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ASSESSMENT OF ANTIOXIDANT ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED USING AQUEOUS EXTRACT OF *SCHIZOPHYLLUM COMMUNE* IN TRIAZOPHOS-INDUCED OXIDATIVE STRESS IN ALBINO RATS

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Keywords:

Silver Nanoparticles, Macrofungal extract, *Schizophyllum commune*, Triazophos, Oxidative stress, Antioxidant activity

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ABSTRACT: The present work includes the green synthesis of silver nanoparticles using aqueous extract of edible macrofungus *Schizophyllum commune*, and to compare the antioxidant potentialities of the aqueous extract and the extract-based silver nanoparticles using Albino Wistar Rats. The synthesized silver nanoparticles showed the SPR (Surface Plasmon Resonance) peak at 431 in the UV-VIS spectrum. The SEM images showed that the nanoparticles have cuboidal and roughly spherical morphology with a size ranging from 80 nm to 110 nm with an average size of 96.13nm. The FT-IR spectrum revealed the surface coating of functional groups of different secondary metabolites present in the macrofungal extract. The results of *in-vitro* assessment of antioxidant activity revealed that the aqueous extract has 21.28 % and the extract-based silver nanoparticles have 33.59% DPPH radical scavenging activity at 100µg/ml concentration. The *in-vivo* analysis involved the assessment of activity of key enzymes like Catalase (CAT), Super Oxide Dismutase (SOD) and Glutathione Peroxidase (GPx) in albino rats and the results showed that in Triazophos-intoxicated rats the CAT and GPx activity significantly ($p=0.05$) increased and activity of SOD was significantly ($p=0.05$) decreased in comparison to the control group of rats. The rats treated with aqueous extract of *S. commune* extract and extract based silver nanoparticles showed a significant ($p=0.05$) decrease in the CAT and GPx activity and a significant ($p=0.05$) increase in SOD activity in comparison to the Triazophos-intoxicated rats showing significant antioxidant activities of both the extract and the extract-based silver nanoparticles.

INTRODUCTION: Nanotechnology is the manipulation of matter on an atomic, molecular and supramolecular scale. In recent years, nanotechnology has gained a lot of attention from researchers because of the wide range of applications of nanoparticles, such as nano-medicines, nano-electronics, bio-based energy production, agri- and consumer products ^{1, 2, 3}.

Nanoparticles have a size range in nanoscale (10^{-9} m) with characteristic surface area to volume aspect ratio, which allows them to interact with other particles in a lot easier way ⁴. Among the metallic nanoparticles, the Silver nanoparticles (AgNPs) occupy a prominent place due to their significant biomedical applications like antibacterial impact, amelioration of skin damage, burns, infertility management, cancer treatment, etc ⁵.

The Silver nanoparticles are also reported to have marked biofunctional role in wound healing, drug-delivery system, catheter modification and bone healing along with significant antibacterial, anti-inflammatory, anti-cancer, anti-viral and anti-

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fungal activities ⁶. The chemical or physical techniques applied for the synthesis of silver nanoparticle are costly, energy consuming and can also have toxic impacts ⁷. But since biological methods of producing silver nanoparticles use plant extracts, microorganisms, fungi, and other less toxic, environmentally friendly, and economical alternatives, they are gaining popularity as a viable approach to producing silver nanoparticles ⁸. The macrofungi, or mushrooms, contain varieties of active biomolecules (secondary metabolites) which can act as reducing agents during the biological synthesis of silver nanoparticles and serve as capping substances, increasing their stability and potentially contributing to their biological activity ⁹.

Schizophyllum commune is an edible macrofungi, which is used as important dietary and traditional medicinal source by the local people of North-Eastern states of India. Kumar et al. (2018) ¹¹ had carried out the qualitative and quantitative analysis of bioactive constituent chemical compounds present in this macrofungus. This macrofungus is also reported to have various pharmacological efficacies like antioxidant, antibacterial, nephroprotective and hepatoprotective activities ¹². The present work was undertaken for the green and ecofriendly, cost-effective synthesis of silver nanoparticles using the aqueous extract of the edible macrofungus *S. commune*. The aqueous extract of the macrofungus reduces the silver ions present in the Silver Nitrate solution, which was confirmed by the color change of the solution. Furthermore, the synthesized silver nanoparticles were characterized by using Ultraviolet Visible (UV-VIS) analysis and Scanning Electron Microscopy (SEM) and the presence of macrofungal bioactive compounds as coating material was analyzed through Fourier Transform Infrared (FT-IR) spectroscopy.

