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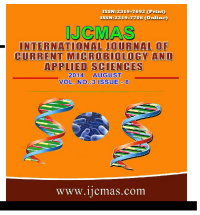

Original Research Article

Isolation and Screening of Mushrooms for Potent Silver Nanoparticles Production from Bandipora District (Jammu and Kashmir) and their characterization

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Keywords

Mushrooms, Silver Nanoparticles (AgNPs), TEM and FT-IR.

A B S T R A C T

In the present study the history of conventional use of mushrooms in ancient times was revealed from tribal and it was found that mushooms were used for their nutritional and medicinal values. The ancient people used mushroom extracts for skin infections even for dreadful disease like diabetes and cancer. The mushrooms in the ancient times have been used as folk medicine throughout the world due to their high nutritious values Aga*ricus bisporus, Ganoderma sps, Pleurotus sps* themselves possess and antibacterial and antifungal properties and the high protein, sterol, macro-elements and low caloric content make these mushroom ideal for cardiovascular diseases. In the present study dry mushroom species of Northern part of Jammu and Kashmir were screened for the production of AgNPs and it was confirmed from color change from light yellow to reddish brown by challenging the mushroom extract with 1mm AgNO3, UV-Vis confirms the AgNPs formation, TEM reveals the size of between 20-44nm, FT-IR reveals the different functional groups involved in stabilization of AgNPs.

Introduction

Mushrooms are the group of fungi which are widely used as food and medicine in different parts of the world since long time. The history of mushrooms being used as food is very old. Mushrooms have been collected and consumed since thousands of years. Archaeological survey revealed the association of edible species with people living 1300 years ago in Chile (Rojas and Mansur 1995). But in China eating of wild mushrooms noted several hundreds of years ago before birth of Christ (FAO, 2004). The Egypt emperor Pharaoh used them for their delicious flavor even nobody was allowed to touch them at that time many believed that mushrooms have properties to produce super human strength and help them to find lost things and lead soul to realm of God. There are more than 14,000 known species of mushrooms nearly 7000 species are well studied to possess varying degree of edibility in the world (Chang and Miles, 2004). The Kashmir Himalaya valley which is stretched between 32°17-37 03 N

latitude and 72°03-80°20 E and covers area of 222235 Km^2 with average rainfall between 60-80cm is a rich repository of mushroom flora. Bandipora which is the northern district of the state is 50km from Srinagar the summer capital of the state. The district occupies an area of 398 km2 with population of 306511(census 2001) stretched at latitude 34.4, longitude 74.6 and the topography represents a mix of beautiful mountains, pastures, streams and is famous for *Chota Amarnath* cave. The lush green forest area of the district covers about 19939 hectares of land and these forests are the common habitation of mushroom species. The Department of Agriculture Government of Jammu and Kashmir provides all the facilities to the farmers for the cultivation of some edible Mushrooms number of cultivations centers supply these cultivated mushrooms out of the district for sale. Mushrooms are nutritionally and medicinally important group of organisms which are being used for therapeutic properties for the treatment of cancer, depression, hypertension and diabetes (Vincent *et al.,* 2000). In China, Japan, U.S.A mushrooms extract is being used successfully for the treatment of gastrointestinal and prostate cancer (Chihara, 1992) Preliminary work carried by Amala Research Institute Thrissur, Kerala on *Ganoderma lucidum, Phelinex rimoses* and *Pleurotus sps* established their medicinal properties. The result revealed antitumor and anti-inflammatory activities of these mushroom species. The ingredients of mushrooms like polysaccharide, lentinan have been found highly effective against a variety of cancer (Ajith and Janardhanan 2007). In Japan and China Kerstin and lentinan anticancer drugs isolated from *Coriolus versicolor* and *Lentinus edodes* are used successfully against tumors along with other anticancer drugs. Several mycologists have reported ethnomycogical usage of this natural resource wealth from some regions of India (Rai et al., 1993, Pandey et al., 1978, Harsh et al., 1993, and Boruah et al., 1997). However, the knowledge about the mushrooms for the production of nano materials from the state of Jammu and Kashmir is absurd and presently no literature is available regarding this work. In the current study we intended to document but hitherto unexplored mushroom flora of this region screened them for their ability for nanoparticles production.

