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Comparative Study Of Antibacterial Activity Of Metallic Nanoparticles Synthesized From Edible Mushroom *Cantharellus* Species

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Abstract

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. In this regard, development of a novel compounds with the potential to fight against the ever-growing number of antimicrobial-resistant pathogenic microorganisms is need of the hour. In this study, we have synthesized Gold and Copper nanoparticles from the extracts of a wild mushroom and their activity against a broad range of bacteria is evaluated.

Key words: Nanoparticles, Green synthesis, Gold, Copper, Antibacterial activity

1. **Introduction-** Antibiotic Resistance (AR) is defined as the ability of bacteria to adjust and thrive despite the use of drugs that previously affected those (Founou *et al.*, 2017). Cases of bacteria surviving antibiotic effects have been observed since the introduction of antibiotics into civilization. One of the most difficult tasks for scientists has been to control and prevent the spread of drug-resistant pathogens. Researchers from all over the world have been striving very hard to develop some new antibiotic and antifungal materials to overcome this problem. Therefore, the creation of a new, safe, and natural agent to combat

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multidrug resistance organisms has become a pressing requirement. Nanotechnology as a whole has emerged as one of the most promising approaches in a variety of fields. The creation of nano-sized materials having distinct physical, chemical, and optical properties than their larger sized counterparts has led to their use in a variety of fields. The use of plants and fungi to synthesize metal nanoparticles is an important field of nanotechnology research, as it is an emerging eco-friendly method for forming nanoparticles of well-defined sizes and forms. Therefore, in present study the extracts of *Cantharellus* sp. Mushroom was used to synthesize Gold and Copper Nanoparticles (AuNPs and CuNPs) and their comparative antimicrobial activity is evaluated against a range of gram positive and gram negative bacteria.

2. Method and materials:

Collection and Identification of Mushroom: mushroom samples were collected from the deep bamboo forests of Balaghat (Madhya Pradesh) during monsoon season. The collected samples were identified on the basis of literature.

Preparation of Extracts: Mushroom fruits body was dried and grinded to prepare fine powder. The extract was prepared in double distilled water using Soxhlet apparatus.

Preparation of Metal Precursor for AuNPs: $HAuCl_4$ was used as a metal precursor for the synthesis of AuNPs. 50 ml of 3 mM $HAuCl_4$ was prepared in deionised water in a flask and covered properly.

Preparation Of Metal Precursor for CuNPs: $CuSO_4$ has been used to provide Cu ions for the preparation of CuNPs. 50 ml of 5 mM $CuSO_4$ was prepared in deionised water in a flask.

Synthesis of AuNPs and CuNPs: The Gold and Copper nanoparticles were synthesized by mixing the respective metal precursor solutions with the mushroom extract in 2:1 ratio (Nachiyar *et al.*, 2015). The mixture was kept in a dark place for 48 hours at room temperature. After 48 hrs, the mixture was observed for change in colour of the solution which is a primary indicator of synthesis of NPs.

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Purification of NPs: Both the solutions were centrifuged separately at 10000 rpm for 10 minutes. The supernatant was removed and precipitate was again centrifuged with de-ionized water at the same RPM. The precipitates found at the bottom of the tubes are purified AuNPs and CuNPs. These were freeze dried and stored for further tests and characterization.

Antibacterial Activity of AuNPs and CuNPs:

The test organism used for the study were MTCC bacterial isolates viz., *Salmonella enterica* (MTCC-3219), *Aeromonas hydrophila* (MTCC-1739), *Listeria monocytogenes* (MTCC-1143), *Micrococcus luteus* **(**MTCC-7950**),** *Bacillus cereus* (MTCC-6909), *Bacillus subtilis* (MTCC-1789), *Staphylococcus aureus* (MTCC-7443), *Escherichia coli* (MTCC-2331), *Klebsiella pneumoniae* (MTCC-9544) and *pseudomonas aeruginosa* (MTCC-3163). The antibacterial activity of both the NPs was evaluated using Agar disc diffusion method (Shameli *et al*., 2012).

3. RESULTS AND DISCUSSION:

Synthesis of AuNPs: In the present study when the 3 mM $HAuCl_4$ was mixed with aqueous extract of *Cantharellus* Sp. mushroom, change in colour of the mixture from yellow to red was observed after 48 hours evident for the synthesis of AuNPs. Change in colour was attributed to the surface Plasmon resonance (Sastry *et al.*, 2003). The synthesis was further confirmed by UV-Vis spectrophotometer which exhibited distinct absorption peaks at 565 nm , which is specific for AuNPs (Daisy, 2010).

