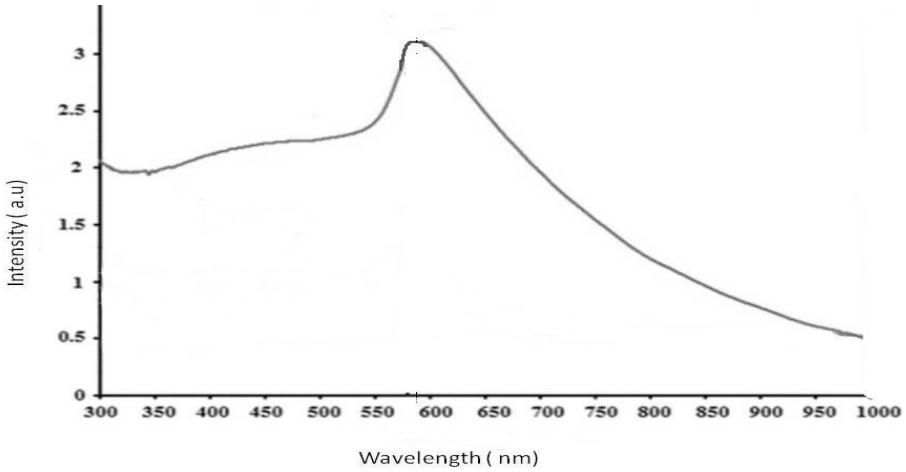
This study was performed to develop a green method to synthesize CuNPs from extracts of *Cantharellus sp.* and estimate their antimicrobial activity. The synthesis of CuNPs was first observed with change in colour from blue to brown (Figure 2) and confirmed by UV-Vis spectroscopy.

Figure 2 : Visual changes indicating CuNPs formation

3.1 Uv-Visible Spectra Analysis:

Purified CuNPs were analyzed in UV-Vis spectrometer. A sharp peak at 583 nm was observed, indicating synthesis of CuNPs. (Figure 3).

Figure 3 : UV-Vis spectra of CuNPs



3.2 Antimicrobial Susceptibility Test:

In this study, Kirby-Bauer method was used to determine antimicrobial activity of CuNPs against 5 gram-positive and 5 Gram- negative bacteria. *Staphylococcus aureus* had the highest zone of inhibition (31±1.2 mm) as compared to other gram positive bacteria. Similarly, *E.coli.* showed highest zone of inhibition amid all gram negative bacteria (33±1.2 mm) (Table 1).

Table 1: Antimicrobial activities of CuNPs

Sl no.	Micro organism	Zone of inhibition (mm)				
		Mycelial extract	$CuSO_4$	CuNPs	CONTROL	
					+VE (Ampicilin)	-VE (Water)
1.	***Staphylococcus aureus***	13±0.5	14±0.5	31±1.2	35±1.4	0
2.	***Bacillus subtilis***	14±0.4	14±0.3	28±1.3	32±1.2	0
3.	***Bacillus cereus***	14±0.3	15±0.4	29±0.9	30±0.9	0
4.	***Micrococcus luteus***	15±0.4	14±0.3	29±1.2	34±1.3	0
5.	***Listcria monocytogenes***	13±0.2	15±0.3	28±0.9	30±1.1	0
6.	***Salmonella enterica***	16±0.3	14±0.4	30±1.1	35±0.9	0

7.	***Aeromonas hydrophila***	14±0.5	14±0.3	29±1.1	34±1.6	0
8.	***Klebsiella pneumonia***	13±0.5	15±0.3	31±0.9	33±1.5	0
9.	***E. coli***	13±0.4	15±0.4	33±1.2	35±1.6	0
10.	***Pseudomonas aeruginosa***	14±0.3	14±0.5	30±1.3	34±1.3	0

