IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
 Journal Volume 11, Iss 12, 2022

Phytofabrication and Antimicrobial Activity of Biologically Synthesized Silver Nanoparticles from *Ganoderma lucidum*

Tuneer Khelkar[1], Ashish Saraf[2*], Kamlesh Kumar Shukla[3] and Meghna Shrivastava[4]

[1,2,4]School of Sciences, MATS University, Raipur, C.G, India

[3]SoS in Biotechnology, Pt. Ravishankar Shukla University, Raipur, C.G, India

Abstract

A straightforward and reproducible technique known as "Green Chemistry" yields nanoparticles with improved stability and high dispersion in an aqueous solution. Plant extracts, bacteria, fungi and algae can all be used to synthesize nanoparticles. A popular medicinal mushroom with unique biological qualities, *Ganoderma lucidum* has antibacterial, antifungal, antioxidant, anti-inflammatory, and anticancer effects. In this study, $AgNO_3$ was reduced to produce silver nanoparticles (AgNPs) using aqueous mycelial extracts of *Ganoderma lucidum*. The hence synthesized nanoparticles on phytofabrication exhibited the presence of alkaloids, flavonoids, saponins and cardiac glycosides. Tested against strains of both Gram-positive and Gram-negative bacteria, the synthesized nanoparticles' antibacterial efficacy was determined. The pathogens were effectively inhibited by the silver nanoparticles, lowering the risk to the environment and public health in the process.

Keywords: *Ganoderma lucidum*, green synthesis, silver nanoparticles, antimicrobial activity

Introduction

The rise of drug-resistant bacteria and their increasing prevalence has made microbial diseases a serious public health concern (Franci *et al*. 2015). This has led to the development of nanotechnology as a means of finding alternative treatments. Among the various applications where nanotechnology can be used to effectively solve problems, metallic nanoparticles are well-known for having strong antibacterial action against human diseases (Roy *et al* 2019, Ronavari *et al*, 2021)

On December 29, 1959, physicist Richard Feynman gave a discussion titled "There's Plenty of Room at the Bottom" at a conference of the American Physical Society, which laid the foundation for the theories and concepts underlying nanoscience and nanotechnology (Feyman, 1960). The so-called "green chemistry" of synthesizing nanoparticles using biological means has numerous benefits over traditional chemical and physical techniques, including lower energy and resource consumption and environmental safety. These

straightforward and reproducible techniques yield nanoparticles with improved stability and high dispersion in an aqueous solution.Regarding its prospective medical uses for early disease detection, treatment, and prevention, it holds great promise for the design and development of numerous unique products (Marambio-Jones and Hoek, 2010).

Among the noble metals, silver has been used since the ancient time for burn wound treatment, dental work, catheters, and bacterial infection control, in forms of metallic silver, silver nitrate, and silver sulfadiazine (Tran *et al*., 2013).Since silver nanoparticles (AgNPs) exhibit optical, electrical, thermal, and biological qualities that make them useful in a variety of industries, food, medicine, and other sectors, they have been the subject of much research. Because AgNPs are so effective in dental applications, bone healing, bone cement, impregnating or coating catheters, and wound healing, their use in the biomedical realm has drawn special attention (Nagrathan*et al* 2022).

Many biological entities such as microorganisms (like bacteria, fungi, etc.), biochemical components (like enzyme, polysaccharide etc.) and plants are being used for the biological synthesis of nanoparticles (Mukherjee *et al*., 2001; Singh *et al*., 2016). However, the use of microorganisms especially the fungi is potentially exciting since they secrete plenty of enzymes and are also easy to handle. These properties of fungi have made them an ideal biological candidate for the synthesis of various metal nanoparticles (Dhillon *et al*., 2012). Comparatively, the higher fungi such as mushrooms in this regard have not received the much attention. Mushrooms are the fleshy fruiting bodies of the Basidiomycetes fungi, typically found above ground in soil, rotten woods, or trees.There have been several reports of *Ganoderma*'s unique biological features, including antibacterial, antioxidant, anti-inflammatory, antiproliferative, anticancer, antitumor, cytotoxic, anti-HIV, antidiabetic, and hepatoprotective effects, and their presence around the world (Kao *et al*, 2013).

Thus, *Ganoderma lucidum*, a significant medicinal mushroom, has been investigated in the current study for the synthesis of silver nanoparticles (Gurunathan *et al*., 2013; Kannan *et al*., 2014). Due to their ability to amass significant amounts of reduced nanoparticles, these fungi make up an ideal subject for nanobiotechnological experiments (Arun *et al.*, 2014). Therefore, it will be safer and more environmentally friendly for medicinal mushrooms, such as Ganoderma lucidum, to biosynthesize Ag-NPs. Using the wild medicinal mushroom Ganoderma lucidum, we have herein demonstrated the production of Ag-NPs and investigated their antioxidant and antibacterial capabilities against human infections. The

characteristics and antibacterial and antioxidant properties of the nanoparticles under synthesis were assessed.

