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Research Article

MYCOSYNTHESIS OF SILVER NANOPARTICLES: CHARACTERIZATION, ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY FROM *PLEUROTUS FLORIDA* (MONT) SINGER: A MACRO FUNGI

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ABSTRACT

Objective: To determine the antioxidant and anti-inflammatory activity of myco-synthesized silver nanoparticle (AgNP) from *Pleurotus florida.*

Methods: Fresh mushrooms were collected, processed, and the AgNPs were synthesized using standard reducing agent 1 mM of silver nitrate. The characteristics of synthesized particles were confirmed using ultraviolet, Fourier transmission infrared, X-ray diffraction, and energy dispersive X-ray analysis. These nanoparticles were subjected for antioxidant activity through 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay and anti-inflammatory activity using bovine serum albumin denaturation assay, respectively, and the percentage of inhibition of AgNPs was calculated with standard error mean.

Results: The AgNPs were recorded the absorbance band at 410 nm was the particle size of 20-50 nm with a rod in shape. In DPPH assay the antioxidant activity was recorded as 96.2% with 50% inhibitory concentration (IC50) value of 85 µg/ml. In anti-inflammatory activity, the activity was recorded as 92.10±0.005% and IC50 value was recorded as 100 µg/ml.

Conclusion: The results indicated the significant antioxidant and anti-inflammatory activity of AgNPs from *P. florida.* Hence, further *in vivo* studies are focused to confirm the anticancer and anti-inflammatory activities.

Keywords: *Pleurotus florida,* Mycosynthesis of silver nanoparticle, Anti-inflammatory, Antioxidant.

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INTRODUCTION

Nanotechnology is an emerging area of modern science due to its promising applications in the field of medical diagnostics; chemicals and materials industry; drug and gene delivery; tissue engineering and biochemical sensors and so on. The nanoparticles play a key role in a multidisciplinary manner due to their variable size, shape, and composition toward human benefits. Nanotechnology deals with the synthesis and fabrication of materials at the nanoscale level (1-100 nm) [1].

Silver nanoparticles (AgNPs) are the important class of nanomaterials required for a wide range of industrial and biomedical applications. AgNPs have been associated with antimicrobial and disinfectant agents due to their detrimental effects on target cells [2].

Several natural resources have been adopted for biosynthesis of NPs. The synthesis of nanoparticles from mushrooms is the emerging field of nanoscience, and the macrofungi have long been used as a valuable food source and as traditional medicines around the world. Mushrooms, the most priced commodity among vegetables, are highly nutritive with low calories good quality protein, vitamins, and minerals [3]. Number of researchers proved that the extracts of these macrofungi demonstrate significant biological properties such as antitumor [4]; anti-inflammatory [5]; antiviral, and immunomodulatory effects [6]. Fruiting bodies of mushrooms, namely, *Volvariella volvacea, Pleurotus sajor-caju, Pleurotus florida,* and *Ganoderma lucidum* have been used for the production of AgNPs. *Inonotus obliquus, Lentinula edodes, Agaricus bisporus,* and *Pleurotus ostreatus,* have been used for the production of AgNPs [7-10]. *P. florida* the most commonly cultivated edible, protein rich variety of oyster mushroom contains essential amino acids including cysteine and methionine; minerals and unsaturated fatty acids (87%), including linoleic acid [11-12] in India. It is also recognized as being an important source of biologically active compounds with possesses antibacterial, anti-inflammatory, antitumor, antiplatelet – aggregating, hyoplipidemic, hepatoprotective, and immunomodulatory activities [13].

