# Synthesis of White Nanoparticals mediate by *Pleurotus tuber-regium* (Rumph. ex Fr.) Extract and Silver Nitrate

Sukumar Dandapat*, Manoj Kumar and Manoranjan P. Sinha.
Department of Zoology, Ranchi University, Ranchi, Jharkhand, India-834008
*corresponding author: Sukumar Dandapat; e-mail: scholar.sukumar27@gmail.com; Mobile No. +91 9905634520

## Abstract

Synthesis of white nanoparticle mediated (WNPs) by ethnomychologially fungal extract is easier, cheaper and ecofriendly. Colour change from pale yellow to dark brown and highest absorption of spectrum at 200nm and a broad spectrum at 474 nm of UV-visible spectroscopy provides the first conformation about the synthesis of white nanoparticles. FT-IR spectroscopy showed broad transmission peak at 3263 $cm^{-1}$ represents hydrogen bonded hydroxyl group (O-H and H- stretch) of alcohols phenols and carboxylic acid, a medium peak at 2395 $cm^{-1}$ 1759 $cm^{-1}$ represents C≡C bond and C=O stretch respectively confirm the presence of compound having carbonyl group. Strong pick at 1384 $cm^{-1}$ corresponding to N-O bend represents as aliphatic nitro compound. Scaning electron microscopy (SEM) showed the spherical and cubical in shapes nanoparticles with diameter of 40nm – 95nm in and the average diameter of the particles were of 50nm.

**Keywords** Drug, Nanoparticles, Plants, Disease, Phytochemical.

## 1. Introduction

Antibiotics, other synthetic drugs and antibiotic chemotherapy have been one of the most important medical achievements, which are used against pathogenic microbes and other diseases since their introduction. However, over the past few decades commonly used antibiotics such as streptomycin, amoxicilin, tetracycline etc. have become less effective due to emergence of multi drug resistant bacteria and are also they associated with various side effects [1, 2].

However, many infectious diseases and disorders, especially intracellular infections, neurological disorder, cancer, etc. remain difficult to treat with the antibiotics and other chemotherapeutic agents because of difficult in transport through cell membrane, low activity inside the cells and negligible bactericidal effects in intracellular matrix of pathogens [3]. It is very challenging to target the drug in the central nervous system and other nervous tissue due to blood brain barrier (BBB), which strictly restricts the delivery of most drugs to the brain because they do not cross the BBB in sufficient amount [4].

In recent times, the applications of nanobiotechnology in pharmacology have been tremendously explored. Nanotechnology in the field of medicine, concerns the size of matters in the range between 1- 100 nm are drug or natural or synthetic polymer loaded material acts as carrier and within this scale materials have unique physicochemical properties including ultra small size with large surface to volume ratio, high reactivity and unique interactions with structural components such as core, emulsion to works as carrier of therapeutic molecules and ligands for targating location of biological systems, which significantly improve the efficacy of the nanoparticles in contrast to the free drug counterparts [5-7].

Within few decades advantages of drug delivery through nanoparticles have gain ground, such as improvement of solubility of drugs in serum, circulation lifetime enhancing and concurrent target base drugs delivery of multiple therapeutic agents to the same cells for therapy [8, 9].

However the delivery and efficacy of many synthetic drugs is often limited to reach the site of therapeutic action and they require few modifications such as changing the molecular structure of the drug or their proper distribution by incorporation in carrier system [10]. Mathur and govind [11] reported that, when the materials are incorporated in to nanocarriers, they are required in low quantity to exert the action in target area and this is useful, when dealing with effective bioactive molecules.

In recent years, biological methods have been used to synthesize nanoparticles without any lethal and expensive chemicals [12, 13]. The reduction of metal ions for synthesis of nano particles mediated by bioactive molecules (*e.g.,* primary and secondary metabolites) found in higher quantity in the extract of medicinal plants and ethanomychologically used fungi [14-16].

*Pleurotus tuber-regium* commonly edible mushroom, belonging to the family pleurotaceae possess various bio active secondary metabolites such as, flavonoids, tannins, saponins, alkaloids, oligosaccharides etc. [17, 18] and these bioactive secondary metabolites are used for medicine production and also used as antitumour, antigenotoxic, antimutagenic, antibacterial and immunomodulatory agent [19-21].

