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Research Article

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In vitro evaluation of antimicrobial activity of Silver nanoparticle from edible mushroom

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Abstract

Objective: This project was designed to determine the effect of edible mushroom with silver nanoparticle against the pathogenic microbes.
Methods : Pathogenic microbes were isolated & identified with routine cultural and biochemical tests from clinical specimen. Silver nanoparticles were prepared using edible mushroom and characterized with UV, FTRI , XRD & the AgNP biomembrane developed to evaluate the antimicrobial efficiency.
Result : The extract has protein and alkaloids, the spectral range of AgNP noted at 340nm. The antimicrobial activity of AgNP biomembrane shows great amount of susceptibility towards bacteria and fungi. Persuding the inhibiting effect of AgNP of MIC Was calculated as 130mcg/ml to 387mcg/ml.
Conclusion : The present work is aimed to investigate the most efficient ecofriendly method for biosynthesis of silver nanoparticles using edible mushroom and to do characterization of the synthesis of AgNP & it shows the biopharmaceutical potential activity against various bacterial & fungal pathogens.

Keywords: edible mushroom, silver nanoparticle, UV, FTRI , XRD, antimicrobial efficiency

Introduction

Nanotechnology is expected to be the basis of many main technological innovations in the 21st century. Research and development in this field is growing rapidly throughout the world. A major output of this activity is the development of new materials in the nanometer scale, including nanoparticles. These are usually defined as particulate materials with at least one dimension of less than 100 nanometers (nm), even the particles could be zero dimension in the case of quantum dots. Metal nanoparticles have been of great interest due to their distinctive features such as catalytic, optical, magnetic and electrical properties (Bar *et al*., 2009; Rassaei *et al*., 2008).Mushroom is the freshy ,spore –bearing fruiting body of a fungus, typically produced above ground on soil or on its food source. The most popular of these, *Agaricus bisporus*, is considered safe for most people to eat because it is grown in controlled, sterilized environment. Several varieties of *A.bisporus, Pleurotus platypus, P.florida and Calocybe indica* are grown commercially, include white, crimini, and Portobello .Nanotechnology is mainly concerned with the synthesis of nanoparticles of variable size, shape, chemical composition and controlled disparity and their potential use for human benefits. In the past decade there has been a tremendous amount of research interest in nanomaterials with respect to its production properties and application. Many varieties of naturally occurring mushrooms are foung to have promising antioxidant and anticancer properties and prolong longevity(Mizuno and Mush 2000).Edible mushrooms are weel known for their antioxidant, antimicrobial, antiinflamatory, antitumor and anticancer activities(ajith and janardhanan,2007).This indicates that mushrooms could be valuable sources of antioxidant(chen et al., 2006) and antitumor compounds .The biological synthesis of silver nanoparticles (AgNPs) by using edible mushroom extract, Agaricus bisporus, as a bioreductant. The biosynthetic method developed in this study for producing silver nanoparticles has distinct advantages over chemical methods such as high biosafety and being ecofriendly and nanotoxic to the environment. Furthermore, these functionalized silver nanoparticles showed a noticeable antimicrobial activity against different clinically important pathogenic microorganisms. Hence, such type of synthesis methods for the production of nanostructured material at lower cost and with natural energy may encourage production of functionalized AgNPs on industrial scale. This is quite handy for using AgNPs on a wide range of applications in the field of nano biotechnology .

Methodology

Preparation of crude extract of *Agaricus bisporus* & *Calocybe indica*

Fresh mushroom (Agaricus bisporus & Calocybe indica) were procured from commercial sources about 5gm \ each of the mushroom was weighed and washed thoroughly with double distilled water then it is cutted into small piece and kept in boiling water bath for 2 hours with intermitted stirring sealed. After 2 hours extract filtered using whatman No.1 filter paper. The filterate kept in oven to get condensed & kept for overnight at room temperature. The result and filterate is the extract of mushroom used for the reduction of Ag+ to Ag0. The extract of mushroom can be preserved for further experiments.