Nanotechnology is a fast growing area of modern science due to its promising applications in the field of medicine due to variable size, shape, chemical composition and controlled disparity and their potential use for human benefits. The most predominately studied nanoparticles are those from the noble metals Ag, Au, Pt, and Pd amongst them silver nanoparticles play significant role in biology and medicine (Phillip, 2009). The biosynthesis of this particle is the thrust area of research in nanotechnology. Few mushrooms (spore bearing fruiting body of fungus) namely *Volvarella volvacea*, *Pleurotus sajor, Pleurotus florida*, *Ganoderma lucidum* and *Micrporus xanthopus* have been used for the production of AgNPs(Phillip 2009, Nithya et al., 2009, Ravishankar et al., 2011, Alka Karwa et al., 2011, Balahanmugam et al., 2013) Many plants like Avinastave, *Aloe vera, Azadiracta indica, Psdium guajava* and fungi like *Verticillium, Fusarium oxysporum*, *Aspergillus flavus*, *Rhizopus stolonifer* and endophytic fungi *Penicillium* have been explored for the production of AgNPs (Ahmad et al., 2003, Afreen et al., 2011, Dattu Singh et al., 2012)

In this work the extract of edible and non-edible mushrooms was used for the biosynthesis of nanoparticles. Silver nanoparticles are being synthesized by using variety of methods like chemical, physical,

ionizing radiation methods etc. However, in chemical methods the toxic substances accumulate in the processes which are harmful and non-friendly to the environment. Biological method is the most reliable method for the biosynthesis of AgNPs which are safe, cost-effective and eco-friendly (Ahmad et al., 2003). The present study includes synthesis of AgNPs from the mushrooms and their characterization by UV-Visible Spectrophotometer, Transmission Electron Microscopy (TEM) and Fourier Infrared Spectroscopy (FT-IR), which appears to be the first work in Jammu and Kashmir region.

Materials and Methods

Collection of Mushrooms from Bandipora District Jammu and Kashmir

The study area of the present investigation is the Bandipora district which is the northern part of Jammu and Kashmir with sub-Mediterranean type of climate. Extensive field surveys were done in different areas and a good number of edible and non-edible mushrooms species were collected. Photographs of the specimens were taken in their natural habitat using digital camera and the specific code names were given to them for identification. The best period for wild mushroom collection in study area starts with the onset of rain the period when the condition is conductive for mushroom growth and their availability is in plenty. During the survey it was found that the most of the species were found on bruised trees rotten wood logs, leaves and dung rich soil and pastures. The proper care was taken in handling transportation and ensures to preserve the features for easy identification and the production of AgNPs. The mushroom samples are documented and identified by authentic keys. (Arora, 1986 and Hawksworth, 1974)

Screening and Biosynthesis of Silver Nanoparticles from Collected Mushrooms

The collected Mushroom species in dry form were screened for the potent silver nanoparticles. About 20gmof dried mushrooms were taken and are being washed thoroughly with double distilled water to remove dust and mud adhering the surface of these mushrooms. The washed mushroom samples were kept in shadow conditions for drying. The mushroom samples are then cut into small pieces with sterile knife and then powdered into fine particles. The mushroom fragments were suspended into 100ml of sterile distilled water and boiled for 10 minutes at 55°C in Erlenmeyer's conical flask. The mushroom extract was filtered twice through Whatman's filter paper No.1 and stored at 4°C for further experiments. The filtrate was used as reducing agent for 1mM of $AgNO_3$ (99.9%). In a typical synthesis of silver nanoparticles the 100ml of mushroom extract was added to 50ml of 1mm $AgNO_3$ solution incubated at room temperature for the reduction. Simultaneously the positive control was maintained with the mushroom extract and de-ionized water used as negative control containing only silver nitrate solution (Narasimha *et al*., 2011).