In the last two decades, a number of plant extract mediated green a number of diagnostic agents based on plant extract mediated green nanoparticles have been developed for the treatment of cancer, diabetes, pain, asthma, allergy, infections etc. [22, 23]. But synthesis of white nano particles mediated by ethanomychologically used fungi has not been reported yet.

Therefore, the present study have been carried out to synthesise of white nanoparticles (without plant extract) using aqueous extract of *Pleurotus tuber-regium* fruting bodies.

## 2. Materials and methods

### 2.1. Collection of fungal material

The fresh fruiting bodies and sclerotium were collected from Assam, washed and disinfected by treating with $HgCl_2$ and washed again and were dried in shade under room temperature for six to seven days, powered and sieved [24].

### 2.2. Extract preparation

50 g of the fine powder was subjected to Soxhlet using distilled water for aqueous extract. The resultant extract was concentrated after filtration, using rotary flash evaporator at 45ºC. The extractability of extract was calculated and the extract was stored in air tight bottles at room temperature for further studies [25].

### 2.3. Synthesis of WNPs

For synthesis of nanoparticles, 1 ml of fruiting body extract was added to 99 ml distilled water and 1 mM of $AgNO_3$ were mixed in a 250 ml conical flask. The mixture was allowed to stir for 2 hours at 90ºC with help of magnetic stirrer. Then the mixture was allowed to cool down and was centrifuged at room temperature at 9000 rpm. The mixture was then washed three times with distilled water to obtain a black powder which was dried overnight in oven at 80ºC [26-29].

### 2.4. Characterisation of silver nano particles

**2.4.1.** *UV-Vis spectra analysis*
The formation of silver ion was monitored using Parkin Elmer Lamda25 UV-Vis spectrophotometer. UV-vis spectrum of reaction mixture was taken after 5 h by dilution of a small quantity of the sample into Milli-Q water.

***2.4.2.*** *FT-IR analysis*
FT-IR analysis was carried out on IPRresting-21 (Shimadzu) in the diffuse reflectance mode operated at a resolution of 4 cm-1 in the range of 400 to 4 000 $cm^{-1}$ to evaluate the functional groups that might be involved in nanoparticle formation.
***2.4.3.*** *SEM analysis of silver nanoparticles*

SEM (Scanning electron microscope) analysis was done using JEOL JSM-6390 LV (Japan) SEM Very small amount of sample was used to prepare thin films of sample on a carbon coated copper SEM grid, which was allowed to dry by putting it in mercury lamp for 5 min and the film was further coated with platinum metal using ion sputter.

## 3. Results and discussion

Synthesis of white nanoparticles mediated by aqueous extract of *P. tuber regium* and $AgNo_3$ solution is presented in figure-1. The colour change from light yellow to dark brown indicates formation of white nanonparticles [26, 29]. Mohan *et al* [32] also reported the change in light colour of solution having $AgNO_3$ and plant extract to dark brown indicates formation of nanonparticles, which exceed with increase in temperature and incubation period.


Figure 1: (A) Photograph of *P. tuber-regium* extract and (B) $AgNO_3$ and *P. tuber-regium* extract mediated silver nano particle solution after 2hours of incubation at 80 ºC.

### 3.1. UV-visible spectra analysis

UV- Vis absorption spectroscopy is an important bio-physical technique to monitor the formation and stability of white nanoparticles with the help of absorption spectrum. Figure-2 represents absorption spectrum of nanoparticles obtained from UV-visible absorption spectroscopy, which showed a broad peak at 474 nm and highest absorption spectra represent highest peak at 200nm, corresponds to the plasmon resonance. Kumar *et al*. [26] reported that the highest absorption spectrum is at 200nm, of alion mediated silver nanoparticles (AgNPs) solution by UV-Vis absorption spectroscopy. Khan *et al*. [29] reported broad peaks at higher and shorter wavelength, depicting increase and decrease in size of nanoparticles respectively.

