Biological properties of Silver nanoparticles synthesized from edible Mushroom- *Pleurotus florida*

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Abstract

Nanoscience refers to the synthesis of small particles termed as nanoparticles, which act as building blocks in a variety of natural systems. The uses of nanoparticles plays an important role in medical science. Silver nanoparticles has stronger antimicrobial potentiality and a wider range of bio applications in various pharmaceutical industries. *Pleurotus florida* (white oyster mushroom) is an edible mushroom that is popular for its nutritional values, low production cost and ease of cultivation. The current investigation shows that the green synthesis of metallic silver nanoparticles using the edible mushroom *Pleurotus florida* as a reducing and stabilizing agent; characterization, antimicrobial and phytochemical activity by studying biosynthesis of silver nanoparticles.

Key words : Nanoscience, Silver nanoparticles, Edible mushroom, *Pleurotus florida*.

Nanotechnology is a significant method where metal nanoparticles show great assurance in improving the treatment and management of various diseases. The nanoparticles in glucose metabolism and their deficiency with diabetes where reported by various studies; strong Plasmon resonance of silver generated a great interest to treat diabetes. The biosynthesis nanoparticles could be another possible drug to treat diabetes mellitus.

Silver nanoparticles were extensively applied as an antimicrobial agent in the treatment of wound, air, medical devices, purification of water, different cosmetics, aqueous paints, etc. Physical, chemical and biological are the various methods used in the preparation of silver nanoparticles. In physical method evaporation and condensation takes place, thus it is time consuming process. In chemical method chemical like sodium citrate, tollens are used to reduce silver ions. These

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methods are toxic, it contains side effects and may cause pollution to environment. In biological methods, the synthesis of nanoparticles are regarded as safe, cheap, sustainable and environment friendly. Microorganisms such as bacteria, yeast and fungi are used in biological methods. For the preparation of the manuscript relevant literature¹⁻⁹ has been consulted.

Pleurotus florida :

White Oyster mushroom is an edible mushroom that gained popularity due to its nutritional values, low production cost and ease of cultivation. Oyster mushrooms do contain lovastatin, a form cholesterol lower in statin. Oyster mushroom are also known as wood fungus. *Pleurotus* sp is an efficient lignin degrading mushroom and can grow well on different types of lignocellulosic materials. Cultivation of these mushroom is very simple and low cost production technology, which gives consistent growth with high biological efficiency.

Collection of dried mushroom :

100 g of dried Oyster mushroom was collected from 'Monday Square' having shelf life of 12 months and it contains no preservatives.

Collection of bacterial strain :

The organism used in the study were Staphylococcus aureus, Klebsiella pneumonia, E. coli and Streptococcus sp.

Preparation of mushroom extracts :

Dried Oyster mushroom was collected

and powdered inti fine particles .4 g powder sample was heated with 100 ml distilled water for 15 min at $50 - 55^{\circ}$ C. After heating the extract was cooled and filtration done through whatman filter paper No.1 and preserved at 4°C for further purposes.

Preparation of Silver nitrate solution :

1 millimolar aqueous solution of silver nitrate solution was prepared by adding 0.0169 g of silver nitrate respectively in 100 ml of distilled water for the synthesis of silver nanoparticles.

Synthesis of silver nanoparticles :

For the synthesis of silver nanoparticles, 50ml of 1 Mm aqueous silver nitrate solution was added into 50 ml of mushroom extract to reduce Ag+ to Ag, and then kept in a shaker incubator under dark condition at 150 rpm in 37°C for 3 days. After incubation observed it for colour change. The colour change of extract from normal to brown or reddish brown indicates the presence of silver nanoparticles. For characterization, the plant extract containing synthesized silver nanoparticles was centrifuged at 10,000 rpm for 45 min and the resulting pellet was dried and converted into powder form.

Antibacterial susceptibility testing :

After synthesis of silver nanoparticle, antibacterial susceptibility testing was done by agar diffusion method using mushroom extract contained synthesized silver nanoparticles. These test was done to check the efficiency of synthesized silver nanoparticles against test organisms.

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Ultraviolet visible- spectroscopy :

The bioreduction of silver ions in the extracts after adding silver nitrate solution was monitoring by using UV visible-spectroscopy at regular intervals between 400-450 nm.

Fourier- Transform Infrared Spectroscopy:

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 7000 rpm for 10 min, and the resulting suspension was redispersed in 10 ml sterile distilled water. The process was repeated 3 times. The purified suspension was freeze dried to obtain dried powder .this dried nanoparticle were analysed by FTIR.

X-Ray Diffractometer measurement :

The freeze dried powder (from 3.8) was smeared on a glass slide of 2 cm \times 2cm according to the method. The dried thin smear of Ag–NPs were collected for the determination of the formation of elemental Ag-NPs by an X-ray Diffractometer operated at a voltage of 40kV and a current of 30 mA.

Scanning Electron Microscopy :

This can be done by coating the sample to the analysed with gold using a sputter coater.

Phytochemical Analysis : Detection of alkaloids :

Extracted were resolved individually in diluted HCl filtered and then subjected to following tests.

Mayer's *test* :

To a few ml of filtrate, a drop of mayers reagent was added by the side of the test tube. A white or creamy precipitate indicate the presence.

Wagner's test :

To a few ml of filtrate few drop of Wagner's reagent were added by the side of the test tube a reddish brown precipitate indicates as positive.