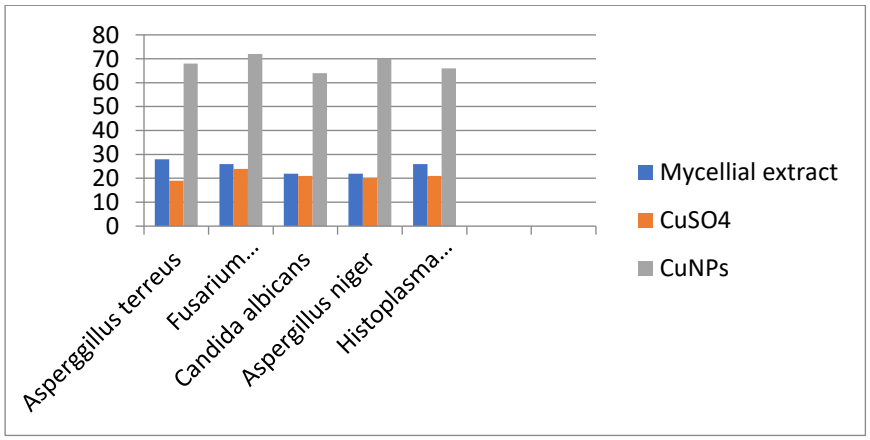
* Values are expressed as mean±SD.

The Findings are in accordance with the results reported by **Usman *et al.* (2013).** They demonstrated antimicrobial activity of conjugated CuNPs against many bacteria. Similar findings were demonstrated by **Abhiman *et al.* (2018),** where CuNPs synthesized from *Azadirachta indica* (neem) leaf extract, exhibited antimicrobial activity against some bacteria. **Giannousi *et al.* (2014)** have also reported antibacterial efficacy of copper based NPs in Gram-positive and Gram-negative bacteria. They also suggested that CuNPs act by degrading plasmid DNA. **Bogdanović *et al.* (2015) and Maqbool *et al.* (2017)** also reported similar outcomes. In other studies, relatively small sized CuNPs were found to be more effective as they can easily penetrate the cell **(Applerot *et al.*, 2012; Azam *et al.*,2012; Kruk *et al.*,2017).**

3.3. Antifungal Activity of CuNPs by Poisoned Food Technique:

The Antifungal activity of CuNPs was determined by Poisoned Food Technique. In present study *Fusarium moniliforme* exhibited 72 % mycelial growth inhibition (Figure 4). The experiments carried out by **Usman *et al.* (2013); Kruk *et al.* (2017) and. Divte *et al.* (2019)** support this result. They observed that antifungal activity increases with the increase in the concentrations of CuNPs. **Quaranta *et al.* (2011)** illustrated this activity of CuNPs was due to the accumulation of NPs inside the cell causing damage to the membrane.

Figure 4 : Antifungal activity of CuNPs



3.4 Characterization of CuNPs:

3.4.1. FTIR Analysis

The CuNPs were analyzed through FTIR in 400 to 4000 cm^{-1} range for presence of

functional groups surrounding them.

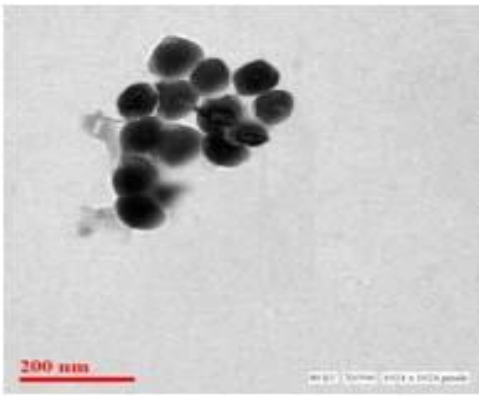
The FT-IR spectra of both the samples represent various functional groups. The extract sample spectra contain 6 significant peaks in the range of 569.59 cm^{-1}, 1107.57 cm^{-1}, 1421.22 cm^{-1}, 1637.72 cm^{-1}, 2934.08 cm^{-1} and 3446.58 cm^{-1}. All the bands signify some functional group present in the extract sample. Bands present at 1107.57, 1421.22, 1637.72 cm^{-1} corresponds to Ester linkages, lignin and cellulose, and amide I group respectively. Band present at 3446.58 cm^{-1} corresponds to proteins, carbohydrates, flavonoids and tannins. When mycelial extract was saturated with Cu^{2+} ions, minor shifts in these bands were observed which might be due to interaction between the cu^{2+} ions and functional groups present in the extract.