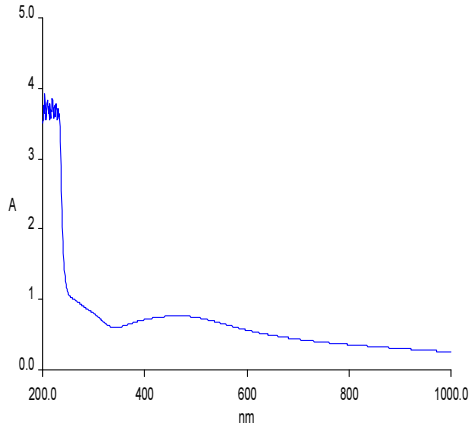


**Figure 2: UV-Visible spectrum of *P. tuber regium* and $AgNO_3$ mediated white nanoparticles.**

### 3.2. FT-IR- Analysis

FT-IR analysis was carried out to analyse the dual role of fungal extract as capping agent and high bioreductant [29] and to analyse common types of molecular bonds and functional groups [33,34]. FT-IR absorption spectra of white nanoparticle mediated *P. tuber-regium* extract and $AgNO_3$ is presented in Figure-3. The spectra showed broad transmission peak at 3263 $cm^{-1}$ corresponding to hydrogen bonded hydroxyl group (O-H and H- stretch) of alcohols phenols and carboxylic acid at high concentration. A medium peak at 2395 $cm^{-1}$ corresponding to C≡C bond and P-H stretch (P= phosphorus). A small pick at 1759 represent C=O stretch and presence of compound having carbonyl group. A strong pick at 1384 $cm^{-1}$ corresponding to N-O bend represents as aliphatic nitro compound and pick at 1072 $cm^{-1}$ corresponding to C=N stretch represents aliphatic amines, 987 $cm^{-1}$ represents =C-H bend represents the alkenes, 825 $cm^{-1}$ corresponding to aliphatic phosphate symmetric P-O-C stretching and chloroalkane ( C-Cl) and 536 $cm^{-1}$ corresponding to C-Br bending [30, 31].

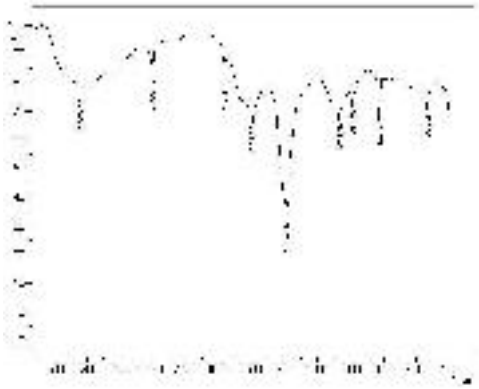

**Figure 3: FT-IR Spectrum of white nanoparticles synthesized from *P. tuber-regium* and $AgNO_3$.**

### 3.3. SEM analysis of white nano particles:

Scanning electron microscopy provided the final confirmation about the morphology of synthesized white nanoparticles. Scanning electron microscopy photograph is presented in Figure-4, which shows that white nanoparticles were of spherical and cubical shapes and were formed in a diameter of 40nm – 94nm and the average diameter of the particles were of 50nm. Kumar *et al.* [26] reported the size of green nanoparticles synthesized from alion of *Aloe vera* and $AgNO_3$ in the range of 287-293nm and average size of nanoparticls were70nm synthesized from plant extract. Firdhouse *et al.* [28] reported the size and shape of green nanoparticle synthesized from plant extract were 20nm -150nm in diameter and spherical respectively.

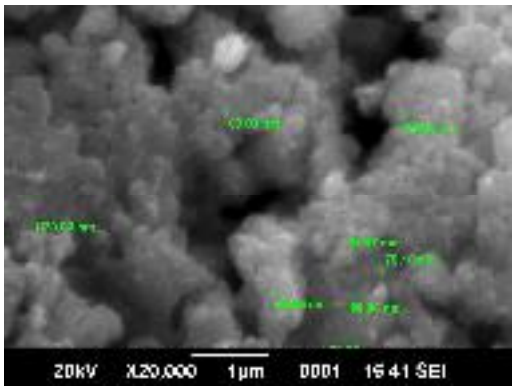


**Figure 4: SEM image of white nanoparticles synthesized from *P. tuber-regium* extract and $AgNO_3$mediated.**

## 4. Conclusion

Nan particles synthesized mediated by aqueous extract of *P. tuber-regium* and $AgNO_3$ is ecofriendly as well as without use of non chlorophilus substances hence, it is called white nanoperticles. This is the first ever reported nanoparticles of any ethnomycologically important edible macro fungi. Thus, white nanoparticles can be used in the prepration of new pharmaceuticals due to its tiny size, capping ability of bioactive compounds, which shows therapeutic efficacy against various disorders and diseases.