Hager's test :

To a few ml of filtrate, few drop of Hager's reagent were added to the side of the test tube, an yellow precipitate indicates as positive.

Reduction of flavonoids :

4ml of 1% NH₃ was added to 0.5 ml extract and then 1 ml of conc. H₂SO₄ was added. Appearance of yellow colour indicates positive.

Alkaline reagent test :

To a few ml of extract, few drops of NaOH were added . Formation of an intense yellow colour which turns into colourless by the addition of few drops of diluted acetic acid indicated the presence of flavonoids.

Detection of Phenol :

1ml of extract was dissolved in 2ml of distilled water . To this few drops of 10% Ferric chloride solution was added. A dark green colour indicates as positive.

Detection of terpenoids :

5ml extract was dissolved in chloroform (2ml) and then 3ml conc. H₂SO₄ was added to the solution, reddish brown colour indicates as positive.

Detection of Glycosides :

To a few ml of extract, few drops of NaOH were added. An intense yellow colour indicates as positive.

Detection of carbohydrate :

1ml of extract were taken and added few drop of Molish's reagent and mix well ,after that add conc. H_2SO_4 by the side of the test tube to formulate the ring at the interface of the two layers.

Detection of protein :

1ml of extract and 0.1 ml of Million's reagent were added a brown colour indicates as positive.

Detection of tannins :

To the 1ml of the extract add 10 ml of distilled water and filtered. To the filtrate added 5% of 1 M FeCl₃ which showed blue black precipitate / greenish precipitate indicates positive result.

Detection of Saponins :

To 1ml of the extract added 5ml of distilled water and boiled. After boiling which allowed to filtrate to that 2.5 ml of filtrate and 1.5 ml of distilled water were added and shake well. The presence of stable and persistent froth indicates as positive.

Detection of Quinones :

To the 1ml of extract added 1 ml of conc. H_2SO_4 , red colour indicates as positive.

Synthesis of nanoparticles :

The colour change of the extracts after adding silver nitrate solution, this indicates the presence of silver nanoparticles in the sample.



Figure 1. Silver nanoparticles

Antibacterial susceptibility testing :

The silver nanoparticles synthesized from the extract shown effective antibacterial activity against the test organisms.

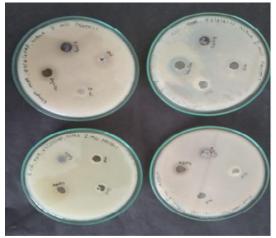


Figure 2. Antibacterial susceptibility testing.

		Silver nano-	Silver	Mushroom	Distilled
S.	Test organisms	particles	nitrate	extract	water
no		(zone size)	solution	(Zone size)	(control)
			(zone size)		(zone size)
1	Streptococcus	5mm	2mm	-	-
2	Klebsiella pneumonia	10mm	2mm	-	-
3	Staphylococcus aureus	3mm	1mm	-	-
4	Escherichia coli	2mm	1mm	-	-

UV characterization :

UV-Vs spectroscopy :

Reaction mixture containing aqueous silver nitrate (1Mm) and mushroom extracts of *P.florida* shown an absorbance peak at around 420nm. (Figure 3).

FTIR analysis :

The two bands at ~ 1642 and ~ 1550 are observed and are recognised as amide 1 and amide 2 are arise due to the carbonyl stretch and -N-H stretch. (Figure 4).

XRD analysis :

XRD pattern of synthesized Ag NPs showed strong peaks at 2 theta degree values of 38.13° , 44.3° and 64.50° . (Figure 5)

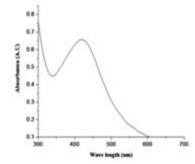


Figure 3. UV- vis absorption Spectrum of Ag NPs

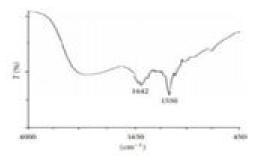


Figure 4. FTIR analysis of Ag NPs

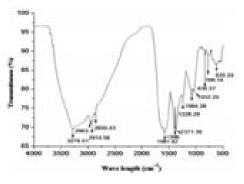


Figure 5: XRD analysis of Ag NPs

SEM analysis :

SEM micrographs revealed that synthesized Ag NPs were approximately spherical in shape and in some places few particles were agglomerated.

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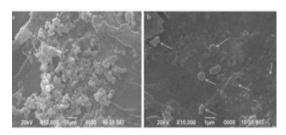


Figure : SEM analysis of Ag NPs

Phytochemical Analysis :

Phytochemical Analysis was done for the mushroom extract that showed the presence of bioactive compounds in the plant.

S.	Phytochemical	Pleurotus
no	test	florida
1	Alkaloids	Positive
2	Flavonoids	Negative
3	Phenol	Negative
4	Terpenoids	Positive
5	Glycosides	Negative
6	Carbohydrate	Positive
7	Protein	Positive
8	Tannins	Negative
9	Saponins	Negative
10	Quinones	Positive
11	Steroids	Positive

This study shows that the synthesis of silver nanoparticles from the edible mushroom *Pleurotus florida* and their characterization. It also shows that the synthesized silver nanoparticles contain effective anti-bacterial property and some bioactive substances. Silver nanoparticles provide an important function in the field of biology and formation of medicine.

It is also eco-friendly in nature. Rapid development of nanotechnology is focused on the synthesis of silver nanoparticles.

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