Materials and Methods

Materials

Silver nitrate (AgNO3) was purchased from Fisher Scientific Pvt. Ltd, India. The six bacterial cultures were obtained from MTCC, IMTECH, Chandigarh, India. Nutrient agar, Mueller-Hinton (MH) agar and antibiotics disc for the antibiotics' susceptibility test were purchased from Hi-Media Laboratories Pvt. Ltd. Mumbai, India.

Collection of Sample

Medicinal wild mushroom *Ganoderma lucidum* was collected from Kanger Velly National Park and forest of Bastar C.G. The identification of sample was confirmed by Dr. Kamlesh Shukla, Assistant Professor, SoS in Biotechnology, Pt. Ravishankar Shukla University, Raipur, C.G.

Preparation of Fungal Extract

The collected fruit body sample of *Ganoderma* sp. was dried in an oven for three days at 40°C after being repeatedly cleaned with deionized water. A mortar and pestle were used to grind the dry sample into a powder. 200 ml of water was used to extract 5 grams of powdered material in filter paper thimble using Soxhlet extractor set to 80 °C for 6 hours. The resulting extract was then concentrated to 100ml at 60°C in a rotary evaporator after being filtered with Whatman No. 1 filter paper. The extract was refrigerated at 4ºC until further analysis.

Phytoconstituent Analysis of Fungal Extract

Terpenoid Test (Salkowski Test): 100 µl of aqueous mushroom extract containing AgNPs was mixed with 2 ml of chloroform and 3 ml of concentrated sulphuric acid to form a layer. A reddish-browncolor at interface is formed to show positive result of the presence of terpenoid and triterpenoids.

Steroid test (Salkowski Test): 100 µl of extract was dissolved in 2 ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish-brown color at the interface is an indicative of the presence of steroidal ring.

Saponin test (Foam test): Add 100 mg of powderedextract to 10 ml of distilled water. Heat the mixture and observe for persistent froth. Formation of froth indicates the presence of saponins.

Flavonoid test (NaOH test): 100 µl of extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes color less on addition of dilute acid, indicates the presence of flavanoids.

Tannin test ($FeCl_3$ test): - 100 µl of extract was treated with few drops of freshly prepared 6% $FeCl_3$. Formation of green color indicates the presence of tannin

Glycosides test (Fehling's Test): - Equal volume of Fehling A and Fehling B reagents were mixed and 2 ml of it was added to the 100 µl of extract and gentlly boiled. A brick red precipitate appeared at the bottom of the test tube indicates the presence of reducing sugars.

Coumarins: 200 µl of extract was mixed with few drops of 10% NaOH. Presence of yellow coloration indicates the presence of coumarins.

Alkaloids (Mayer's Reagent): One ml extract was taken in test tube and 2 ml of 2N HCL was added to it then solution was shaken vigorously to mix and kept for 5 minutes. Few drops of Mayer's reagent ($HgCl_2$+ KI in water) were added to it. Formation of creamy colour precipitation indicates the presence of alkaloids.

Phytofabrication of Silver Nanoparticles

For the synthesis of Silver nanoparticles, 10 milliliters of mushroom extract were added to 150 milliliters of a conical flask that held 90 milliliters of a 1 mM silver nitrate solution. The flask was then kept at 60 degrees Celsius in the dark, with periodic stirring of the reaction solution.

The consequent reduction of silver ions (Ag+) was monitored periodically for 24 h. After 4 hours of incubation, the colour of the reaction mixture changed from light yellow to pale yellow colour, further the colour was changed into dark brown indicating the formation of Ag-NPs (Gurunathan *et al.*, 2013).

Purification of Silver Nanoparticles

The Ag-NPs formed were collected by centrifugation at 10,000 rpm for 30 min at 4ºC. The clear supernatant was discarded and the pellet of colloidal silver was washed three times with double distilled water to remove impurities and the unbound extract components. Finally, Ag-NPs were dried at 60ºC in the hot air oven and were used for further analysis.

Antibacterial Assay

Antibacterial activity of the extracellular silver nanoparticles, synthesized by *Ganoderma lucidum* was tested by well diffusion method. Bacteria including *Klebsiella pneumoniae, Escherichia coli, salmonella typhi, Bacillus subtilis, Bacillus cereus and Staphylococcus aureus* were used as hosts to test the antimicrobial activity.