Characterization of Silver Nanoparticles. UV-Visible Spectroscopy

The preliminary indication of silver nanoparticles production by using mushroom extracts is confirmed by the color change from yellow to dark brown within 24h. Further it has been characterized by UV-Visible Spectroscopy (UV-1650 PC Shimadzu).The process of reaction between $AgNO_3$ and mushroom extract was monitored by UV-Visible spectra with resolution of 2.0 nm between the wavelength 200 to 700 nm.

Transmission Electron Microscopy (TEM)

The silver nanoparticles synthesized from the mushroom extract were characterized by TEM. Produced AgNPs solution is centrifuged to about 3000 rpm for 15 min. Supernatant obtained was taken for TEM analysis. A drop of aqueous solution containing silver nanoparticles was placed on carbon coated grids and air dried under infrared lamp. TEM micrographs were taken by analyzing the prepared grids to know the size and shape of AgNPs.

Fourier Transmission Infrared Spectroscopy (FTIR)

The crude suspension of AgNPs by mushrooms was initially centrifuged at 3000 rpm for 15 min to remove the unwanted impurities and then supernatant is again centrifuged to 10000 rpm for 15 min the resulting solution was repeated. Pellets obtained were washed with deionized water to get the pure AgNPs. The sample was completely air dried at room temperature; the collected powdered AgNPs were taken for FTIR analysis in the range of 450 to 4500 cm^{-1}

Results and Discussion

Collection of Mushrooms from Bandipora District Jammu and Kashmir

During the extensive field survey fifteen different mushroom species such as *Agaricusbisporus, Helvella lacunose. Fomes fomentarius, Tremates versicolor, Pleurotus florida ,Ganoderma applanatum* and the unidentified mushroom species bearing the code no VM-3,VM-4,VM-6,VM-7,VM-8,VM-9,VM-10,VM-11,VM-12,VM-1 were collected from Bandipora district Jammu and Kashmir .

Screening and Biosynthesis of Silver Nanoparticles from Collected Mushrooms

Out of fifteen screened dried mushroom only five species viz*: Agaricus bisporus, Helvella lacunose, Fomes fomentarius, Pleurotus florida. Ganoderma appalanatum* were found potent producers of AgNPs (Table 1 and Fig 1). Among these mushroom species few species(*Agaricus bisporus,Plearotus florida* are already explored for production of silver nanoparticles.The preliminary confirmation of silver nanoparticles from these mushroom species was the color change from yellow to dark brown by challenging the 100ml mushroom extract with 50ml of 1mM Solution of AgNO3 due to deposition of AgNPs (Fig 2). The color change is due to Surface Plasmon resonance of silver nanoparticles in the solution and it is due to the excitation of free electrons present in AgNPs which intensifies into brown color after 24 hours. Our results are similar with works of Dattu et al., (2013) and Shivaraj et al., (2014).

The mushroom species found positive for the production of silver nanoparticles are rich in proteins, riboflavin anti-oxidants and are medicinally important group of fungi .The exact mechanism behind the conversion of $AgNo_3$ to silver nanoparticles by mushroom extract is not known. Reductase enzymes and riboflavin in mushrooms acts as catalyst for reduction-oxidation reactions which converts the silver ions into silver nanoparticles.

Characterization of Silver Nanoparticles

UV-Visible Spectroscopy

The visual study of AgNPs production from the mushroom extracts was confirmed by UV-1650PC Shimadzu

spectrophotometer by recording the absorbance from 200-700nm and the strong Plasmon absorbance band was observed at 420-430nm in positive mushroom samples indicating the production of silver nanoparticles from the dried mushroom extracts which co relates with the work of Balashanmugam *et al* 2013. In the study they got the absorbance peak at 425nm of AgNPs biosynthesized from macro mushroom *Micrococus xanthopus* species.

Formation of silver and gold nanoparticles from 1mm solution of silver nitrate and auric acid has already being confirmed by UV-vis spectroscopic analysis AgNPs and AuNPs have free electrons which give rise to surface Plasmon resonance (SPR) absorption band due to combined vibration of these electrons of metal nanoparticles in response to Surface Plasmon resonance in AgNPs was 578nm with brow- yellowish pink to red color (Noginov et al 2007, Nath et al 2007). The surface Plasmon resonance at 420nm of silver nanoparticles from *Ganoderma lucidum Agaricus bisporus* has already been reported. However, Surface Plasmon resonance at 435nm and 300nm of AgNPs from *Plearotus florida* and *Pleurotus platypus* and has been observed (Sujatha *et al* 2013).