## 5. Acknowledgement

The authors acknowledged the facilities provided for the whole experiment by the EAEBCEPMA, DBT-NER Twinning project sponsored by DBT, NER-BPMC, New Delhi (BT/462/NE/TBP/2013) and Department of Zoology, Ranchi University, Ranchi, Jharkhand, India. Authors also thankful to CIF, BIT Mesra, Ranchi for their co-operation in UV-Vis Spectroscopy, FT-IR and SEM analyses.

## 6. References

[1] R.F. Service, Antibiotics that resist resistance, Science. 270 (1995) 724-27.

[2] O.A. Olowe, A.B. Olayemi, K.I.T. Eniola, A.O. Adeyeba, Aetiological agents of iarrhoea in children under 5 years of age in Osogbo. Afr. J. Clinical and Experimental Microbiol, 4(3), (2003) 62 – 6.

[3] L. Zhang, D. Pornpattananangkul, C-M.J. Hu, C-M. Huang, Development of nanoparticles for antimicrobial drug delivery, Current Medicinal Chem. 17 (2010) 585-94.

[4] W.M. Pardridge, Blood - brain barrier delivery, *Drug Discovery Today*, 1-2 (2007) 54–1.

[5] S.M. Moghimi, A.C. Hunter, J.C. Murray, Nanomedicine; Current status and future prospect, The FASEB J. 19 (2005) 311-330.

[6] A. Shoaib, Nano technology in drug delivery, introduction and recent developments, The International J. Nano Technol. 2(1), (2007) 54-56.

[7] L .Zhang, F.X. Gu, J.M. Chan, A.Z. Wang, R.S. Langer, O.C. Farokhzad. Nanoparticles in medicine: therapeutic applications and developments, Clinic Pharmacol. Ther. 83 (2008) 761-769.

[8] D. Peer, J.M. Karp, S. Hong, O.C. Farokhzad, R. Margalit, Langer R. Nanocarriers as an emerging platform for cancer therapy. Nat. Nanotechnol. 2 (2007) 751-760.

[9] M.E. Davis, Z.G. Chen, D.M. Shin, Nanoparticle therapeutics: an emerging treatment modality for cancer. Nat. Rev. Drug Discov. 7 (2008) 771-782.

[10] L.R. Atmakuri, S. Dathi, Current trend in herbal medicines. J. Pharma. Sci. 3(1), (2010) 109-113.

[11] M. Mathur, V. Govind, Role of nanoparticles for production of smart herbal drug−An overview *Ind J Nat Prod Resourc.* 4(4), (2013) 329-38.

[12] A. Ahmad, P. Mukherjee, S. Senapati, D. Mandal, M.I. Khan, R. Kumar, M. Sastry, Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*, Colloids Surfaces B. Biointerfaces. 28 (2003) 313-318.

[13] J. Huang, Q. Li, D. Sun, Y. Lu, Y. Su, X. Yang, H. Wang, Y. Wang, W. Shao, N. He, J. Hong, C. Chen, Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf, Nanotechnology. 18 (2007). 105,104

[14] M. Sastry, A. Ahmad, M.I. Khan, R. Kumar, Biosynthesis of metal nanoparticles using fungi and actinomycete. Current Science. 85 (2003) 162-70.

[15] H. Korbekandi, S. Iravani, S. Abbasi, Production of nanoparticles using organisms, Critical Rev. Biotechnol. 29 (2009) 279-06.

[16] S. Iravani, Green synthesis of metal nanoparticles using plants, Green Chem. 13 (2011) 2638-2650.