For the preparation of well, sterile cutter (6mm diameter cork borer) was used to prepare five wells in pre determined bacterial lawn petri plate, approximately of 6 mm in diameter, the cutter was placed in hot air oven at 180^{O}C for sterilization. Pure cultures of bacterial pathogens were sub cultured in the nutrient broth for overnight and turbidity of the culture was maintained by comparing with 0.5% McFarland standard. Overnight cultures of each bacterial strain were swabbed uniformly onto individual plates using sterile cotton swabs then wells were formed which loaded (25µl in each well) with different test samples in each cultured agar plates. After incubation at 37±2ºC for 24 h, the diameter of the zone of inhibition which appeared as a clear zone around the well was measured using antimicrobial sensitivity zone scale (HiMedia). Three sets of antimicrobial experiments were performed.

Result and Discussion

Survey and Collection of Samples

Antibacterial and anti-fungal compounds are essential for the survival of mushrooms in their natural habitat. Therefore, it is not surprising that more or less potent antimicrobial compounds have been isolated from many mushroom species and that they may be beneficial to humans.

The central part of India is a cultural habitat for many mushroom species due to the climatic conditions in the state. However, due to various modern developments, human ignorance and the indiscriminate harvesting of mushrooms, important species have become extinct and some are on the brink of extinction. Hence, there is an urgent need to prepare an

antimicrobial active mushroom map for the central part of India. The purpose of this study is to evaluate the antimicrobial activity of the wild mushrooms of the central India followed by biochemical synthesis of silver nano-particles using most potent species. To this end, extensive and regular surveys were conducted in important forests and tribal areas of central India during the monsoon season 2019-2020. Special visits were made to local markets to monitor and determine the edibility of the mushrooms. Large quantities of mushrooms available on the market were collected and isolated into pure culture.

Synthesis and UV-Vis spectrophotometric determination of AgNPs

Various extracts of *Ganoderma* sp. were prepared in different solvents viz. Hot water, ethanol and ethyle acetate. Water has a polar arrangement of oxygen and hydrogen, this property makes water capable of dissolving most of the substances as compared to other solvents. When various extracts of *Ganoderma* fruit body were mixed at different concentrations with 1mM $AgNO_3$ Solution, and incubated at room temperature for 6h so that different degree of AgNPs synthesized by different extracts of AgNPs but Hot water extract was able to synthesize good quality of **AgNPs**. It was evident from change of colour from yellow to red and then dark brown (fig.1), indicating the formation of AgNPs Change in colour was attributed to the surface Plasmon resonance (Ahmad and Beg, 2003). Purified **AgNPs** were analysed in UV-Vis spectrometer where they showed sharp absorbance at 440 nm which is specific for **AgNPs** (Daisy, 2010) (Fig. 2). Control Sets showed no change in colour and did not show any absorption at these wavelengths.

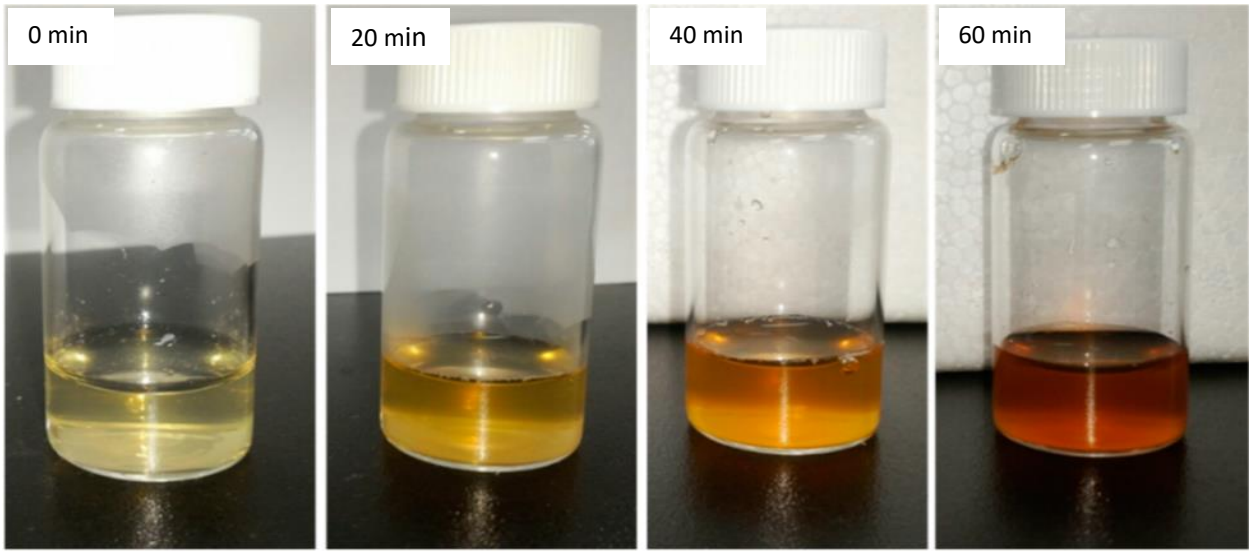

Fig. 1. Time dependent variations in the colour of colloidal solution of silver nanoparticles synthesized using aqueous extract of *Ganoderma lucidum*