Transmission Electron Microscopy (TEM)

TEM analysis is the most reliable method for determining the size of the nanomaterials.TEM provides the insights into the morphology, stabilization and the size of silver nanoparticles. TEM measurement was carried out to determine the size and morphology of silver nanoparticles extracellular synthesized from the mushroom extracts. TEM micrographs revealed nano sized and well dispersed silver nanoparticles with the size of 20-44 nm our study correlates with the work of many authors Narasimha et al., (2011) biosynthesized AgNps extracellularly from *Agaricus bisporus* in the range of 8-55nm.

Fourier Transmission Infrared Spectroscopy (FTIR)

The FT-IR characterization of AgNPs was carried out according to the previous methods (Phillip 2009). It was carried out to identify the molecules present in mushroom extracts thought to be responsible for the reduction of silver ions to silver nanoparticles and the capping reagent for the stability of this bio reduced nano metal. FT-IR measurement was carried out the spectra obtained was between 450 to 4500cm^{-1} of silver nanoparticles which showed the absorption band centered at 2250 – 2100, 1500 – 1700 and 1000 of these 2250-2100 represents C=C Alkyne (stretch), 1500 – 1700 for C=C amide (stretch) and 1000 for C-O Alcohols, Ethers, Esters and Carboxylic acid (stretch). The infra- red spectra shows the bands which clarifies the presence of N-H, H-O, C-N, C-H.C-O amide linkages and for nitro compounds that may be present between the silver nanoparticles as stabilizing caps along with the proteins and amino acid residues.

Fig.1 Positive Mushroom spp. of Bandipora District for the production of AgNps.

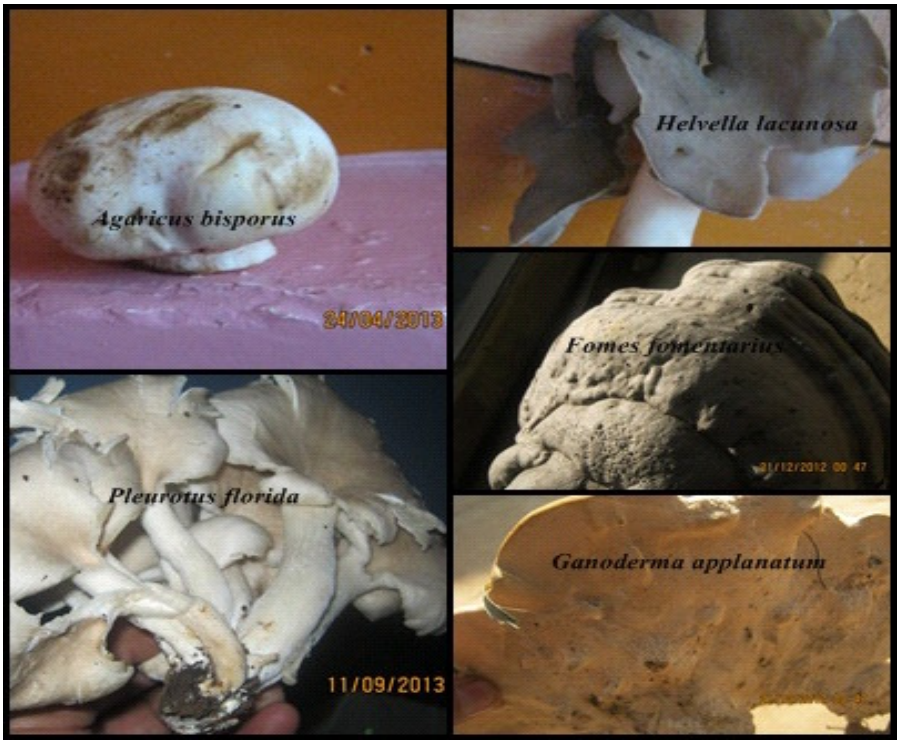

Fig.2 (A) Filtrate of mushrooms shows the yellow color before $AgNO_3$ (B) The picture shows the change of color from yellow to dark brown after challenging mushroom extract with 1mm $AgNO_3$ solution.