Inflammation is termed as the complex function of the immune system on infection and injury that leads to removal and restoration of tissue structure and physiological function [14]. Inflammation is known as complex biological response of vascular tissues which act against aggressive agents, namely, pathogens, irritants, or damaged cells [15]. It is mainly addressed by majority of the drugs groups, namely, the steroidal anti-inflammatory drug and the non-steroidal anti-inflammatory agents and these conventional drugs are associated with numerous side effects which compelled the need for identification of alternative substances [16]. Purified natural compounds from plants have aided in the synthesis of new generation anti-inflammatory drugs with higher therapeutic value and lower toxicity and application of nanotechnology for drug development and delivery could be an alternative method to develop newer anti-inflammatory agents with sustained release and better efficacy [17]. This study has been aimed to determine *in vitro* antioxidant, and anti-inflammatory activity of AgNPs synthesized from *P. florida.*

METHODS

Sample collection

For biosynthesis of nanoparticles, the fresh mushrooms of *P. florida* were collected from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Preparation of mushroom extract

A total 20 g of fresh mushrooms of *P. florida* were washed repeatedly with double distilled water and transferred to 100 ml of sterile distilled water and boiled for 10 minutes and then filtered through Whatman No. 1 filter paper. The extract was stored at 4°C for further experiments.

Mycosynthesis of AgNPs

The filtrate was reduced and stabilized using the standard reducing agent for silver nitrate ($AgNO_3$) (1 mM). In a typical synthesis of AgNPs, the mushroom extract was added with 50 ml of 10^{-3} $AgNO_3$ aqueous solution (prepared in deionized water) and kept in a shaker at 150 rpm at 37°C which resulted in a change in color from orange to dark orange color within 80 minutes. Simultaneously, a positive control was maintained with mushroom extract deionized water was used as a negative control containing only $AgNO_3$ solution [18].

Characterization of AgNPs

Ultraviolet-visible (UV-vis) spectroscopy analysis

UV-visible spectroscopy analysis was performed on a JASCO V-530, UV-visible absorption spectrophotometer with a resolution of 2.0 nm between 200 and 600 nm possessing a scanning speed of 300 nm/minute, reactions between metal ions and mushroom extract were monitored by UV-vis spectra of AgNPs in aqueous solution [19].

Fourier transmission infrared (FT-IR) spectroscopy measurements

The residual solution after the reaction was centrifuged at 10,000 rpm for 15 minutes and the resulting suspension was centrifuged repeatedly for 3 times, after that the purified suspension was washed with deionized water to get a pure form, i.e., free of proteins/enzymes which may not able to cap with the AgNPs. The sample was completely dried at 60°C. Finally, the dried nanoparticles were analog by FT-IR (Thermo Nicolet nexus 670 spectrometer of resolution 4 cm^{-1}).

Scanning electron microscopy (SEM)

For SEM, the AgNP synthesized using mushroom (*P. florida*) was allowed to dry completely and ground well to a powder. Since the specimen was at high vacuum, fixation was usually performed by incubation in a solution of a buffered chemical fixative, such as glutaraldehyde. The dry specimen was mounted on a slide using an adhesive epoxy resin of electrically – conductive double-sided adhesive tape and sputter coated with gold palladium alloy before examination in the microscope.

X-ray diffraction analysis (XRD)

The XRD technique was used to analyze the metallic nature of particles. After bioreduction, AgNPs solution thus obtained was purified by repeated centrifugation at 5000 rpm for 20 minutes followed by re-dispersion of the pellet of AgNPs into 10 ml of sterile deionized water. After freeze drying of the purified silver particles, the structure and composition were analyzed by XRD. The dried mixture of AgNPs was collected for the determination of the formation AgNPs by INEL X-ray diffractometer.

Antioxidant activity

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay

Free radical scavenging activity of methanolic extract of nanoparticle synthesized from *P. florida* was performed using DPPH assay. 0.1 mM solution of DPPH was prepared in 100% methanol, and 1 ml of this solution was added to 4 ml of sample in 40% methanol at various concentrations (10-160 µg/ml). This reaction mixture was shaken thoroughly and incubated for 30 minutes at 37°C in the dark. The reduction of the DPPH radical was measured by continuous monitoring of the decrease of absorption at 517 nm using a blank containing the same concentration of sample without DPPH. L-ascorbic acid was taken as standard [20]. Percentage of inhibition was calculated using the following equation:

%inhibition={ A control−A sample/A control}×100

A control=Absorbance of control (which contain equal volumes of DPPH solution and methanol without any test compound).