ISSN PRINT 2319 1775 Online 2320 7876
 Journal Volume 11, Iss 12, 2022

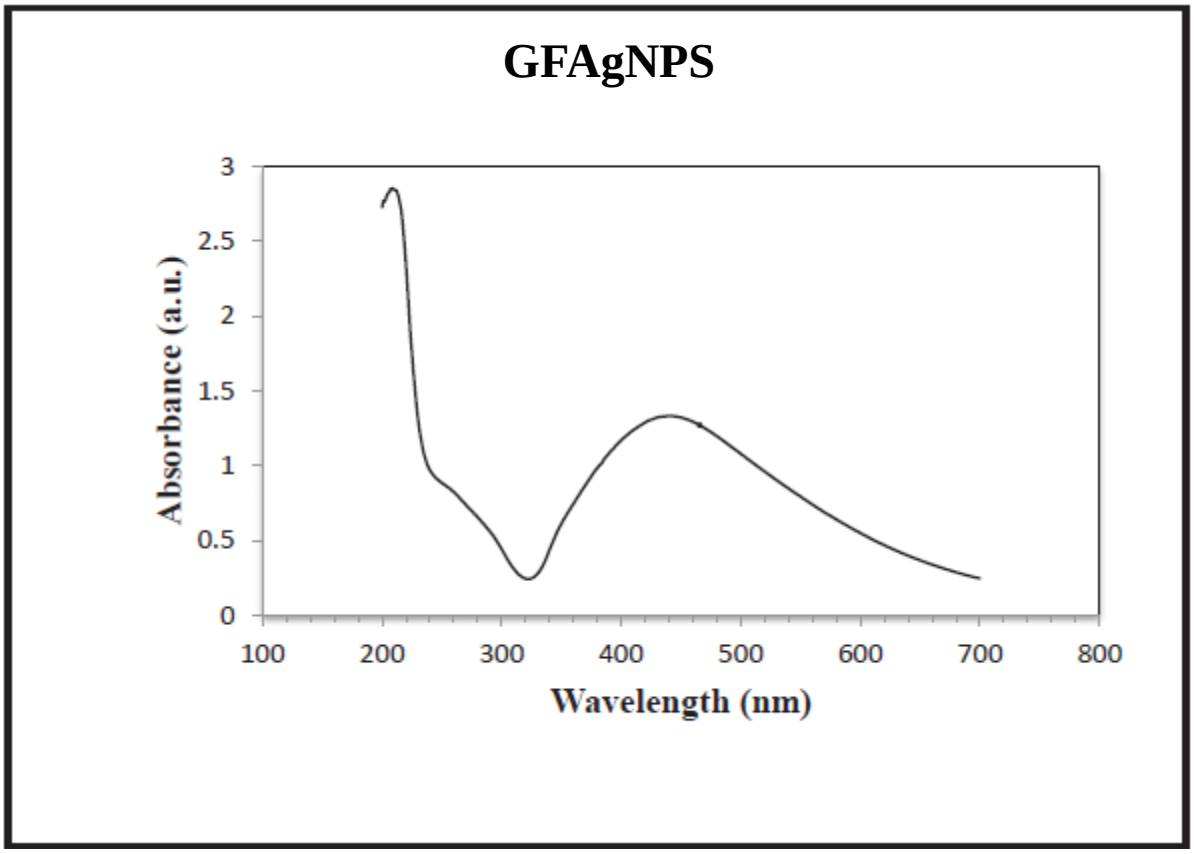

Fig. 2.2. Spectrophotometric determination of AgNPs synthesized using Hot Water Extract of *Ganoderma lucidum*

Preliminary phytochemical analysis

Antibacterial and anti-fungal compounds are essential for the survival of mushrooms in their natural habitat. The central part of India is a cultural habitat for many mushroom species due to the climatic conditions in the state. A preliminary phytochemical examination of *Ganoderma lucidum* aqueous extract revealed the presence of polyphenolic components such as tannins and flavonoids, as well as other metabolites such as alkaloids, carbohydrates, glycosides, terpenoids, and proteins. This result was in accordance to the previously reported literature (Shamaki *et al*., 2012) and is represented in Table-1.The presence of these phyto-constituents in fungal extract formed the basis for using the mushroom as a good source of AgNP synthesis.