(A) **(B)**

Fig.3 UV-Visible Spectroscopy of AgNPs

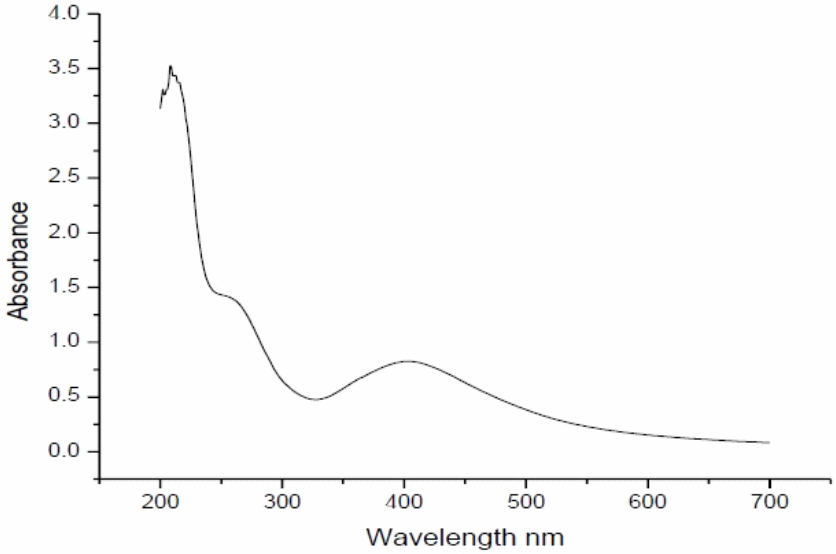

Fig.4 TEM Micrographs of AgNPs biosynthesized from dry *Agaricus bisporus*

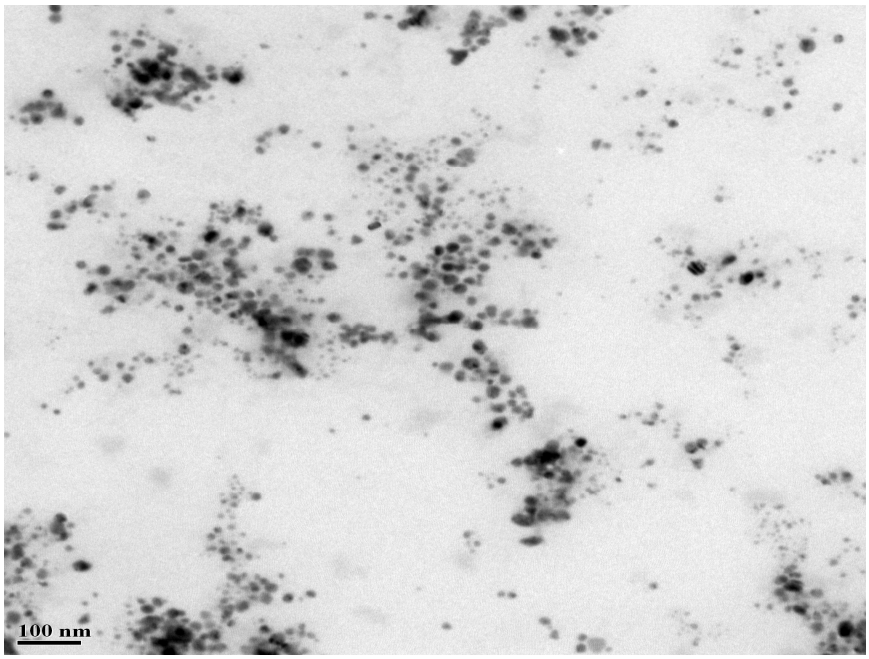

Fig.5 FT-IR Photograph of AgNPs synthesized from *Agaracus bisporus*

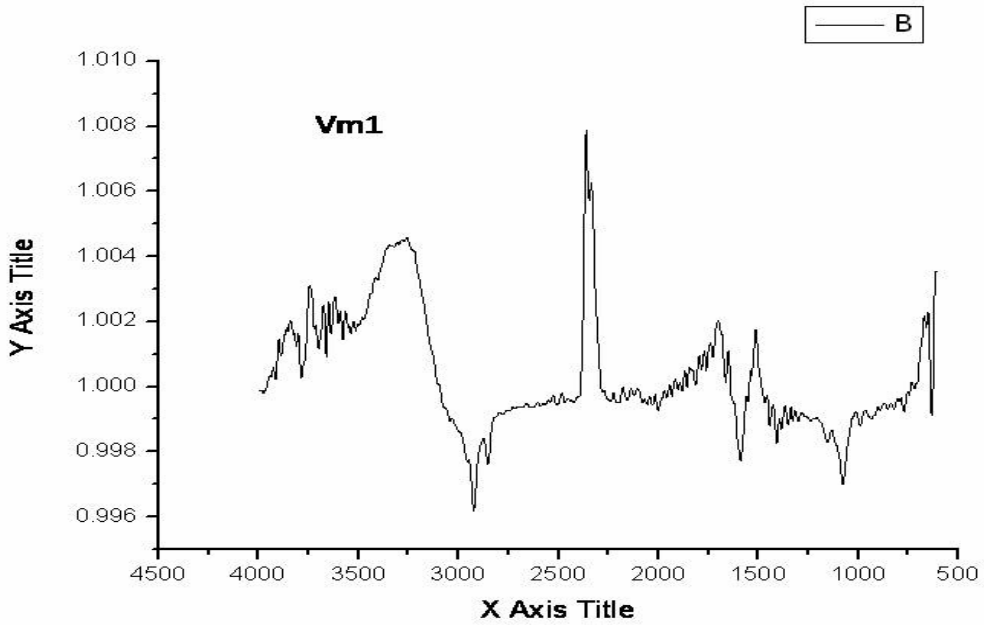

Table.1 Screening of Mushrooms for silver nanoparticles production from, Bandipora district Jammu and Kashmir

Code	Mushrooms	Area of collection	Edible	UV-Vis Spectroscopy Analysis
VM-1	*Agaricus bisporus*	Arin	Yes	Positive Peak at 424nm
VM-2	*Helvela lacunose*	Nathpora	Yes	Positive Peak at 425nm
VM-3	*Morchella esculenta*	Dardapora	Yes	Negative No peak
VM-4	*Morchella vulgaris*	Arin-Sheep farm	Yes	Negative No peak
VM-5	*Fomes fomentarius*	Chontimullah	No	Positive Peak at 430nm
VM-6	VM-6 (Unidentified)	Arin	No	Negative No peak
VM-7	*Tremetes versicolor*	Chontimullah	No	Negative No peak
VM-8	VM-8(Unidentified)	Dardapora	No	Negative No Peak
VM-9	VM-9(Unidentified)	Sumlar	Yes	Negative No peak
VM-10	*Ramariopsis kunzei*	Lundisulban	Yes	Negative No peak
VM-11	VM-11(Unidentified)	BaporaArin	No	Negative No peak
VM-12	VM-12(Unidentified)	Shamthan	No	Negative No peak
VM-13	*Pleurotus florida*	Dardapora	Yes	Positive Peak at 430nm
VM-14	*Ganoderma appalanatum*	Arin	No	Positive Peak at 420nm
VM-15	VM-15(Unidentified)	Bapora	No	Negative No Peak

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References

Afreen Banu, Vandana Rathod, E. Ranganath. 2011. Silver nanoparticle production by *Rhizopus stolonifer* and its antibacterial activity against extended spectrum β-lactamase producing (ESBL) strains of Enterobacteriaceae. Materials Research Bulletin. 46(9) 1417-1423.

Ahmed A, Mukharjee P, Senapati S, Mandal D, Khan MI, Kumar R, *et al.* 2003. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloid surf B 28:313-8.