The biochemical reactions taking place in our body continuously produces free radicals like Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (NOS) which include potentially damaging chemical species like Hydroxyl radical, Super Oxide anion radical, Alkoxyl radical, Hydrogen peroxide and Nitric oxide ^{11, 13}. These free radicals have one or more unpaired electrons, making them highly unstable and highly reactive chemical

entities that can react with and damage key biomolecular components of the cell such as DNA, proteins, enzymes, membrane phospholipids etc., resulting in cellular injury which may ultimately result into several diseases ¹⁴. The damaging impact of these free radicals is neutralized by specific molecules called Antioxidants which are either synthesized within the cells and/or supplied with the dietary sources ¹⁵. When the balance between antioxidants and free radicals is disrupted in any way, the cells and, as a result, the body, experience oxidative stress, which can have major health consequences and may even prove lethal ¹³. Therefore, the dietary sources which are rich in antioxidants are more likely to be preferred by the individual and edible mushrooms are one of the most important dietary sources which contain such antioxidant molecules in plenty ¹⁵. The present experimental macro fungus *Schizophyllum commune* is found to contain many biochemical constituent compounds as their secondary metabolites, like Alkaloids, Tannins, Flavonoids, Phenolics etc. in significant amounts and it's aqueous extract has been found to have significant antioxidant activity against several free radical species ¹¹.

Several previous works have reported that the silver nanoparticles, synthesized from extract of natural products like plants or macrofungi, have significant antioxidant properties ^{16, 17}. Since the aqueous extract of the present experimental macrofungus i.e. *Schizophyllum commune* has been found to have significant *in-vitro* antioxidant activity in the previous work of the author, therefore the author has extended this work to assess the *in-vitro* as well as *in-vivo* antioxidant potentiality of silver nanoparticles synthesized using aqueous extract of the Macrofungus *Schizophyllum commune* in albino wistar rats.

MATERIALS AND METHODS:

Preparation of Macrofungal Extract: Fresh fruiting bodies of *S. commune* were collected from Assam state of India and brought to Jharkhand for further experimentation. The sample was disinfected with HgCl₂, followed by repeated washing with distilled water. The sample was then allowed to dry under shade. The fully dried samples were then powdered and subjected to

solvent extraction through Soxhlet apparatus using water as solvent¹⁸.

Synthesis of Silver Nanoparticles: To synthesize the extract-based AgNPs, 1 ml of aqueous extract of *S. commune* was added to 99 ml of AgNO₃ solution of 10⁻³M concentration. The resulting mixture solution was subjected to continuous stirring at 90°C for 2 hrs. The mixture solution was then allowed to cool down. After 2 hrs, the mixture was subjected to centrifugation at 10000 rpm for 15 minutes at normal room temperature. The resulting supernatant was discarded, and the pellet was separated. The sediment is then washed with distilled water three times and then dried in an oven at 80°C overnight^{19, 20}.

Characterization of synthesized Silver Nanoparticles: The characterization of the synthesized silver nanoparticles was done by analysis of UV- visible spectrum, Fourier Transform Infrared Spectroscopy (FTIR) spectrum and Scanning Electron Microscopic (SEM) analysis.

UV-Vis Analysis: The UV-Vis analysis was done by using a Perkin Elmer, Lambda 25 UV-visible spectrophotometer (USA). An aliquot of the sample mixture solution was diluted with Milli-Q water. After 5 hrs, the reaction (reduction of Ag⁺ ions) between silver ions (Ag⁺) and the bioactive constituent compounds present in the macrofungal extract was monitored by UV-Vis spectrum.

FT-IR Spectroscopy Analysis: The sediment or pellet obtained after centrifugation was completely dried, and then the FT-IR spectrum of the dried sample was recorded by using the Shimadzu IR-prestige-21 (Shimadzu Corpn., Japan) spectrophotometer of resolution 4 cm⁻¹. The FT-IR spectrum was used to analyse the presence of the functional groups of bioactive compounds of macrofungal extract that might have coated or acted as capping agents on the synthesized silver nanoparticles.