Table 1: Preliminary phytochemical analysis of different solvents extract of *Ganoderma lucidum* fruit body.

S. N.	Phytoconstituents	Observation		
		Ethanol	Ethyl Acetate	Hot Water Extract (D/M)
1.	Alkaloids	-	-	+
2.	Carbohydrates	+	+	+
3.	Coumarins	-	-	-
4.	Flavonoids	+	-	+
5.	Glycosides	+	+	+
6.	Polyphenols	+	-	+
7.	Proteins (Amino Acids)	-	-	+
8.	Saponins	-	-	-
9.	Tannins	+	+	+
10.	Terpenoids	+	+	+
11.	Resins	-	-	-

'+'= Present and '-'= Absent

Evaluation of Antimicrobial Activity

During the course of the investigation potential antimicrobial activity of aqueous and organic extracts of the test isolates viz., *G. lucidum* N5/48b was determined. It is evident from the data recorded in Table 2 that extracts obtained from the test fungi showed significant activity against a wide range of bacteria. Antimicrobial potential of the test strains were further determined by well diffusion method.

It is clearly indicates that phytofabricated AgNPs from using hot water extract of *G. lucidum* N5/48b fruit body showed very strong inhibitory activity against *E. coli* at 1mM of concentration. It was followed by *B. brevis*, *B. cereus*, *B. megaterium* and *A. faecalis*. Inhibition was significantly declined against *S. typhimurium* and *P. putida* at same concentration. More or less similar trend was also recorded with silver nanoparticles synthesized with ethyl acetate (EAc-AgNPs) fraction against, *E. coli*., *B. brevis*, *B. megaterium* and *A. faecalis* while failed to inhibit *P. aeruginosa*. The plane AgNPs extract was also showed inhibitory activity against bacteria which was maximum against *B. brevis* followed by *B. megaterium* and *E. coli* while very less effective against *S. typhi* .

Significant inhibitory effect of extracts of *G. lucidum* N5/48b against yeast had also recorded during the investigation. GFAgNPs fractions of aqueous extract were highly effective against *Rhodotorula rubra*, *S. cervisiae* and *Schizosaccharomyces* sp. (Table 2). In comparison to

GFAgNPs fraction of hot water extract showed maximum sensitivity against these yeasts similar trends were also recorded with AgNPs in case of *R. rubra*. Minimum effect was recorded against *Schizosaccharomyces* (13.80mm).

Aqueous fraction gave considerable activity against test bacterial and fungal strains. There was no appreciable activity recorded against *C. glutamicum C. xerosis, E. Amylovora, V. heamoparalyticus, S. Dysentery, S. Sonnei, Candida albicans* and *Candida Utilis*. The macro fungi (*G. lucidum*) differ significantly in their activity against tested organisms. These differences may be attributed to fact that the cell wall composition of different microorganisms is different for instance yeast cell wall is quite complex than bacteria. Variation in effectiveness of *G. lucidum* N5/48b extracts and its nano particles against the target microorganism use may be due to differences in antimicrobial compounds present in these extracts. According to the findings of earlier workers, variety of extracts of *Ganoderma* and other macro fungus was effective (Benedict and Brady, 2012) but from the present study it is clear that antimicrobial activities of biologically synthesized silver nano-particles from fruit body extract (*G. lucidum*) and aqueous fraction were higher as compare to the ethanolic and ethyle acetate extracts which indicate that the antimicrobial compounds are extractable with hot water and efficacy of the molecule induced when turns into the nano particles.

There have been several reports available which showed that NPs don't have a single action mechanism of antibacterial action. According to Thill et al. (2006), the NPs adhere to the cell wall and alter its integrity, similarly in 20131, Kon and Rai the bacterial cell wall may be essential for the transportation of nanoparticles (NPs) into biofilm matrixes. Porins, peptidoglycans, and lipopolysaccharide compounds that are present on the outside of bacteria could similarly modify the action of NPs. According to Giannousi *et al*. (2014), NPs may destroy plasmid DNA. Some other factors like precursor metal, size, and the types of pathogen are also affect NPs activity. The experiment performed by Kon and Rai (2013), suggested that *E. coli* is more susceptible to silver nano particles (AgNPs), than *S. aureus* and *Bacillus subtilis*.