[17] I.I. Ijeh, I.A. Okwujiako, P.C. Nwosu, H.I. Nnodim, Phytochemical composition of *Pleurotus tuber regium* and effect of its dietary incorporation on body /organ weights and serum triacylglycerols in albino mice. J. Med. Plants Res. 3(11), (2009) 939-943.

[18] E.N. Agomuo, Proximate phytochemical and mineral elements analysis of the sclerotium of *plerotus tuber regium,* Int. Sci. Res. J. 3 (2011) 104-107.

[19] S.C. Jong, J.M. Birmingham, S.H. Pai, Immunomodulatory substances of fungal origin, J. Immunopharmacy.3 (1991) 115-122.

[20] M. Fillipie, A. Umek, Screening of basidomycetes mushroom extracts for antigenotoxic and biomutagenic activity, Die Pharmazie. 57 (2002) 416-20.

[21] S.H. Hu, V.C. Chia, J.L. Lien, K.S. Chen, M.Y. Lee, J.C. Wang, Antihyperlipidaemic and antioxidant effects of extracts of *Pleurotus cetrinopileatus*, J. Agic Food Chem. 54 (2006) 2103-1210.

[22] L. Brannon-Peppas, J.O. Blanchette, Nanoparticle and targeted systems for cancer therapy, Adv. Drug Delivery Rev. 56 (2004) 1649–59.

[23] E.S. Kawasaki, A. Player, Nanotechnology, nanomedicine, and the development of new, effective therapies for cancer, Nanomedicine. 1 (2005) 101–109.

[24] S. Dandapat, M. Kumar, A. Kumar, M.P. Sinha, Therapeutic efficacy and nutrotional potentiality of

Indian Bay leaf (*Cinnamomum tamala* Buch.-Ham.). Int. J. Pharm. 3(4), (2013) 779-85.

[25] S. Dandapat, M. Kumar, A. Kumar, M.P. Sinha, Antipathogenic efficacy of methanolic leaf extract of *Cinnamomum tamala* and *Aegle marmelos* (L.) with their nutritional potentiality. The Bioscan. 8(2) Supplement on Medicinal Plants (2013) 635-41.

[26] T.V.C. Kumar, T.N.V.K.V. Prasad, K. Adilaxmamma, M. Alpharaj, Y. Muralidhar, P.E. Prasad, Novel synthesis of nanosilver particles using plant active principle aloin and evaluation of their cytotoxic effect against *Staphylococcus aureus*, Asi. Pecif J. Tropic. Diseas. 4 Suppl-1 (2014) S92-S6.

[27] J.Y. Song, B. Kim, Rapid biological synthesis of silver nanoparticles using plant leaf extracts, Bioprocess Biosys.t Eng. 32 (2008) 79-84.

[28] M.J. Firdhouse, P. Lalitha, S.K. Sripathi, Novel synthesis of silver nanoparticles using leaf ethanol extract of *Pisonia grandis* (R. Br). Der. Pharma. Chemica. 4(6), (2012) 2320-2326.

[29] M. Khan, M. Khan, S.F. Adil, M.N. Tahir, W. Tremel, H.Z. Alkhathlan, A. Al-Warthan, M.R.H. Siddiqui, Green synthesis of silver nanoparticles mediated by *Pulicaria glutinosa* extract. Int. J. Nanomedicine. 8 (2013) 1507–1516.

[30] R.M. Silverstein., G.C. Bassler., T.C. Morrill, Spectrometric Identification of Organic Compounds. 4th Edition. John Wiley and Sons publications. New York, 1981.

[31] B. Stuart. Infrared Spectroscopy: Fundamentals and Applications. John Wiley & Sons. Ltd. New York. 2004.

[32] K.K. Mohan, M. Sinha, B.K. Mandal, A.R. Ghosh, K.K. Siva, R.P. Sreedhara, Green synthesis of silver nanon particles using *Terminalia chebula* extract at room temperature and their antimicrobial studies. *Spectrochim.* Acta A Mol. Biomol. Spectrosc. 91 (2012) 228-33.

[33] S. George, Infrared and Raman Characteristic group frequencies: Tables and charts. John Wiley & Sons publications, New York .2004.

[34] P. Larkin, Infrared and Raman Spectroscopy; Principles and Spectral Interpretation. Elsevier; 2011.