SEM Analysis: The completely dried pellet was ground into powdered form. The dried sample was then mounted on a slide by using a double-sided adhesive and electrically conductive resin tape, followed by sputter coating by gold-palladium alloy. Now the sample was examined in the SEM

(JEOL JSM-6390 LV machine, Japan). This machine provides accessory software which, in synchronization with the magnification employed, can measure the size of nanoparticles using on-screen instructions and methods.

Dynamic Light scattering Analysis (DLS): Light scattering analysis of nanoparticles is an essential method for determining nanoparticles size in solution. It quantifies the light scattered from a laser travelling through a colloidal solution by analyzing the variation of scattered light as a function of time. The results of light scattering study show a size distribution by number, intensity, and volume. In the present study light scattering analysis of nanoparticles were carried on Malvern Nano ZS (U.K.).

In-vitro Assessment of Antioxidant Activity: The determination of antioxidant potentiality of the macrofungal extract based silver nanoparticles was done by following the previously established method of Moon and Terao (1998)²¹ with slight modifications^{11, 22}. In this method variable concentrations like 10, 50 and 100µg of samples were taken in Dimethyl sulfoxide (DMSO) and the volume was adjusted upto 500µl by adding Methanol. Then 5 ml of 0.1 mM methanolic solution of 1,1-diphenyl-2-picryl hydrazyl (DPPH) (Sigma –Aldrich, Bangalore, India) was added to the tubes containing the samples and the mixture was shaken vigorously. A control solution was prepared which didn't contain the sample but contains an equivalent amount of methanol. The tubes were kept at room temperature for about 20 minutes and then the absorbance was measured at 517 nm wavelength using Butylated Hydroxy Anisole (BHA) as reference standard. The percentage free radical scavenging activity of the sample was calculated using following formula:

$$\% \text{ radical scavenging activity} = (\text{control OD} - \text{sample OD}) / \text{control OD} \times 100$$

In-vivo Assessment of Antioxidant Activity:

Animals: Wistar Albino rats (*Rattus norvegicus*) of weight 175-200 gm were used for the present study. The rats were acclimatized and maintained at room temperature of 27±3°C and relative humidity of 50±15% under a dark-light cycle of 12 hrs duration. The rats were fed with commercial pellet diet and water *ad libitum*.

The present work has been approved by the ethical committee of Ranchi University (page no 137, proceedings no 46).

Acute Toxicity Studies: The oral toxicity studies and dose determination of the aqueous extract and extract-based silver nanoparticles were done by following the Organization for Economic Co-operation and Development (OECD) guidelines (2004)²³ for testing of chemicals. Two groups of 10 rats each were formed, and each group received different doses of the aqueous extract and extract-based silver nanoparticles orally through an oral feeding tube. In each case, mortality was not observed up to the dose of 1500 mg/kg BW (body weight) within 48 hrs.

Induction of Oxidative Stress: After acclimatization for 10 days, the rats were divided into four groups and the experiment was carried out as follows:

Group 1: Served as control, fed with olive oil.

Group 2: Intoxicated with 10% dose of LD₅₀ value (82 mg/Kg Body Weight) of an organophosphorus pesticide Triazophos, O,O-diethyl-1-H-1,2,4-triazol-3-yl Phosphorothioate, (TZ) dissolved in olive oil (Sharma and Sangha, 2014)²⁴.

Group 3: Received 500mg/Kg BW of aqueous extract of *S. commune*.

Group 4: Received 500 mg/Kg BW of extract based AgNPs of *S. commune*.

Collection of Blood and Assessment of Blood Parameters: The experiment was carried out for 21 days. At the end of the experimental time

period, the rats were kept fasting overnight, and then the blood was collected by puncturing the retro-orbital sinus under light ether anaesthesia without sacrificing the animals. The blood was centrifuged at 3000 rpm for 15 minutes to get the clear serum. Further, the serum was analyzed for different biochemical parameters.

Superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) are the three primary enzymes of the body's defence system against oxidative stress. Their activities have been evaluated to assess antioxidant potentiality^{25, 26, 27}. The SOD activity was measured by following the method of Sun *et al.* (1998)²⁸ which involves the inhibition of Nitroblue Tetrazolium (NBT) reduction with a superoxide generator like xanthine-xanthine oxidase. One unit of SOD activity is expressed as the amount of protein that inhibits the rate of reduction of NBT by 50%. The CAT activity was assayed by following the method of Aebi (1984)²⁹, in which the CAT activity is measured by decrease in absorbance per unit time at 240 nm (UV range) using a UV spectrometer (Shimadzu UV-1800), which in turn reflects the rate of decomposition of hydrogen peroxide. The GPx activity was measured by following the method of Beutler (1975)³⁰ and Yalcin *et al.* (2020)³¹, in which the GPx activity was measured by the decrease in absorbance at 340 nm due to the conversion of oxidised glutathione into its reduced form associated with the oxidation of NADPH to NADP⁺. The experimental data was analyzed with statistical tools like calculation of Mean, Standard deviation and t-test (for comparison of means of two comparative data sets).

RESULTS AND DISCUSSION:

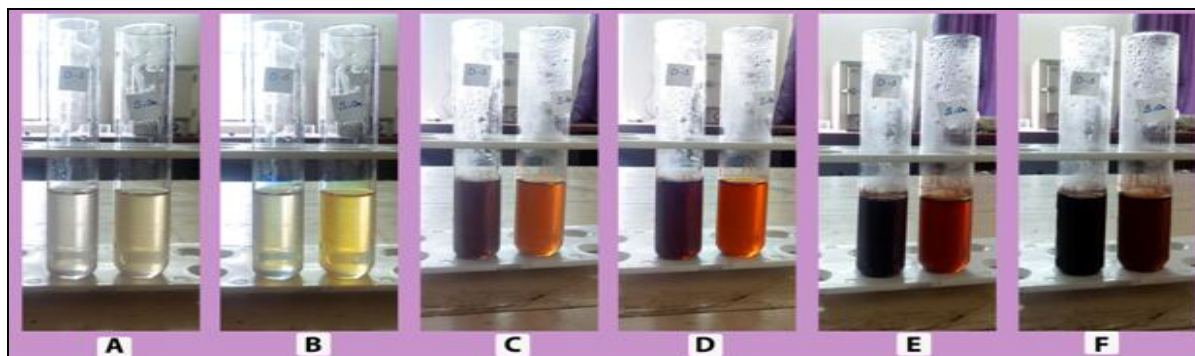


FIG. 1: FIGURE SHOWING GRADUAL COLOR CHANGE IN AQUEOUS SAMPLE MIXTURE SOLUTION GRADUALLY FROM A TO F

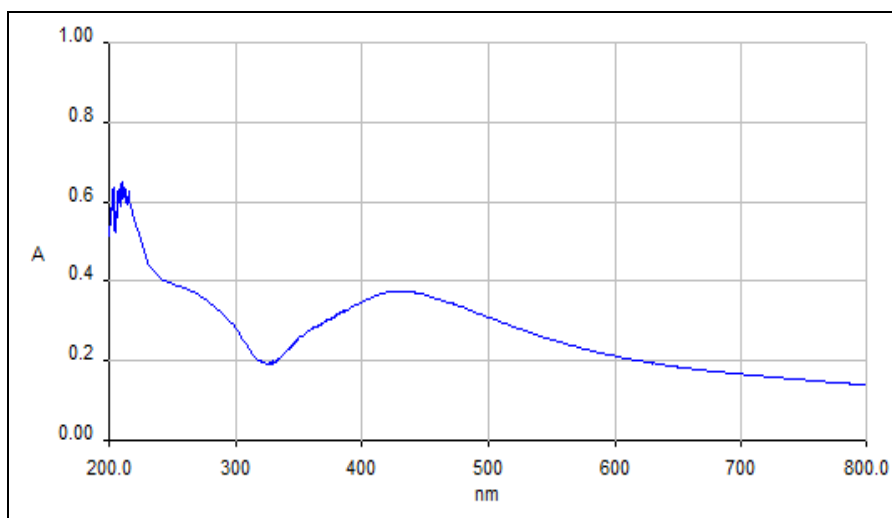


FIG. 2: UV-VIS SPECTRUM OF SILVER NANOPARTICLES SYNTHESIZED FROM *S. COMMUNE* EXTRACT SHOWING PEAKS AT 431 NM

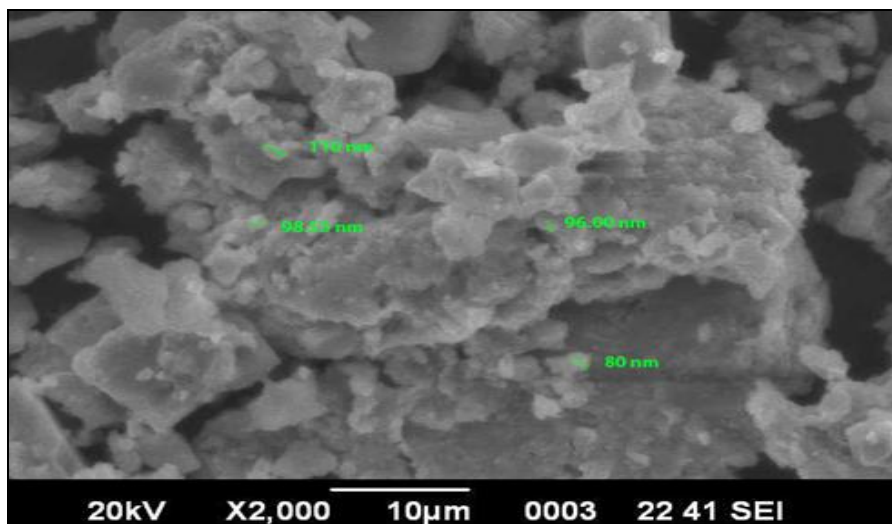


FIG. 3: SEM IMAGE OF *S. COMMUNE* EXTRACT MEDIATED SYNTHESIZED SILVER NANOPARTICLES

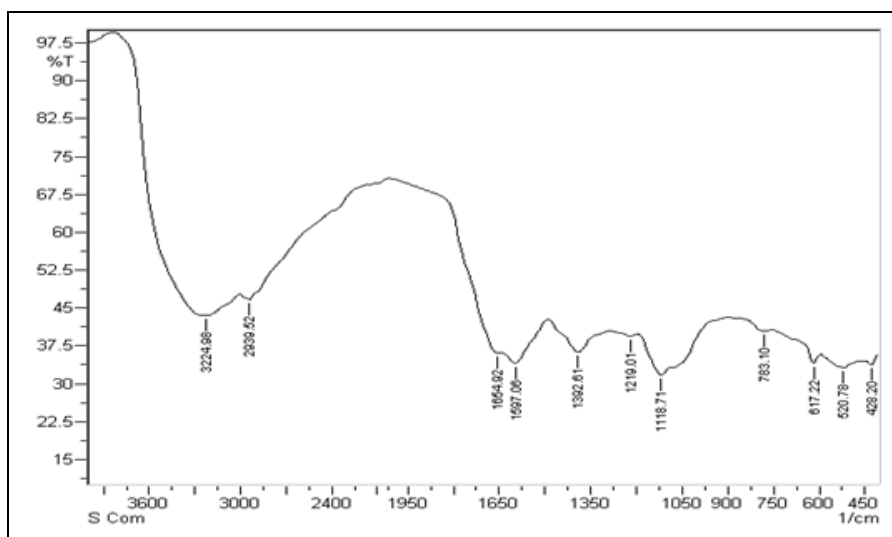


FIG. 4: FT-IR SPECTRUM OF *S. COMMUNE* EXTRACT MEDIATED SYNTHESIZED SILVER NANOPARTICLES

Antioxidant Activity:
In-vitro Assessment:

TABLE 1: % AGE FREE RADICAL SCAVENGING ACTIVITY OF AQUEOUS EXTRACT AND EXTRACT BASED SILVER NANOPARTICLES OF *S. COMMUNE*

Concentration	Aq. Extract of <i>S. commune</i>	Extract based Silver NPs	BHA
10	2.14	3.36	42.65
50	10.32	14.96	73.69
100	21.28	33.59	98.36