Screening of aqueous extract for phytochemical analysis

Screening for the phytochemical activity shows the antimicrobial effect of the extract.

Screening of protein by millon’s test :The few ml of extract added with few drops of millon’s reagent and heated.

Screening of alkaloids by Dragendorff’s test : The few ml of extract added with few drops of dragendroff’s reagent.

Screening of carbohydrate by Benetic’s test : Few ml of benetic’s reagent with few ml of mushroom extract andheated.

Screening of Flavonoids by Shinoda’s test : Few ml of extract with 1gm of magnesium and

few drops of concentrated HCL was added and mixed well.

Screening of terpenoids by salkowski test : Few ml of extract with few drops of concentrated sulphuric acid andchloroform was added.

Biosynthesis of silver nanoparticles from edible mushroom extract

10ml of mushroom extract were mixed with 40ml of 0.30mM AgNo3. The mixture turned to brown color which indicates the formation of silvernanoparticles. The nanoparticles are then pelleted and dried in oven at 50^{o}C. similar procedure adopted for both the mushroom extract i.e for button and milk mushroom.

Characterization of silver nanoparticles

I .UV-vis spectrophotometer

Silver nanoparticle exihibit strong absorption of electromagnetic waves due to surface Plasmon reasonce in the visible range. Thus, UV-vis spectroscopy is used to investigate the formation and stability of silver nanoparticle in solution wavelength ranges of 200-800nm. Dark brown color production indicates the presence of silver nanoparticlein the solution.

II. Fourier transform infrared spectroscopy(FTIR) analysis

When infrared radiation is pass through sample,specific wavelength are absorbed which causes the chemical bonds present in the material to undergo vibrations. The colloid of AgNPs-250 to 4500cm^{-1}

III.X-ray Diffraction (XRD) analysis

XRD is a conventional technique used to establish the crystalline nature ,domainsize, and structure of nanoparticles.It can be used to look at a single crystal or polycrystalline materials

Detection of silver nanoparticle using agarose gel electrophoresis

Agarose gel electrophoresis is a method used in biochemistry and molecular biology to separate the molecules by size.This achived by moving negatively charged particles through an agarose matrix with an electric field.Shoter molecules were move faster and migrate further than larger once. Agarose gel prepared 10ulnof BM-AgNPs &MM-AgNPs loaded in each well,in 50vto100v for 30 minutes electrophoresised when the front wave approach the end of the gel.The electrophoresis is stopped ,it is now possible to visualize the AgNPs with UV transilluminator.The AgNP moves towards negative due to positive charge on its core. The brown band of AgNP documented.

Development of Agnp biocompatible membrane

Initially the bioamembranepreparation standardized with biopolymers such as Pectin (food grade) with % range from 0.5%,1.5% and 3% respectively with the cross linker such as 1 % PEG as plasticizer separately and noted for its efficient film formation and peeling tendency and solubility in sterile water at 37 ℃ .On concluding the biopolymer with significant character to be pectin along with plasticizer 1 % PEG.The experiment further proceeded with standardized formulation for biomembarne preparation and used for Anti microbial activity for both bacteria and fungi obtained from laboratory culture (MTCC and ATCC).

Antimicrobial activity of AgNPs Biocompatible membrane

Since the several years silver has been known as a strong disinfecting agent and has been founds many uses in traditional medicinal practices. Several silver based compounds have been utilized effectively as antimicrobial specialists. Compounds have been utilized effectively as antimicrobial specialists. compounds of silver are also used in the medical field to treat burnt wounds and various other types of infection. Nanoparticles of silver have aptly been investigated for their antimicrobial property because the silver nanorticle have high specific area than their volume. Which will lead to excellent antimicrobial activity as compared with bulk silver metal.