Table 2. Antimicrobial activity of fruit body extract of *G. lucidum* N5/48b through WDM

Tested microorganism	EAAgNPs	Hot Water Extract (D/M)	EAcAgNPs	Ref.
Aeromonas hydrophilla (MTCC 966)	13.00±0.00	15.83±0.16	11.00±0.32	30.20 ± 0.04

Alcaligenes faecalis (MTCC 2763)	12.16±0.06	30.00±0.01	16.85±0.10	31.00 ± 0.03
Alacalignes eutrophus *	14.00±0.22	27.83±0.45	16.30±0.05	34.00 ± 0.01
Bacillus subtilis (MTCC 1789)	13.16±0.05	25.80±0.15	14.00±0.00	32.50 ± 0.01
B. cereus (MTCC 633)	9.00±0.05	31.00±0.01	18.00±0.00	29.50 ± 0.07
B. brevis *	20.00±0.04	30.67±0.15	18.20±0.07	31.60 ± 0.07
B. megaterium (MTCC 2343)	18.00±0.00	30.50±0.74	17.30±0.15	32.00 ± 0.02
Corynebacterium glutamicum *	-	-	-	29.80 ± 0.10
C. xerosis *	-	-	-	28.20 ± 0.04
Enterobacter aerogenes *	13.50±0.02	26.63±0.05	16.20±0.20	30.10 ± 0.02
E. facalis *	12.00±0.00	22.00±0.05	13.50±0.04	34.50 ± 0.02
E. faecium *	14.00±0.11	21.83±0.16	13.00±0.05	28.50 ± 0.01
Erwinia amylovora	-	-	-	28.00 ± 0.23
Escherichia coli (MTCC 1591)	16.00±0.06	31.16±0.07	20.50±0.04	26.18±0.05
Klabsiella pneumoniae (MTCC 2405)	8.67 ±0.07	19.60±0.18	8.00 ±0.08	27.10±0.02
Proteus vulgaris (MTCC 842)	-	-	-	30.12±0.01
Pseudomonas aeruginosa (MTCC 779)	-	13.83±0.03	-	25.58±0.00
P. fluorescens (MTCC 1748)	-	11.24±0.10	-	27.00±0.01
P. putida	ND	10.50±0.08	-	29.30±0.15
Salmonella typhi (MTCC 531)	11.00±	17.00±0.05	7.30±0.01	28.00±0.15
S. Paratyphi	ND	11.83±0.07	-	29.15±0.19
S. typhimurium (MTCC 1008)	-	8.0±0.03	ND	28.15±0.08
Staphylococcus aureus (MTCC 187)	11.00±0.11	17.83±0.12	14.67±0.11	32.00±0.34
Staphylococcus epidermidis	-	15.83±0.16	-	30.50±0.02
*Staphylococcus sp.**	17.63±0.20	27.50±0.04	14.00±0.05	31.83±0.19
Vibrio cholerae (MTCC 1168)	10.50±0.05	18.50±0.06	-	27.50±0.16
V. heamoparalyticus	-	-	-	28.00±0.00
S. dysentery *	-	-	-	25.00±0.01
S. sonnei *	-	-	-	29.10±0.09
Candida albicans MTCC 1022	-	-	-	NT
Candida Utilis MTCC 847	-	-	-	NT
Rhodotorula aurantocia FGCC# SH-7(P)	10.80±0.03	18.50±0.22	-	NT
Rhodotorula rubra *	13.00±0.02	22.0±0.06	12.50±0.40	NT
Saccharomyces cerevisiae MTCC1732	9.50±0.02	17.50±0.03	12.67±0.06	NT
Schizosaccharomyces sp.*	7.80±0.05	13.80±0.06	7.50±0.09	NT

- Data are multiple of three observations
- Values ± standard error (SEM)
- WEM: Well diffusion method

- Ref.: Reference antibiotic
- *Microbial culture obtained from Chandrakar Pathology Lab. Raipur.
- ND: Not detectable, -: No activity, NT: Not tested