A sample=Absorbance of the sample.

The extract concentration providing 50% inhibition (IC50) was calculated from the graph.

Anti-inflammatory activity

Bovine serum albumin (BSA) denaturation assay

The synthesized nanoparticles were dissolved in methanol to obtain a stock solution with the concentration of 10 mg/ml. Different volumes (100-500 µl) were diluted with methanol to obtain a final volume of 1 mL. To 50 µL of each dilution, there was added 5 mL BSA 0.2% in Tris buffer saline, pH=6.8 and the final concentrations of samples in the test tubes were 10 µg/mL, 20 µg/mL, and 50 µg/mL, respectively. A mixture of 50 µL methanol and 5 mL BSA 0.2% was used as a control. The samples and the control were incubated at 37°C for 20 minutes and then at 72°C for 5 minutes. Finally, the samples and the control were cooled for 10 minutes, and the turbidity was measured at 660 nm in reference to Tris buffer saline solution. Diclofenac (0.1 mg/mL) was used as the standard anti-inflammatory drug being processed in a similar manner with the samples [21]. The inhibition of protein denaturation (%) was calculated using the following formula:

Inhibition of denaturation (%)=(A control−A sample/A control)×100,

Where, A control=Absorbance of the control; A sample=Absorbance of the tested compounds.

RESULTS

Biosynthesis of mushroom AgNPs

The preliminary confirmation of AgNPs from *P. florida* was obtained by the color change from yellow to dark brown by adding 1 mM solution of $AgNO_3$ after 24 hr.

Characterization of AgNPs

UV-vis spectroscopy

The biosynthesis of AgNPs from mushroom sample was characterized by a visual study using UV-vis spectrophotometer by recording the absorbance band at 410 nm which recorded the positive results in the mycosynthesis of the AgNPs (Fig. 1).

FT-IR spectroscopy (FT-IR)

FT-IR absorption spectra of myconanoparticles of *P. florida* revealed the various functional groups involved in the bioreaction of nanoparticles. Accordingly, the FT-IR spectrum showed the presence of various functional groups, namely, carboxylic acids, amines, ketones, esters, ethers, alkenes, alkynes, and alkyl halides (Table 1 and Fig. 2).

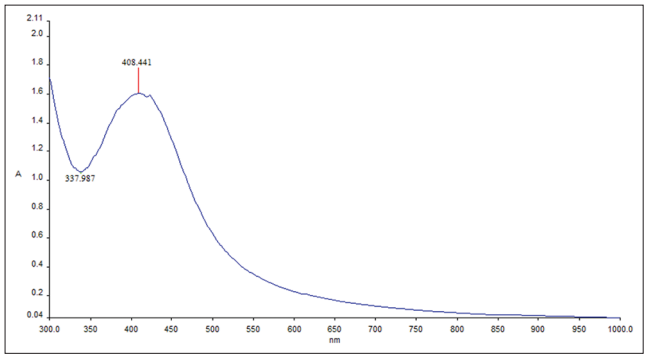

Fig. 1: Ultraviolet-visible spectrum of silver nanoparticle from *Pleurotus florida*