In-vivo Assessment:**TABLE 2: ACTIVITY OF CAT (CATALASE), SOD (SUPEROXIDE DISMUTASE) AND GPX (GLUTATHIONE PEROXIDASE) IN DIFFERENT ANIMAL GROUPS**

Animal Groups	CAT	SOD	GPx
Group 1	19.52±1.02	11.56±0.92	3.27±0.19
Group 2	28.89±0.93 ^a	7.26±0.42 ^a	5.92±0.21 ^a
Group 3	26.14±1.26 ^{ab}	9.78±0.36 ^{ab}	4.28±0.17 ^{ab}
Group 4	22.46±0.88 ^{ab}	10.35±0.75 ^{ab}	4.18±0.12 ^{ab}

^aSignificant when compared to Group 1, ^bSignificant when compared to Group 2; CAT (μ mole of H₂O₂ decomposed/minute/mg Protein), SOD (U/ mg Protein), GPx (U/mg Protein).

Reduction of Silver Ions Produces a Color Change: The mixing of macrofungal extract with the AgNO₃ solution has resulted in a gradual colour change in the mixture solution **Fig. 1**, which preliminarily indicates the reduction of silver ions and formation of silver nanoparticles ³². No further colour change was observed after 2 hrs, which indicates the completion of reduction of silver ions in the reaction mixture. Further confirmatory characterizations were done through analysis of SEM images, UV-Vis and FT-IR spectra.

UV-Vis Spectrum: The UV-VIS spectrum of the *S. commune* extract-based silver nanoparticles has been shown in **Fig. 2**. Previous studies have established that the plasmonic properties like Surface Plasmon resonance (SPR) in the visible range are correlated with the morphology of the silver nanoparticles, and therefore they can provide useful information regarding the silver nanoparticle synthesis by UV-Vis spectroscopy ^{35, 36}. Mie's theory predicts that spherical nanoparticles will have a single Surface Plasmon Resonance (SPR) band in their absorption spectra, whereas anisotropic particles may have two or more SPR bands, depending on their form ³⁷. The resulting band in the UV-Vis absorption spectra is due to the phenomenon of SPR, which in turn depends upon the size of the nanoparticles and the refractive index of the solution containing the nanoparticles ³⁸. Several previous studies showed that the SPR peak of silver nanoparticles ranges from 400 to 500 nm ³⁹. Borthakur *et al.* (2017) ⁴⁰ had studied the macrofungi-mediated silver nanoparticle synthesis and reported that the UV-Vis spectrum showed the

SPR peak at 400-450 nm. In our previous work ⁴¹ we had synthesized silver nanoparticles using *Punica granatum* leaf extract and found the SPR peak at 420 nm. Athira *et al.* (2017) ⁴² had synthesized silver nanoparticles using the macrofungus *Pleurotus florida* and reported the absorbance peak at 410 nm. In the present work we had observed the SPR peak at 431 nm, which infers the synthesis of silver nanoparticles in the present sample. Further, in the present work we got a single SPR peak (at 431 nm); hence it may be inferred that the silver nanoparticles synthesized are roughly spherical in shape.

SEM Analysis: The SEM image **Fig. 3** showed the shape and the dimensions of the synthesized nanoparticles. The nanoparticles were found to have cuboidal and roughly spherical morphology with a size ranging from 80 nm to 110 nm; average size was found to be 96.13 nm. Mustafa (2019) ⁴³ reported that the silver nanoparticles synthesized using *Pleurotus* (oyster mushroom) have a spherical shape and a size ranging from 2 to 100 nm. Adeyemi and John (2018) ⁴⁴ have synthesized silver nanoparticles using *Lentinus edodes* extract and reported that the nanoparticles had a size range of 50-100 nm.