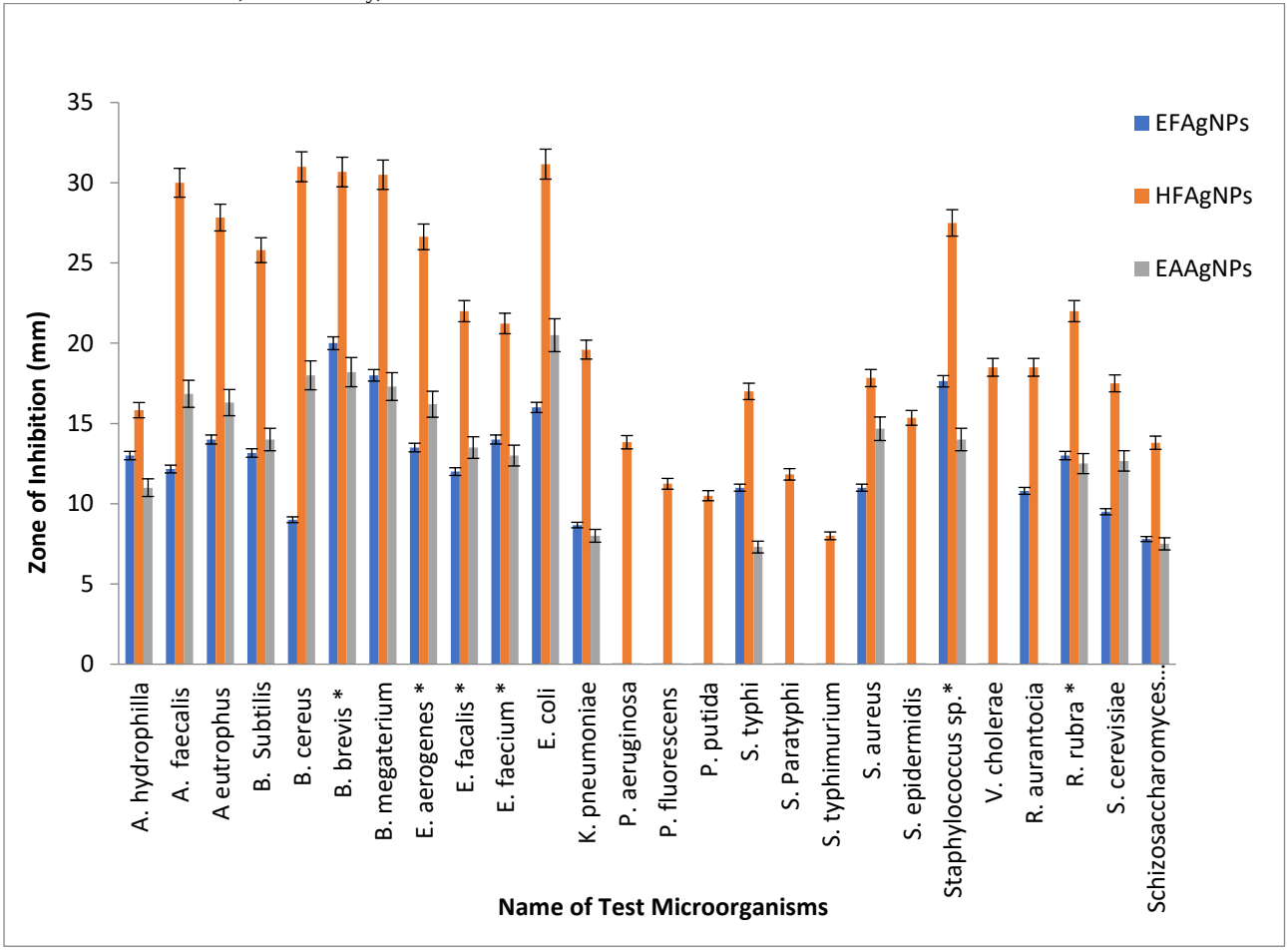

Fig.3. Graphical presentation of antimicrobial activity of fruit body extract of G. lucidum

Conclusion

The Present study reports the Green synthesis of AgNPs using fruit body extracts of wild edible mushroom *Ganoderma lucidum*. This method is environment friendly, cost effective, less toxic and proficient. It overcomes the problems associated with chemical and physical methods of nanoparticles production. This method can be used for large scale production of metal NP as it's very fast and efficient. AgNPs synthesized using this method showed significant antimicrobial activity against a range of microorganisms. The phytofabricated AgNPs ability to inhibit both Gram-positive and Gram-negative bacteria suggests that the active ingredients are broad-spectrum molecules. Nevertheless, before any phytoconstituents be utilised in the creation of pharmaceuticals, extensive research is required to identify those

responsible for the antibacterial action. The presence of carbohydrates, glycosides, triterpenoids, phenolic compounds, and tannins was revealed by preliminary phytochemical analysis. The presence of these phytoconstituents in the extract may account for the antibacterial activity. This study supports the claims made about the use of *G. lucidum* in the traditional medical system to treat a variety of infectious diseases brought on by microbes.

References:

[1.] Ahmad, I. and Beg, A.Z. (2003). Antimicrobial and Phytochemical studied on 45th Indian medicinal plants against multi-drug resistant human pathogens. *Journal of Ethnopharmacology*, 76: 113-123

[2.] Arun, G., Eyini, M. and Gunasekaran, P. (2014). Green synthesis of silver nanoparticles using the mushroom fungus Schizophyllum commune and its biomedical applications. *Biotechnology and Bioprocess Engineering*, 19: 1083-1090.