Alka Karwa, Swapnil Gaikwad and Mehander Rai. 2011. Mycosynthesis of silver nanoparticles using Lingzhi or Reishi Medicinal Mushroom *Ganodermalucidum* (W.Curt.Fr) Karst and their Role as Antimicrobials and Antibiotic Enhancers. International Journal of Medicinal Mushrooms 13(5): 483-491.

Armendariz V, Herrera I, Peralta-Videa J R et al., 2004. Size controlled gold nanoparticle formation by Avena sativa biomass: use of plants in nanobiotechnology. Journal of Nanoparticle Research. vol. 6, no. 4, pp. 377–382.

Arora, D. 1986. Mushroom Demystified. A comprehensive guide to fleshy fungi. 2nd ed. ten spectrums of species of mushrooms. Speed Press, Berkeley, California. pp. 936.

Balahanmugam P, Santhosh S, Giyaullah H, Balakumaran M D, Kalaichelvan P T,. 2013. Mycosynthesis, Characterisation and Antibacterial activity of Silver nanoparticles from *Microsporusxanthopus*: A Macro Mushroom. Int. J. Inov. Res. in Sci. Eng. & Tech. Vol.2, Issue 11.

Boruah, P, Kailta, P, Bordoloi, D, Gogi, P., Adhikary, R.K. 1997. Some fleshy fungi of ethnobotanic use from north east India. Advances in Forestry Research in India. 16: 165-171.

Chandran S P, Chaudhary M, Pasricha R, Ahmad A, and Sastry M. 2006. Synthesis of gold Nano triangles and silvernanoparticles using Aloe vera plant extract. Biotechnology Progress. vol. 22, no. 2, pp. 577–583.

Chang, S.T., Miles, P.G. 2004. Mushrooms- cultivation, nutritional value, medicinal effect and environmental impact. CRC FAO Press, Washington, D.C. pp. 451.

Chihara G. 1992. Immunopharmacology of Lentinan, a polysaccharide isolated from Lentinusedodes: its application as a host defence potentiator. International Journal of Oriental Medicine, **17**: 57-77.

Dattu Singh, R. Vandana, N. Shivaraj, H Jyothi, S. Asish kumar, and M. Jasmine. 2014. Optimization and characterization of silver nanoparticles by endophytic fungi *Penicillium* sp. isolated from *Curcuma longa* (turmeric) and application studies against MDR *E. coli* and *S. aureus*. Bioinorganic Chemistry and Applications. vol. 2014, Article ID 408021, 8Pages.

Dattu Singh, Vandana Rathod, Shivaraj Ninganagouda, Jyothi Hiremath, Prema Kulkarni. 2013. Biosynthesis of silver nanoparticle by endophytic fungi *Penicillium* sp. isolated from *Curcuma longa* (turmeric) and its antibacterial activity against pathogenic gram negative bacteria.

Journal of pharmacy research. 7 (2013) 448-453.

FAO. 2004. Non wood forest products, wild edible fungi: A global overview of their use and importance. (Ed. Boa, E). Publication, Rome, pp. 17-147.

Harsh, N. S. K, Rai, B. K., Ayachi, S. S. 1993. Forest fungi and tribal economy- a case study in Baiga tribe of Madhya Pradesh. J. Trop. Forest. 9: 270-279.

Harsh, N.S.K., Tiwari, C.K., Rai, B.K. 1996. Forest fungi in the aid of tribal women of Madhya Pradesh. Sustainable Forestry. 1: 10-15.

Hawksworth, D.L. 1974. Mycologist Handbook. An Introduction to the Principles of Taxonomy and Nomenclature in Fungi and Lichen. CMI, Kew, Surrey, England.

Janardhanan K K and Nayana J. 2000. Antioxidant and antitumour activity of Pleurotus florida. Current Science. vol. 79, no. 7, pp. 941–943, 2000

Jyothi Hiremath, Vandana Rathod, Shivaraj Ninganagouda, Dattu Singh and Prema Kulkarni. 2014. Antibacterial Activity of Silver nanoparticles from *Rhizopus* spp against Gram negative *E.coli*-MDR strains. Journal of Pure and Applied Microbiology. vol.8 (1) pp.555-562 (2014).