FT-IR Spectrum: The analysis of FT-IR spectrum **Fig. 4** infers the presence of the functional groups from the bioactive chemical constituent compounds present in the macrofungal extract that act as reducing as well as coating or capping agent during the synthesis of silver nanoparticles. The FT-IR spectral peaks and their corresponding functional

groups have been listed in **Table 3**. The different characteristic peaks obtained in the FT-IR spectrum of *S. commune* extract-based silver nanoparticles have been compared with previously established

references for the presence of different functional groups^{45, 46}. The resultant peaks corresponding to different functional groups are as follows:

TABLE 3: THE PEAKS OF FT-IR SPECTRUM AND THEIR CORRESPONDING FUNCTIONAL GROUPS

Sl. no.	Peaks (cm ⁻¹)	Corresponding functional group
1.	3224.98 cm ⁻¹	Presence of hydrate (H ₂ O), Hydroxyl (-OH) and Amino groups, which refers to the presence of phenolic compounds and alcohols
2.	1392.61 cm ⁻¹ to 1654.92cm ⁻¹ , 1118.71 to 1219.01 cm ⁻¹ and 617.22 to 783.10 cm ⁻¹	presence of -OH groups
3.	1654.92 and 1597.06 cm ⁻¹	presence of double bonds and aromatic rings, which are found in flavonoid and tannin compounds
4.	1219.01 and 1392.61 cm ⁻¹	presence of proteins, nucleic acids and other carbon related compounds
5.	1118.71 cm ⁻¹	presence of C-O stretch which may be found in alcoholic compounds or ethers.
6.	783.10 cm ⁻¹	presence of C-H bond in aromatic ring with C-H, 1-3 (meta) distribution
7.	617.22 and 428.20 cm ⁻¹	presence of disulfide stretch (-S-S-)

The peaks obtained in the present FT-IR spectrum belong to the mid-IR region. The spectrum showing the broad spectral peaks at 3224.98 cm⁻¹ corresponds to hydrates (H₂O), hydroxyl (-OH) and amino groups, which refer to the presence of phenolic compounds and alcohols. The presence of -OH groups is further confirmed by the associated peaks in the range of 1392.61 cm⁻¹ to 1654.92cm⁻¹, 1118.71 to 1219.01 cm⁻¹ and 617.22 to 783.10 cm⁻¹. The peak in the range of 1654.92 and 1597.06 cm⁻¹ indicates the presence of double bonds and aromatic rings, which are found in flavonoid and tannin compounds. The peaks in the range of 1219.01 and 1392.61 cm⁻¹ refer to the presence of proteins, nucleic acids and other carbon-related compounds. The peak at 1118.71 cm⁻¹ region indicates the presence of C-O stretch, which may be found in alcoholic compounds or ethers. The peak at 783.10 cm⁻¹ refers to the presence of a C-H bond in an aromatic ring with C-H, 1-3 (meta) distribution. The peaks at 617.22 and 428.20 cm⁻¹ refer to the presence of disulfide stretch (-S-S-). The peak at 520.78 cm⁻¹ indicates the presence of aliphatic organohalogen compounds.

In-vitro Assessment of Antioxidant Activity: The result of the comparative *in-vitro* assessment of the antioxidant activity of crude extract and extract-based AgNPs of *S. commune* has been shown in **Table 1**. The results revealed that the extract-based silver nanoparticles showed comparatively more free radical scavenging activity in comparison to

the crude extract. The aqueous extract of *S. commune* showed 2.14, 10.32 and 21.28% DPPH radical scavenging activity at 10, 50 and 100 µg concentrations respectively, whereas the extract-based AgNPs of *S. commune* showed 4.36, 17.96 and 33.59% DPPH radical scavenging activity at 10, 50 and 100 µg concentrations, respectively. Athira Ragunath *et al.* (2017)⁴² had reported that the extract-based AgNPs of macrofungus *Pleurotus florida* showed increasing DPPH radical scavenging activity with increasing concentration and found to show 96.48% DPPH radical scavenging activity at 200 µg concentration. Aegun *et al.* (2020)⁴⁷ have reported that the silver nanoparticles synthesized from Reishi mushroom showed 76.45% DPPH radical scavenging activity at 250 mg/L concentration. Several works to date have reported that silver nanoparticles synthesized from plant or mushroom extracts exhibited marked antioxidant activities⁴⁸. The results of the present experimental work clearly infer that the extract based AgNPs have more antioxidant potentiality in comparison to the aqueous extract of *S. commune*.

