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Biosynthesis of Gold nanoparticles from *Laetiporus versisporus* and its Anticancer activity against Human Breast cancer cells

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Abstract

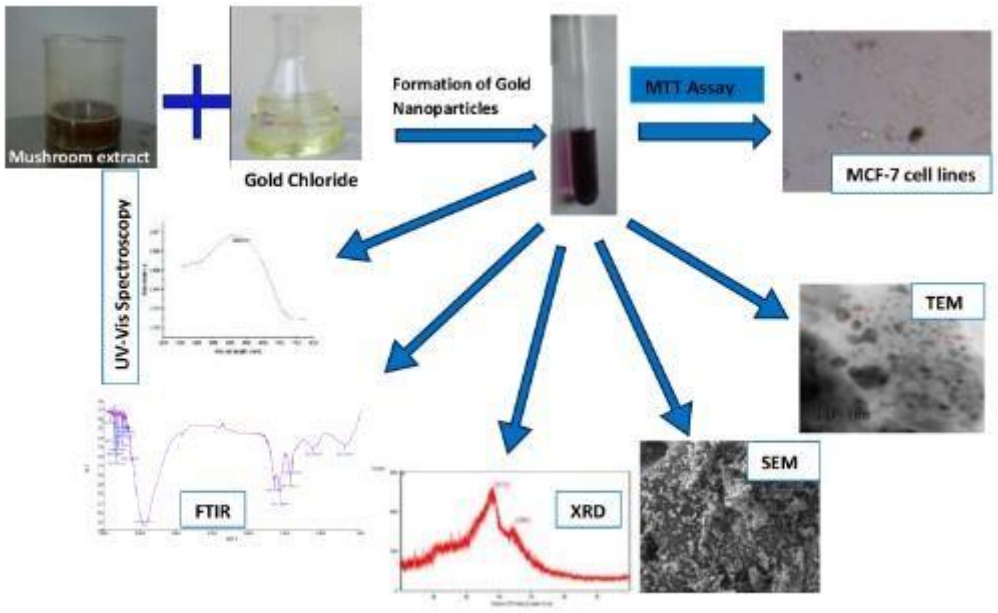
Nanotechnology brought everlasting wonders in the field of health science and medicine. It extends its role in other fields such as textile industries, engineering and electronics, defense and security, cosmetics and energy storage. This vast field is steered up by the involvement of nanoparticles. Metal nanoparticles are distinctive and they show a great range of advantages in diverse disciplines. Nanoparticles are obtained via different approaches like physical, chemical and biological methods. Synthesis of metal nanoparticles by biological method is an ecofriendly access to move in to the extraordinary uses of the same. Noble metal nanoparticles are highly beneficial especially in the drug delivery and therapeutic techniques. Gold nanoparticles, especially have indigenous medicinal features in curing different diseases. One pot green synthesis of nanoparticles are common nowadays and it turns down the side effects created by other usual treatments. Various Biosystems are being used for the synthesis of nanoparticles including prokaryotes and eukaryotes. Edible mushrooms are the richest source of nutritional constituents principally antioxidants, fibers and proteins. *Laetiporus versisporus* is one such mushroom which was used for the synthesis of gold nanoparticles in the current study. Earlier studies reported the isolation of Lanostane Triterpenoids and Saponins from *Laetiporus versisporus*. Synthesized gold nanoparticles were characterized by UV-Vis Spectroscopy, X-ray diffraction (XRD), Fourier Transform –Infrared spectroscopy (FT-IR), SEM and TEM. They were then investigated for their anticancer activity against breast cancer cells (MCF-7) and found to be having greater cell inhibition percentage.

Keywords: Anticancer activity, *Laetiporus versisporus*, Mushrooms, Nanoparticles, Nanotechnology.

Highlights

- Gold nanoparticles were synthesized from wild edible mushroom *Laetiporus versisporus*.
  - The mushroom act as a reducing agent and Gold (III) chloride was the precursor.

- The mushroom mediated gold nanoparticles were characterized by UV- Visible Spectroscopy, FTIR, XRD, SEM and TEM.
- Anticancer activity against the Human breast cancer cell lines (MCF-7) was done with the synthesized gold nanoparticles.



1. Introduction

Nanotechnology is a vast discipline which provides enormous applications that we can employ in health sciences, agriculture and food, electronics, optics and communication engineering, metallurgy and textile industries, defense and energy storage. It's been a cutting edge in the nutrient delivery systems especially in plants. Nanoparticles, being the miniscule elements, surprise the planet with numerous explorations in every nook and corner. Its peculiarity relies mostly on the size and shape of nanoparticles. Invention of nanoparticles eases the detection of oncogenes and proteins at the initial stages of the disease [18]. They are synthesized using diverse methods of which the green synthesis is rather better in all ways when compared to other conventional techniques. Green biosynthesis includes not only the whole prokaryotes and eukaryotes but also the constituents from them. Noble metal nanoparticles have some specificity over others as they are highly stable; they make up their use in the field very intensely. Gold nanoparticles are multifunctional and possess high specific surface area which supports the photo thermal conversion capability. Stability of gold nanoparticles goes in hand with the optimum pH, concentration and type of salt ions.