Kamat, N. 1999. Ecotheological dimensions of termite hill. *Govapuri.* Bull. Inst. Menezes Braganza 1: 3.

Narasimha B. Parveen.K. Mallikarjuna, B. Deva Prasad Raju. 2011. Mushrooms (*Agaricus bisporus*) mediated biosynthesis of silver nanoparticles, characterization and their antimicrobial activity. International Journal of Nano Dimentions.2 (1); 29-36.

Nath, S.S., Chakdar, D. and Gope, G. 2007. Quantum dots and their applications in electronics, Nanotrends J. Nanotechnol. Appl. 2.

Nithya R and Ragunathan R. 2009. Synthesis of silver nanoparticle using *Pleurotus sajor* caju and its antimicrobial study. Digest Journal of Nanomaterials and Bio structures, vol. 4, no. 4, pp. 623–629.

Noginov, M.A., Zhu G., Bahoura M., AdegokeJ.,SmallC., Ritzo B.A., Drachev V.P. and Shalaev.V.M.2007.The effect of gain and absorption on Surface Plasmons in metal nanoparticles Appl. Phys. 86:455 460.

Pandey, G., Singh, B.K. 1978. Indian Mushroom Sciences I: 383-388.

Philip D. 2009. Biosynthesis of Au, Ag and Au-Ag nanoparticles using edible mushroom extract. Spectrochemica Acta—Part A: Molecular and Biomolecular Spectroscopy. vol. 73, no. 2, pp. 374–381.

Priyabrata Mukherjee, Absar Ahmad. Deendayal Mandal, Satyajyoti Senapti and Sudhakar R Sainkar et al., 2001. Fungus Mediated synthesis of silver nanoparticles and their immobilization in the mycelia matrix; A novel biological approach to Nanoparticle synthesis. Nano. Lett, vol.1, No 10.

Rai, B.K., Ayachi, S.S., Arvinder, R. 1993. A note on ethnomycomedicines from central India. Mycologist 7: 192-193.

Ravishankar Bhat, Raghunandan Deshpande, Sharanabasava V G, Do Sung Huh, Venkataraman A. 2011. Photo-irradiated biosynthesis of silver nanoparticles using edible mushroom Pleurotus florida and their antibacterial activity studies,

Bioinorganic Chemistry and Applications. http://dx.doi.org/10.1155/2011/650979.

Rojas, C., Mansur, E. 1995. Ecuador: "Informaciones generalssobreproductos non madereros en Ecuador. In *Memoria, consulta de expertossobreproductosforestales no maderero*spara" *America Latina Y el Caribe*, pp. 208-223. Serie Forestal. Santiago, Chile, FAO Regional Office for Latin America and the Caribbean.

Shivaraj N, Rathod Vandana, Jyoti H, Singh Dattu, Prema K, Manzoor-ul-Haq. 2013. Extracellular biosynthesis of silver nanoparticles using *Aspergillus flavus* and their antimicrobial activity against gram negative MDR strains. Int J Pharma Bio Sci. Apr; 4(2):222-229.

Shivaraj N, Vandana Rathod, Dattu Singh, Jyothi Hiremath, Ashish Kumar Singh, Jasmine Mathew and Manzoor ul Haq. 2014. Growth Kinetics and Mechanistic Action of Reactive Oxygen Species Released by Silver Nanoparticles from *Aspergillus niger* on *Escherichia coli*". *BioMed Research International*, Volume 2014, Article ID 753419, 9 pages.

Sujatha S, Tamilselvi S, Subha K. and Panneerselvam A. 2013. Studies on biosynthesis of silver nanoparticles using Mushroom and its antibacterial activities. International Journal of Current Microbiology and Applied Sciences. 2(12); 605-614.

Vincent, E., C, Ooi and Fang Liu. 2000. Immunomodulation and anti-cancer activity of polysaccharide- protein complexes. Current Medicinal Chemistry. **7**: 715-729.