Table 1: FT-IR spectral analysis of AgNPs from *Pleurotus florida*

S. No.	Functional group assignment	Group frequency cm^{-1} of the sample
1.	-H-C-H asymmetric and symmetric, carboxylic acids	2983.88 cm^{-1}, 2931.80 cm^{-1}
2.	-C=O stretch, ketones, carboxylic acids	1728.22 cm^{-1}
3.	-N-H bend, primary amines	1600.92 cm^{-1}, 1579.70 cm^{-1}
4.	-H-C-H bend, alkanes	1446.61 cm^{-1}
5.	N=O bend, nitromethane	1392.61 cm^{-1}, 1367.53 cm^{-1}
6.	-C-O stretch, esters, ethers	1284.59 cm^{-1}
7.	-C-N stretch, aliphatic amines	1170.79 cm^{-1}, 1126.43 cm^{-1}, 1072.42 cm^{-1}, 1039.63 cm^{-1}, 1016.49 cm^{-1}
8.	-C-H aromatics	893.04 cm^{-1}, 864.11 cm^{-1}
9.	-C-Cl stretch, alkyl halides	744.52 cm^{-1}, 704.02 cm^{-1}, 563.21 cm^{-1}
10.	-C=C-H, alkynes	619.15 cm^{-1}

FT-IR: Fourier transmission infrared, AgNPs: Silver nanoparticle

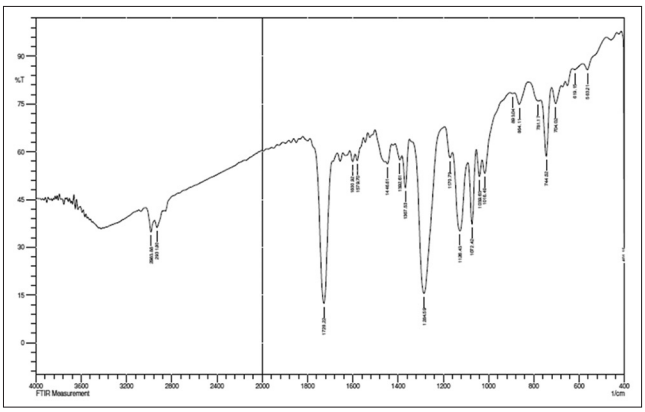

Fig. 2: Fourier transmission infrared spectrum of silver nanoparticle from *Pleurotus florida*

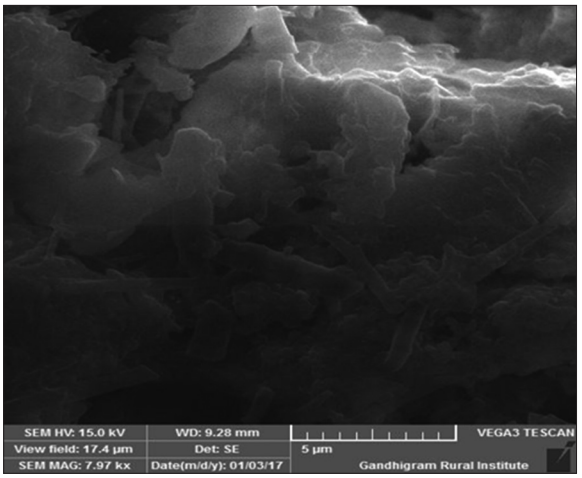

Fig. 3: Scanning electron microscopy image of silver nanoparticles from *Pleurotus florida*

SEM and energy dispersive X-ray analysis (EDAX)

SEM micrographs showed the rod-shaped AgNPs with a diameter range of 20-50 nm. EDAX further confirmed the significant presence of silver along with carbon and oxygen (Figs. 3 and 4).

XRD measurement

Determination of crystalline size was carried out using the above tool.

Average crystalline size of silver was calculated using the Scherrer's formula:

$D=k\lambda/\beta\cos\theta$

D=Average crystalline size, k=Constant, λ=X-ray wavelength, β=Angular FWHM value of the XRD peak at the diffraction angle, θ=Diffraction angle.