The synthesis of nanoparticles became a breakthrough in the world of nanotechnology by supporting the plan of action in wide range. The bottom –up and top-down approach equally

favors the production process albeit the former is considered to be the pre- eminent of the two. Among the chemical vapor deposition, sol gel processes, Laser and spray pyrolysis and other similar techniques, biological methods are considered to be a boon as it is extremely ecofriendly. Prokaryotes, Eukaryotes and their extracts, DNA and even membranes are being used in the green synthesis. It surpasses the other conventional methods with its expeditious results, and mere use of toxic chemicals. The cutting edge of nanotechnology and nanoparticles made a great progress and development in different fields including the diagnosis such as diagnostic devices and drug carriers [12] and [13]. It increases the chemical activity inside the cells as it has high surface area [15]. Dental medicine has a wide application of nanoproducts especially in bone grafting, mandible implant, nanorobots etc. [21].

Microbes usually grow rapidly, secretes enzymes which favor the reaction rate, produce different enzymes and manipulate genes [16]. Earlier studies figured out the concept of microbial reduction which is an important phase in the formulation of nanoparticles production [5]. Nanoparticles synthesized from mushrooms show greater stability and also the dimensions of which are different from those obtained from other living sources [1]. The surface of nano carriers is modifiable and so they can be applied as therapeutic carriers in dreadful diseases arises as a result of mutation [20]. Although there are many advances in device implantation, the dark side of it shows a steady decrease in the remediable path because of device centered infections. This can be controlled by the use of nano products which can assist in anti-infection [7]. Mushrooms, one of the key sources in nanomaterial synthesis contributes high stability Nano metals. Green synthesis of nanoparticles using mushroom was initially done in silver which fortunately engenders more experiments in the same [2] and [8]. Edible mushrooms are consumed for their nutritional and medicinal value. *Laetiporus versisporus* is a ubiquitous edible mushroom. Five lanostane triterpenoids and three saponins were isolated by [6]. Those kinds of triterpenoids from other mushrooms were examined for their anticancer activity.

Cancer, one of the deadliest diseases ever is common in every parts of the world regardless of the causative agent, gender and age. Various types have been explored and many leads to an increased fatality rate. Different stages of each type is a key factor to observe, failing which leads to amputation or even worse ends with death. Medications differ for each phase and regular therapies after surgery protects organs from metastasis. Series of counter measures impact the individuals physically and cast down the body to its ultimate. Anticancer drugs cure the tumors to maximum extent though there are some side effects. This can be overcome by entangling the nanoparticles with the desired drugs [9]. Metal nanoparticles bound with the drugs are nontoxic and elimination of nanoparticles are supervised properly [10]. The top three types of cancers which are most commonly seen worldwide are breast cancer, colorectal cancer and lung cancer with no specific cause for its development. Breast cancers are leading in the list which can be acquired even by a genetic source. It can be non-invasive, invasive or metastatic. Gold

nanoparticles obtained from mushrooms showed anticancer activity and other biological activities [14].

2. Materials and methods

2.1. Preparation of mushroom extract

Fresh fruiting bodies of *Laetiporus versisporus* were obtained from the hills of Kodaikanal. The mushrooms were washed repeatedly with double distilled water to get rid of the impurities present on the surface. They were then chopped into small pieces, shade dried and powdered. It was then boiled for a while and the extract was filtered, separated and allowed to cool at room temperature.

2.2. Biosynthesis of gold nanoparticles

The fresh mushroom extract was added to desired concentration of gold chloride solution. The experimental reaction took place in dark condition along with the control. The color change was observed visually. The sample was then centrifuged at 8000 rpm for 10 minutes. The obtained precipitate was dried in a hot air oven for 24 hours which leads to the formation of gold nanoparticles in powder form.