In case of FTIR spectrum of extracts with CuNPs, 5 main peaks at 1097.44 nm, 1398.69, 1656.45, 2921.72, 3437.25 are present. Presence of these groups suggests that they are acting as capping agent, preventing NP's agglomeration **(Bansal *et al.*, 2006).** The conversion of metal ion to a reduced state by mycelial extract can be attributed to the activity of various biomolecules present in the extract **(Ogundipe *et al.*, 2001; Marshall *et al.*, 2007)**.The findings of study conducted by **Mohamed (2020),** supports the same which showed presence of phenolic group on the surface of the nanoparticles, acting as a capping agent.

3.4.2. TEM Analysis

Sample for TEM study was prepared by coating the carbon coated copper grids with aqueous CuNPs. Extra sample was soaked with bloating paper. This grid was dried completely and analyzed in TEM. The result showed that the CuNPs were spherical with the average size of 72.4 nm.

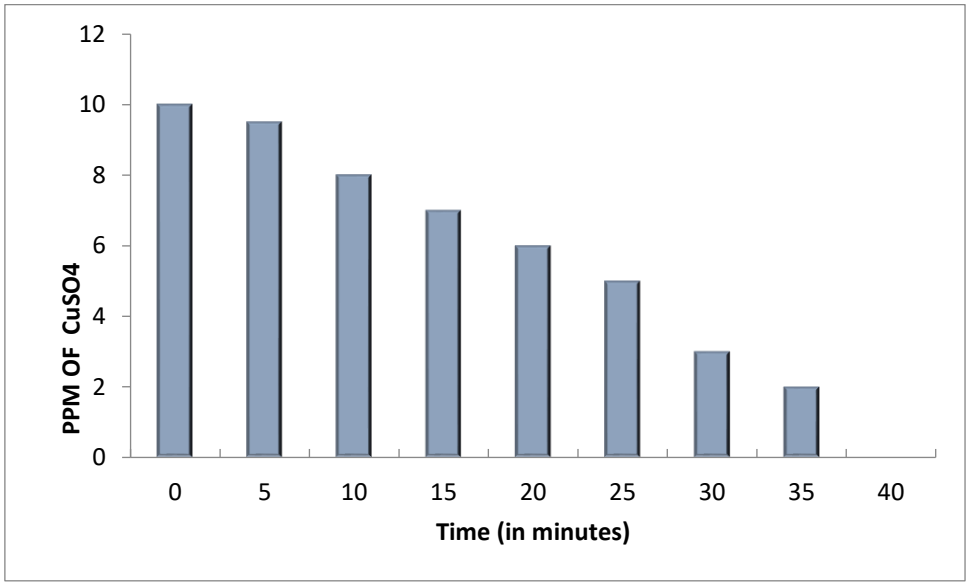
Figure 5: Transmission electron microscopic image of CuNPs



3.4.3. AAS analysis

AAS was used to determine copper ion concentration. The gradual reduction in the concentration of Cu^{2+} ions indicates the formation of Copper Nanoparticles. The Cu^{2+} ion concentration was measured before and after being reduced to Cu^{0} ion by mycelial extract.

Figure 6: AAS analysis



4. Conclusion

This study focuses on the novel technology to synthesize the cost effective and environment friendly Copper nanoparticles from naturally available edible mushroom. These nanoparticles showed antimicrobial potential against some bacteria and fungi. Further studies can be carried out to evaluate the in vivo effects of the nanoparticles which will help to bridge the gap between increasing antimicrobial resistance and decreasing trends of discovering novel antimicrobial agents.

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