Applying XRD data in Scherrer's formula, the average size of particle was recorded approximately as 22.27 nm (Fig. 5)

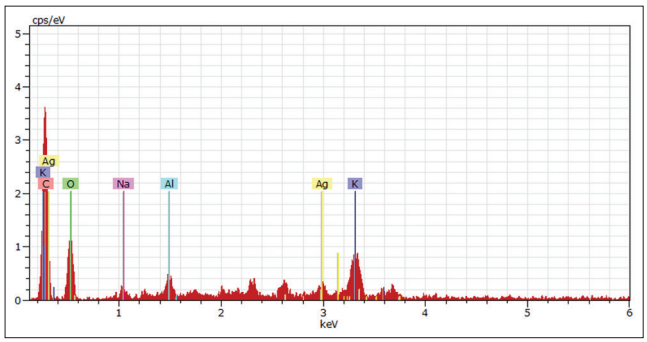

Fig. 4: Energy dispersive X-ray analysis spectrum of silver nanoparticles from *Pleurotus florida*

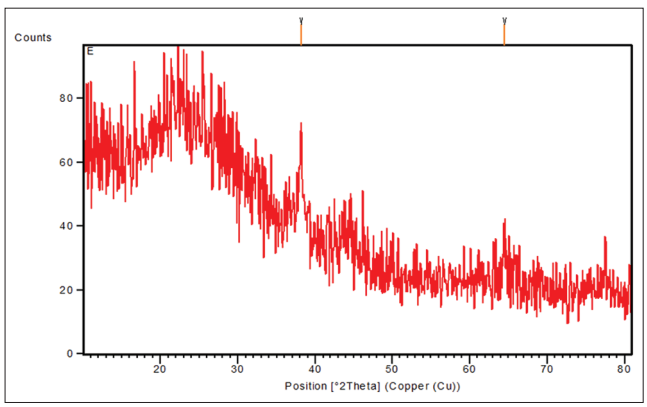

Fig. 5: X-ray diffraction spectra of silver nanoparticles from *Pleurotus florida*

Antioxidant activity

DPPH Assay

The antioxidant potential of AgNPs synthesized from *P. florida* was determined using DPPH assay and the results revealed maximum antioxidant effect of *P. florida* at 96.48% (200 µg/ml) when compared with 96.2% 200 µg/ml recorded in standard ascorbic acid with an IC50 value of 85 µg/ml (Fig. 6).

Anti-inflammatory activity

BSA denaturation assay

Albumin denaturation assay indicated the anti-inflammatory activity. The anti-denaturation activity of AgNPs synthesized from *P. florida* at different concentrations (100-500 µg/ml) is presented in. It was

Table 2: The albumin denaturation activity of AgNPs from *Pleurotus florida*

S. No.	Concentration (µg/ml)	Inhibition of albumin denaturation (%) Mean±SEM
1.	100	71.05±0.01
2.	200	76.31±0.005
3.	300	84.21±0.01
4.	400	89.47±0.005
5.	500	92.10±0.005

AgNPs: Silver nanoparticle, SEM: Standard error of the mean

observed that the inhibition of BSA denaturation was increasing with the concentration and the maximum anti-denaturation activity was observed as 92.10±0.005% at 500 µg/ml. Diclofenac, the standard drug showed the maximum inhibition of 97.36±0.015% at 500 µg/ml. The concentration required for 50% of the inhibition (IC50) was 100 µg/ml (Fig. 7and Table 2).

DISCUSSION

Nanotechnology deals with the synthesis and fabrication of materials at the nanoscale level (1-100 nm). Nanoparticles have a wide range of applications in fluorescent biological labeling, cancer treatment, drug and gene delivery, bio-detection of pathogens, detection of proteins, tissue engineering, etc. [22]. Among the various nanoparticles available, AgNPs are gaining more importance due to their diversified biological properties and potential applications. Silver has been used since ancient times for the treatment of wounds and inflammation for their in antibacterial, antifungal, antiviral, and anti-inflammatory activity [23]. In this study mycosynthesis of AgNPs using edible mushroom, *P. florida* was confirmed by the color change from yellow to red within 24-48 hr using 1 mM $AgNO_3$ as reducing agent. The reduction of silver ions to AgNPs by the aqueous extract of the above macrofungi was confirmed by the formation of darkening of the brown color of the extract after adding 1 mM $AgNO_3$ solution. This change in color was due to the excitation of the surface plasmon resonance of the AgNPs in the solution and also excitation of the presence of free electrons in AgNPs which intensified the brown color after 24 hr [24]. The synthesis of AgNPs from various edible and non-edible mushroom species such as *Helvella lacunosa, Trametes versicolor,* and *Ganoderma applanatum* and some unidentified mushrooms by their color change from yellow to red with 24-48 hr of incubation [25]. During the process of formation of AgNPs, the silver ions get trapped on the surface of secondary metabolites and antioxidant compounds of the mushroom and reduced by their proteins leading to the formation of silver nuclei which lead to form the accumulation of silver nuclei and subsequently grow in size that resulted in the formation of the AgNPs and the AgNPs are further capped by the major phytocompounds that prevented their aggregation and made them stable [26].