2.3. Characterization of gold nanoparticles

Characterization by UV visible spectroscopy is the commonly used way to confirm the formation of gold nanoparticles. Gold nanoparticles were characterized by UV spectrum in the range of 400 to 800 nm. The functional groups of few biomolecules that are responsible for converting chloride ions to gold nanoparticles were analyzed by Fourier Transform Infrared Spectroscopy (FTIR) between the wavelength ranges of 4000 to 500 cm^{-1}. XRD measurements of the gold nanoparticles was done on XPERT – PRO diffractometer system with certain parameters including the generator settings as 30 mA and 45kV.TEM and SEM analysis shows the size and shape of the synthesized gold nanoparticles

2.4. Anticancer activity

2.4.1. Cell culture

The human breast cancer cell line (MCF-7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37° C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week. **2.4.2. MTT Assay**

The monolayer cells were detached with trypsin-ethylene diamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microliters per well of cell suspension were seeded into 96- well plates at plating density

of 10,000 cells/well and incubated to allow for cell attachment at 37° C, 5% CO_2 , 95% air and 100% relative humidity. After 24 hours the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethyl sulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 hours at 37° C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

3. Results and Discussion

3.1. Synthesis and characterization studies of Gold Nanoparticles (AuNPs)

The gold nanoparticles were synthesized by one pot green synthesis method. Aqueous extract of mushroom *Laetiporus versisporus* was used which acted as a reducing agent whereas gold chloride acts as a precursor simultaneously. The formation of gold nanoparticles was initially observed by a color change of yellow to pale purple before and after the reaction respectively. The formation of gold nanoparticles started at 15 minutes which was represented as a change of pale purple color and deep purple color in an hour (Fig. 1).

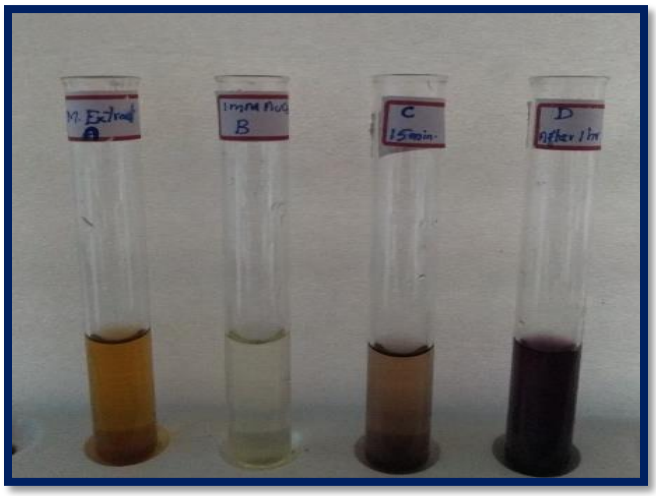


Fig 1: Image showing the color change of gold nanoparticles after the reduction of gold ions

3.2. UV Visible Spectroscopy of gold nanoparticles

Optical spectroscopy is one of the simplest and easiest ways to determine the geometrical properties of metal nanoparticles including their size and shape. Optical property of gold nanoparticles was analyzed by JASCO UV VIS NIR V-670 Spectrophotometer. One of the unique features of metal nanoparticles is Surface Plasmon Resonance which is exploited in

optical spectroscopy to estimate the size and distribution of nanoparticles. UV Visible spectroscopic analysis confirmed the formation of gold nanoparticles. Fig. 2 shows the UV Visible spectra obtained for the mushroom mediated synthesis of gold nanoparticles. The Surface Plasmon Resonance (SPR) peak wavelength of gold nanoparticles were estimated at different times with different samples and found to be in the range of 515 to 572 nm. The maximum absorption spectra were observed at 566 nm. Similar SPR peak of gold nanoparticles was studied by [22].

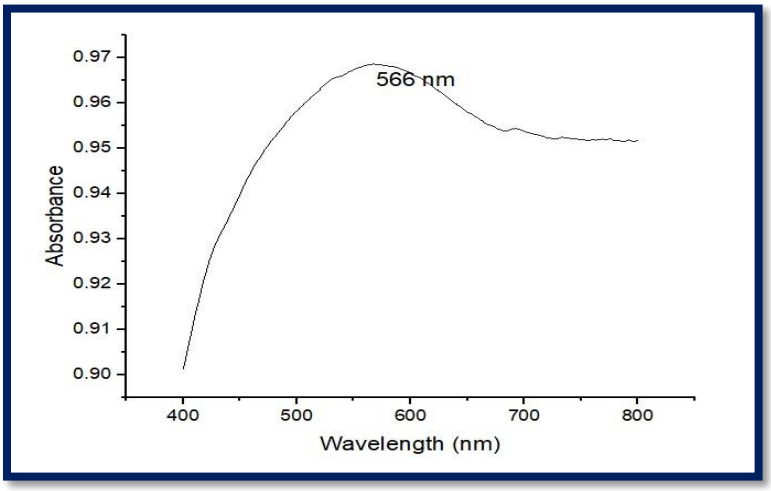


Fig 2: Image of UV – vis spectroscopy showing a maximum band at 566 nm

3.3. XRD

The crystalline nature of gold nanoparticles was confirmed by XRD analysis. Fig. 3 shows the XRD spectra of gold nanoparticles having 2 diffraction peaks in the 2 theta ranges 38.2^0 and 44.3^0 from 10^o to 70^0 which can be indexed to (111) and (200) that shows the face centered cubic structure of gold nanoparticles (JCPDS no. 04-0784). This represents the crystalline nature of the gold nanoparticles.