Antibacterial activity og AgNP biocompatible membrane

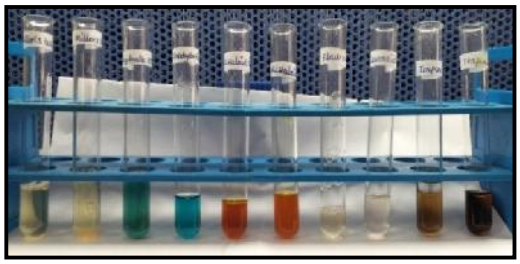
Silver nanoparticles showed potential antibacterial activity against bacteria *(Ecoli, Klebsiella, Streptococcus, Bacillus).* The inhibitory mechanism of silver nanoparticle is only partially understood. mullerhinton agar prepared swabbed with bacterial & loaded with AgNP in different concentration and incubated at 37 for overnight.

Antifungal activity of AgNP biocompatible membrane

Silver nanoparticles showed potential antibacterial activity antifungal activity against the fungal pathogens(*A.flavus, A.niger, C.albicans*, Mucor). The potatodextrose agar plates were prepared and inoculated with respective fungal culture the AgNP biocompatible membrane placed on the fungal plate and incubated at 28 for 48-96 hours. The zone of inhibition were observed.

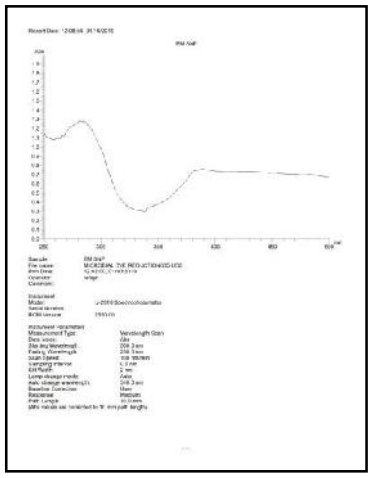
Results

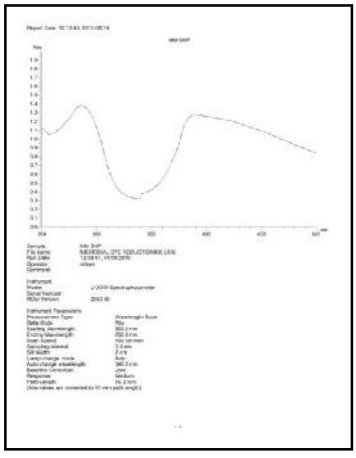
The most popular mushroom Button, & Milk mushroom is considered safe for most people to eat because it is grown in controlled, sterilized environment. Many varieties of naturally occurring mushrooms are found to have promising antioxidant and anticancer properties and prolong longevity. Edible mushrooms are well known for their antioxidant, antimicrobial,anti-inflammatory, antitumour and anticancer activities. This indicates that mushrooms could be valuable sources of antioxidant and antitumour compounds. The extract has protein and alkaloids, the spectral range of AgNP noted at 340nm. The antimicrobial activity of AgNP biomembrane shows great amount of susceptibility towards bacteria and fungi. Persuding the inhibiting effect of AgNP of MIC Was calculated as 130mcg/ml to 387mcg/ml.