Characterization of AgNPs

The UV-visible spectra of AgNPs of *P. florida* showed a specific absorption band at 410 nm after 2 hr of reaction time. The spectral characterization study using UV on AgNP of some species of mushrooms, namely, *Trametes, Ganoderma* and *Pleurotus* at 420-430 nm [27]; the surface plasmon resonance at 420 nm of AgNPs from *G. lucidum, A. bisporus* and the plasmon resonance at 435 nm of AgNPs from *P. florida* and *Pleurotus platypus* have already been reported [28].

FT-IR

FT-IR with a spectral range of 400-4000/cm was carried out to identify the functional groups of biomolecules present in mushroom extracts that are responsible to reduce the silver ions to AgNPs and also capping and stabilization of the bioreduced nano-metal. The absorbance band exhibited the prominent peak values at various levels such as 1728.22/cm, 1284.59/cm, 1126.43/cm, 1072.42/cm, and

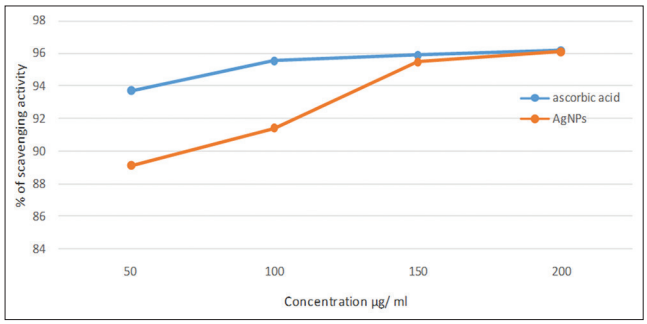

Fig. 6: Free radical scavenging assay of silver nanoparticles from *Pleurotus florida*

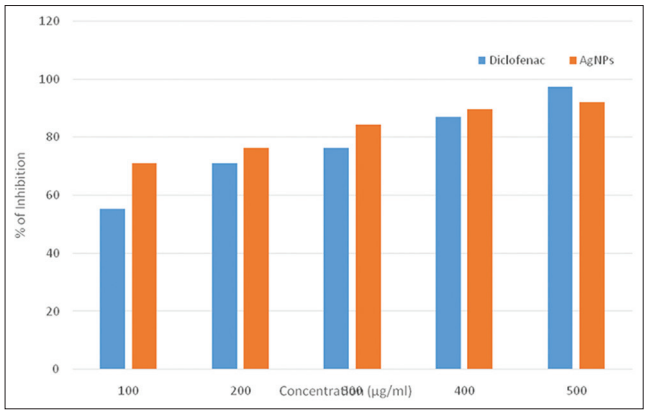

Fig. 7: Inhibition of albumin denaturation activity of AgNPs from *Pleurotus florida*

744.52/cm; the spectra showed the sharp and strong absorption band at 1728.22/cm assigned to the stretching vibration of (NH) –C=O group and carboxylic acids. Peaks at 1284.59/cm indicated that the mushroom extract contains -C-O stretch, esters; and 1126.43/cm indicated that the sample possesses -C=O stretch, ketones, and carboxylic acids; 744.52 and 1072.42/cm band indicated the presence of alcohol and carboxylic acids and clearly expressed that the AgNPs are capped with the phytocompounds with various functional groups having characteristic peaks in the spectrum. The synthesized AgNPs of *Pleurotus roseus,* showed strong absorbance peak from 3446.58 cm to 673.97 cm, representing various functional groups in FT-IR spectrum [29].