[3.] Benedict, R.C. and Brady, L.R. (2012). Antimicrobial activity of mushroom metabolites. *Journal of Pharmaceutical Science*, 61: 180-182.

[4.] Daizy, P. (2010). Green synthesis of gold and silver nanoparticles using *Hibiscus rosa* sinensis. *Physica E: Low-dimensional Systems and Nanostructures*, 42(5): 1417–1424.

[5.] Dhillon, G.S., Brar, S.K., Kaur, S. and Verma, M. (2012). Green approach for nanoparticle biosynthesis by fungi: current trends and applications. *Critical Reviews in Biotechnology*, 32: 49-73.

[6.] Feynman, R. P. (1960). There's plenty of room at the bottom—An invitation to enter a new field of physics. *Caltech Engineering and Science*, 23: 5.

[7.] Franci, G., Falanga, A., Galdiero, S., Palomba, L., Rai, M., Morelli, G. and Galdiero, M. (2015). Silver nanoparticles as potential antibacterial agents. *Molecules*, 20(5): 8856-8874.

[8.] Gurunathan, S., Raman, J., Malek, S. N. A., John, P. A. and Vikineswary, S. (2013). Green synthesis of silver nanoparticles using Ganoderma neo-japonicum Imazeki: a potential cytotoxic agent against breast cancer cells. *International Journal of Nanomedicine*, 43: 4399-4413.

[9.] Kannan, M., Muthusamy, P., Venkatachalam, U. and Rajarajeswaran, J. (2014). Mycosynthesis, characterization and antibacterial activity of silver nanoparticles (Ag-NPs) from fungus Ganoderma lucidum. *Malaya Journal of Biosciences*, 1: 134-142.

[10.] Kao, C., Jesuthasan, A. C., Bishop, K. S., Glucina, M. P., & Ferguson, L. R. (2013). Anti-cancer activities of *Ganoderma lucidum*: active ingredients and pathways. *Functional Foods in Health and Disease*, 3(2): 48-65.

[11.] Kon, K. and Rai, M.(2013). Metallic nanoparticles: mechanism of antibacterial action and influencing factors. *J. Comp. Clin. Pathol. Res*., 2: 160–174.

[12.] Marambio-Jones, C. and Hoek, E. M. (2010). A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. *Journal of Nanoparticle Research*, 12: 1531-1551.

[13.] Mukherjee, P., Ahmad, A., Mandal, D., Senapati, S., Sainkar, S. R., Khan, M. I. and Sastry, M. (2001). Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis. *Nano Letters*, 1(10): 515-519.

[14.] Naganthran, A., Verasoundarapandian, G., Khalid, F. E., Masarudin, M. J., Zulkharnain, A., Nawawi, N. M. and Ahmad, S. A. (2022). Synthesis, characterization and biomedical application of silver nanoparticles. *Materials*, 15(2): 427.

[15.] Rónavári, A., Igaz, N., Adamecz, D. I., Szerencsés, B., Molnar, C., Kónya, Z. and Kiricsi, M. (2021). Green silver and gold nanoparticles: Biological synthesis approaches and potentials for biomedical applications. *Molecules*, 26(4): 844.

[16.] Roy, A., Bulut, O., Some, S., Mandal, A. K. and Yilmaz, M. D. (2019). Green synthesis of silver nanoparticles: Biomolecule-nanoparticle organizations targeting antimicrobial activity. *RSC Advances*, 9(5): 2673-2702.

[17.] Shamaki, B.U., Geidam, Y.A., Abdulrahma, F., Ogbe, A.O. and Sandabe, U. K. (2012). Evaluation of phytochemical constituents and invitro antibacterial activity of organic solvent fractions of *Ganoderma lucidumm* ethanolic extract. *International Journal of Medicinal Plant Research*, 1(3): 026-031.

[18.] Singh, R., Wagh, P., Wadhwani, S., Gaidhani, S., Kumbhar, A., Bellare, J. and Chopade, B.A. (2013). Synthesis, optimization, and characterization of Acinetobacter silver calcoaceticus nanoparticles from and their enhanced antibacterial activity when combined with antibiotics. *International Journal of Nanomedicine*, 8: 4277-4290.

[19.] Thill, A., Zeyons, O., Spalla, O., Chauvat, F., Rose, J., Auffan, M., and Flank, AM. (2006). Cytotoxicity of CeO2 nanoparticles for *Escherichia coli*. Physico-chemical insight of the cytotoxicity mechanism. *Environmental Science & Technology*, 40(19):6151-6.

[20.] Tran, Q. H. and Le, A. T. (2013). Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, 4(3): 033001.