In-vivo Assessment of Antioxidant Activity: The *in-vivo* assessment of antioxidant activity of extract-based silver nanoparticles of *S. commune* involved the measurement of the activity of three key enzymes among other enzymes of the antioxidant defence system of the body, viz., CAT, SOD, and GPx (results are shown in **Table 2**) CAT is found in almost all the organs of the body and is

responsible for the breakdown of H_2O_2 into H_2O and O_2 , thereby protecting the cells from oxidative damage by the Reactive Oxygen Species (ROS) ⁴⁹. SOD catalyzes the disproportionation or dismutation of superoxide anion (O_2^-) into O_2 and H_2O_2 and is found in three forms in mammals: SOD1, SOD2 and SOD3, out of which SOD3 is extracellular ⁵⁰. Superoxide radical is formed in the body as a byproduct of the metabolism of oxygen and is responsible for causing oxidative damage if not regulated ⁵¹.

The H_2O_2 produced as a result of SOD activity, i.e., dismutation of superoxide radical is also damaging, and hence it is decomposed by the activity of other enzymes like catalase. The GPx is a family of enzymes which reduces H_2O_2 into H_2O and lipid hydroperoxides into their corresponding alcohols, thereby protecting the body against oxidative damage ⁵². GPx3 is one of the members of this enzyme family abundantly found in blood plasma ⁵³. Previous studies reported that a decrease in GPx levels might be responsible for increased life expectancy in mice ⁵⁴. In fact, under normal physiological conditions there exists a very delicate balance between the rate of formation of H_2O_2 by the diproportionation of superoxide radical, and the rate of removal of H_2O_2 by the activity of GPx and CAT.

Previous studies reported that the triazophos-induced oxidative stress in rats results in an increase in the activity of CAT and GPx associated with a decrease in the activity of SOD ⁵⁵. The results of the present study showed that in triazophos-intoxicated rats (Group 2), the CAT and GPx activity significantly ($p=0.05$) increased, and the activity of SOD was significantly ($p=0.05$) decreased in comparison to the control group of rats (Group 1). The rats treated with aqueous extract of *S. commune* (Group 3) showed a significant ($p=0.05$) decrease in the CAT and GPx activity and a significant ($p=0.05$) increase in SOD activity in comparison to the triazophos-intoxicated rats (Group 2). The rats treated with extract-based silver nanoparticles also showed a significant ($p=0.05$) decrease in the CAT and GPx activity and a significant ($p=0.05$) increase in SOD activity in comparison to the Triazophos-intoxicated rats (Group 2). Further, the decrease in CAT activity in extract based silver nanoparticles treated rats

(Group 4) was found to be significantly ($p=0.05$) more than the aqueous extract treated rats (Group 3) in comparison to the normal control group of rats (Group 1). But the increase in SOD activity and decrease in GPx activity in extract-based silver nanoparticles treated rats (Group 4) and the aqueous extract treated rats (Group 3) were not found to be significantly different. These results revealed that the aqueous extract as well as the extract-based silver nanoparticles has significant antioxidant activities, which were also found in the *in-vitro* assessment results.

CONCLUSION: The silver nanoparticles have significantly important applications in the biomedical field, and the green synthesis of silver nanoparticles using macrofungal extract is cost-effective and environment friendly. The silver nanoparticles synthesized using *S. commune* extract showed marked stability and significant antioxidant activity against the DPPH radical. The *in-vivo* assessment of antioxidant activity of aqueous extract and extrac- based silver nanoparticles also showed significant antioxidant activities. The present work is one of the few works going on in this field, and it could further be elaborated and extended towards new research areas in the field of green synthesis and biomedical applications of nanoparticles. Moreover, oxidative stress is a causative factor of several diseases and the ageing process in the body; therefore, any agent which is found to possess significant antioxidant activity might be developed as an option in the amelioration of such biomedical problems.

Declarations:

Ethical Approval: The present work is approved by the Institutional Ethical Committee of Ranchi University (page no 137, proceedings no 46).

Authors' Contributions: The idea, execution and manuscript writing is done by the main author Amar Kumar. Manoj Kumar and Rakesh Ranjan have assisted in Laboratory and animal handling. M.P. Sinha has guided the whole team with his valuable inputs time to time.

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