SEM

SEM analysis revealed that the particle size of the AgNP was at 20-50 nm with a clear rod in shape and was well dispersed without any aggregation. The biosynthesized AgNPs from *A. bisporus* are spherical in shape, mono dispersed, uniform in size and with the size range of 15-20 nm [30]. SEM images conformed the biosynthesized AgNPs of *Nelumbo nucifera* with the particle size of 25-80 nm and triangle, pentagon, and rod in shape [31].

XRD measurement

XRD studies were conducted to confirm the crystalline nature of the synthesized particles. The spectra of the nanoparticles derived from *P. florida* extract suggest the formation of metallic silver and two XRD peaks were observed corresponding to the (111) and (220) planes at 2θ angles of 38.13° and 64.46° which agrees with the Bragg's reflection of silver nanocrystals. Using Scherrer's equation the average crystalline size was recorded as 22.27 nm. The intensities of the (111) diffractions were much stronger than (220) diffraction. The XRD pattern thus clearly showed that the AgNPs were essentially crystalline in nature. The average size of AgNPs synthesized from *Persea americana* (avocado) as 27.42 nm using XRD data [32].

The XRD pattern of AgNPs produced by leaf extract of *N. nucifera* with intense peaks at 2θ values of 38.08°, 53.62°, 65.67°, and 76.67° corresponding to (111), (200), and (311) [35].

Antioxidant activity

DPPH radical scavenging assay

Oxidative stress is an important parameter that increases cancer risk. Free radicals are known to induce oxidative damage in biomolecules and play an important role in aging, cardiovascular disease and unbalanced immune system [33]. Antioxidant activity is manifested in a wide variety of actions such as inhibition of oxidation enzymes, chelating of transition metals, and enzyme detoxification of reactive oxygen species [34]. The scavenging activity of the AgNPs of mushroom *P. florida* was compared with L-ascorbic acid as standard. The DPPH radical scavenging activity was increased with increasing concentration (50-200 µg/ml), and the antioxidant effect of *P. florida* was maximum at 96.48% in 200 µg/ml in sample concentration. The EC50 value of the nanoparticle was 85 µg/ml. The antioxidant potential of AgNPs of the macrofungi *I. obliquus* (Chaga mushroom) was found to increase with an increase in the concentration, showing maximum inhibition (76.57%) at 1 mM and minimum inhibition (60.98%) at 0.125 mM solution [35].

Anti-inflammatory activity

BSA anti-denaturation assay

The agents that can prevent the denaturation of protein can be used for the treatment of inflammatory diseases because protein denaturation is considered as one of the causes of inflammation. Inflammation also termed as the protective mechanism of the local microcirculation to tissue injury which is caused by physical trauma, noxious stimuli by chemical agents, heat, antigen-antibody reaction, and microbial effect [36]. Any compound that inhibits the protein denaturation more than 20% could be considered as a potential anti-inflammatory drug [37]. The denatured BSA expressed antigens associated to Type III hypersensitive reaction which is related to diseases such as serum sickness, and glomerulonephritis [38]. BSA denaturation assay indicated the anti-inflammatory activity of AgNPs of *P. florida* and the maximum anti-denaturation activity was observed as 92.10±0.005% at 500 µg/ml. The concentration required for 50% of the inhibition (IC50) was 100 µg/ml. Similar anti-inflammatory activity of methanolic extract of *P. florida* by protein denaturation method with IC50 at 233.91 µg/ml was also reported [39].

CONCLUSION

The updated information of this research work revealed that the AgNPs of edible macrofungi *P. florida* with its significant in antioxidant activity and anti-inflammatory activity could play a crucial role in mushroom therapeutics. In depth and high-tech approaches of *in vivo* studies need to be carried out for deeper exploration.

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