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Figure 3.1: *Cantharellus* sp. Mushroom collected from the forest of Balaghat, Madhya Pradesh, India.

Figure 3.2 : Colour change indicating synthesis of AuNPs

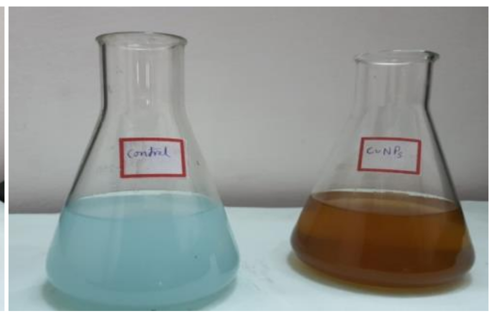

Figure 3.3: Colour change indicating synthesis of CuNPs

Synthesis of CuNPs: The change in colour was observed from blue to brown indication the synthesis of CuNPs. The presence of CuNPs in the mixture was further confirmed using UV-Vis spectrophotometer which exhibited absorption peaks at 585 nm, specific for CuNPs (Kaur *et al.*, 2016).

Antibacterial Activity of AuNPs and CuNPs: The antibacterial potential of AuNPs and CuNPs synthesized using green method were evaluated by disc diffusion method (Shameli *et al.*, 2012) against above mentioned 5 gram positive and 5 gram negative bacteria.

S. No.	**Microorganism**	Zone of Inhibition (mm)				
		Hot water extract	CuNPs	AuNPs	Control	
					+VE	-VE

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1.	*Staphylococcus aureus*	14±0.4	29±1.2	26±0.8	35±1.3	0
2.	*Bacillus subtilis*	13±0.5	27±0.4	27±0.4.	32±1.1	0
3.	*Bacillus cereus*	13±0.5	25±0.5	25±0.6	30±0.9	0
4.	*Micrococcus luteus*	12±0.3	26±0.4	25±0.7	34±1.2	0
5.	*Listeria monocytogenes*	13±0.5	27±0.5	24±0.7	30±1.1	0
6.	*Salmonella enterica*	13±0.5	28±0.3	25±1.2	35±1.4	0
7.	*Aeromonas hydrophila*	12±0.4	26±0.5	24±0.8	34±1.3	0
8.	*Klebsiella pneumoniae*	13±0.4	29±0.9	23±0.9	33±1.3	0
9.	*E. coli*	14±0.3	26±0.4	24±0.8	35±1.5	0
10.	*Pseudomonas aeruginosa*	13±0.5	27±0.5	26±0.5	34±1.5	0

In present study, the antimicrobial effect of AuNPs and CuNPs were evaluated against 5 Gram positive bacteria and 5 Gram negative bacteria. In case of AuNPs, among gram positive *Bacillus subtilis* showed highest zone of inhibition i.e. 27±0.4. Among gram negative bacteria, *Pseudomonas aeruginosa* was found to be more susceptible (26±0.5).

When treated with CuNPs, among gram positive bacteria *Staphylococcus aureus* exhibited highest zone of inhibition i.e. 29±1.2. Among gram negative bacteria, *Klebsiella pneumonia* showed maximum zone of inhibition (29±0.9). Our results are in line with findings of Usman *et al.* (2013) and Abhiman *et al*. (2018). They reported the similar antibacterial activity of conjugated CuNPs. The action mechanism of NPs may vary depending on many factors. Ahmad *et al* (2013) noted that AuNPs demonstrate remarkable antifungal activity that is dependent on their size, effectively killing *Candida* isolates. Moreover, the concentration of NPs also seems to be related to their antimicrobial effect. However, several studies disagree with findings of another, clearly indicating

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that there is no exact mechanism of NPs toxicity to microorganisms (Bolla *et al.*, 2011; Lara *et al.*, 2011; Musee *et al.*, 2011). Various studies have also shown that the rate of growth of a microorganism may also influence the action of NP (Mah and O'Toole 2001; Brown *et al.*, 2012). Consequently, antibacterial effects highly depend on the particular strain as well.

4. **Conclusion**: Synthesis of nanoparticles is usually the bottleneck in the research of nanotechnology as physical and chemical methods adopted for this process include the use of hazardous chemicals, the generation of damaging by-products, and excessive energy usage. But green synthesis of nanoparticles using mushroom (*Cantharellus* sp.) bypasses all these issues. Further studies can be undertaken to optimize this green synthesis method for large scale production of nano particles. The results of the study could act as a baseline for future exploration of green synthesized nanoparticles as antimicrobial agents which can be used in infective diseases.

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