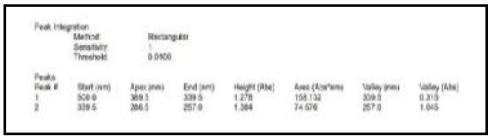
Phytochemical test of mushrooms

Mushroom extract in silver nitrate

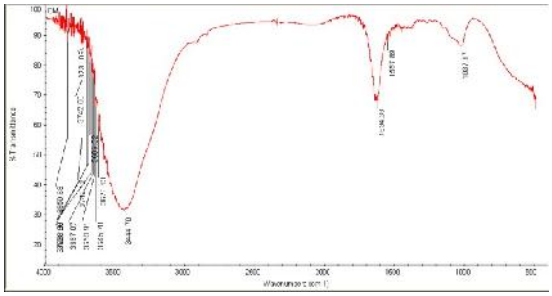




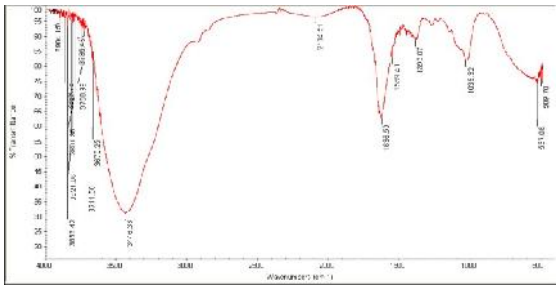
Detection of AgNp by UV scans of BM



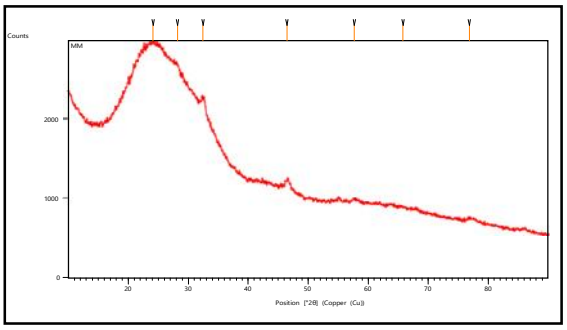
Detection of AgNp by UV scans of MM



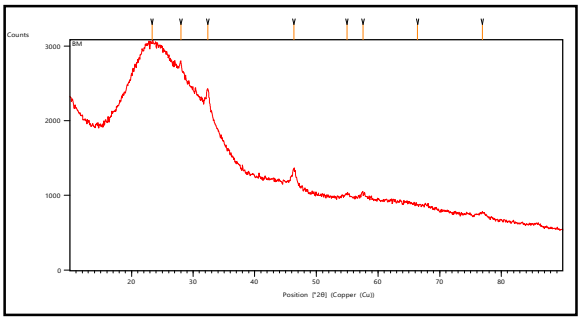
FT-IR stretching of BM AgNP



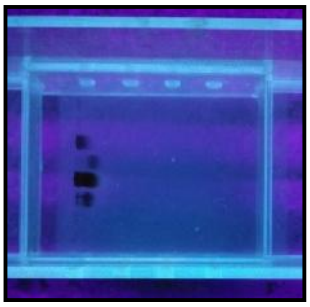
FT-IR stretching of MM AgNP



XRD spectrum BM AgNP



XRDspectrum-MM AgNP



Agarose gel electrophoresis

Antimicrobial activity of SNP (BM & MM)

Bacteria-BM	50	150	250	350	MIC (mcg/ml)
Klebsiella	13	13	14	14	130.69
Streptococci	14	14	16	16	130.08
E.coli	12	12	13	13	131.10
Pseudomonas	13	14	14	14	130.59
B.subtilis	12	13	13	14	130.90
Bacteria-MM	**50**	**150**	**250**	**350**	**MIC (mcg/ml)**
Klebsiella	12	13	14	14	130.80
Streptococci	14	14	16	16	130.08
E.coli	14	14	15	15	130.29
Pseudomonas	14	15	16	16	129.98
B.subtilis	14	14	15	16	130.19
Fungi-BM	**50**	**150**	**250**	**350**	**MIC (mcg/ml)**
C.albicans	14	15	15	16	130.08
A.flavus	22	24	24	24	126.66
Aniger	22	25	27	27	125.98
Mucor	16	16	17	18	129.37
Fungi-MM	**50**	**150**	**250**	**350**	**MIC (mcg/ml)**
C.albicans	15	15	16	16	129.88
Aflavus	23	24	24	24	126.56
A.niger	25	26	26	27	387.31
Mucor	16	16	17	18	129.37

Conclusion

The present work is aimed to investigate the most efficient ecofriendly method for biosynthesis of silver nanoparticles using edible mushroom and to do characterization of the synthesis of AgNP & it shows the biopharmaceutical potential activity against various bacterial &fungal pathogens. However whether such extract will acts as effective. Therapeutic agent remain to be investigated, the identification of the bioactive compounds and study of the mechanism of action are necessary prior to application.

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