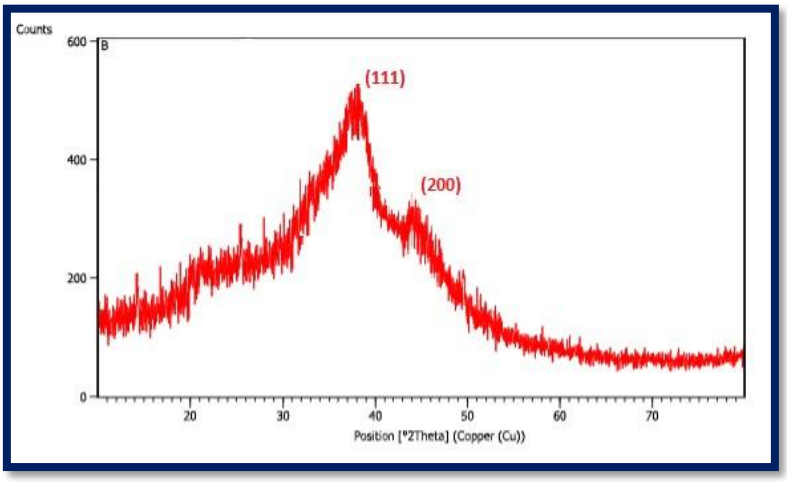


Fig 3: XRD spectra of synthesized gold nanoparticles

3.4. Fourier Transform Infrared Spectroscopy (FT-IR) studies

FT-IR analysis of gold nanoparticles was analyzed to understand the biomolecules responsible for the reduction of gold ions to gold nanoparticles. Surface chemistry of the nanoparticles plays a vital role in the biological reactions occur in biosystems in vitro and in vivo. [4]. FTIR spectra give a detailed study of the surface chemistry of residues present in the mushroom which aids in the reduction. [3]. The spectrum Fig (4.1) exhibits bands at 3399 cm $^{-1}$ (N-H stretching of amines and amides), 2927 cm $^{-1}$ (C-H stretch of alkanes), 1637 cm $^{-1}$ (-C=C- stretch of alkynes), 1412 cm $^{-1}$ (C-C stretch in aromatics), 1147 cm $^{-1}$ (C-H of alkyl halides), 1082 cm $^{-1}$ and 1035 cm $^{-1}$ (C-N stretch of aliphatic amines), 928 cm $^{-1}$ (O-H bend of carboxylic acid), 850 cm $^{-1}$ (C-Cl stretch of alkyl halides) for the mushroom *Laetiporus versisporus*.

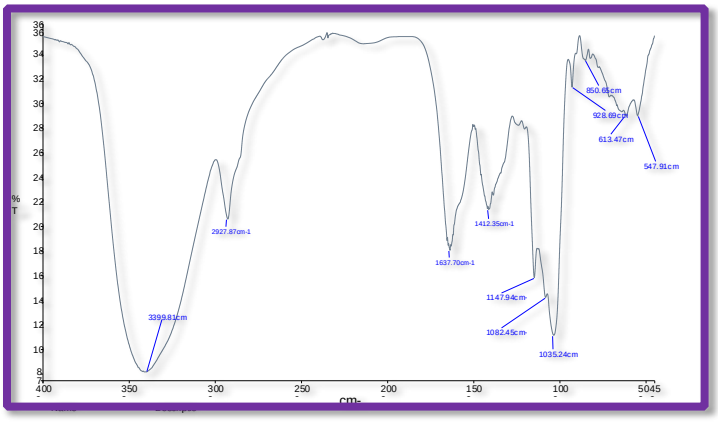


Fig 4.1: FTIR spectra of *Laetiporus versisporus*

The bands observed for gold nanoparticles Fig (4.2) are 3444 cm $^{-1}$ (O-H stretch of alcohols and phenols), 1634 cm $^{-1}$ (C=O stretch of carboxylic acids), 1557 cm $^{-1}$ and 1417 cm $^{-1}$ (C=C stretch of aromatics), 1093 cm $^{-1}$ (C-N stretch of aliphatic amines) and 659 cm $^{-1}$ (C-H bond of aromatics). The broad intense peak observed at 3399 cm^{-1} in the mushroom extract was shifted to 3444 cm^{-1} representing the N-H bond stretching of amines. By comparing the spectra of the source mushroom and gold nanoparticles, it was understood that there was a change in the N-H stretch of amines at the peak 3399 cm^{-1} which was happened by the reduction of gold ions and it was shifted to 3444 representing the O-H stretch of alcohols. Similarly the peaks 1412 and 1082 is reduced to 1417 and 1093 cm^{-1}.

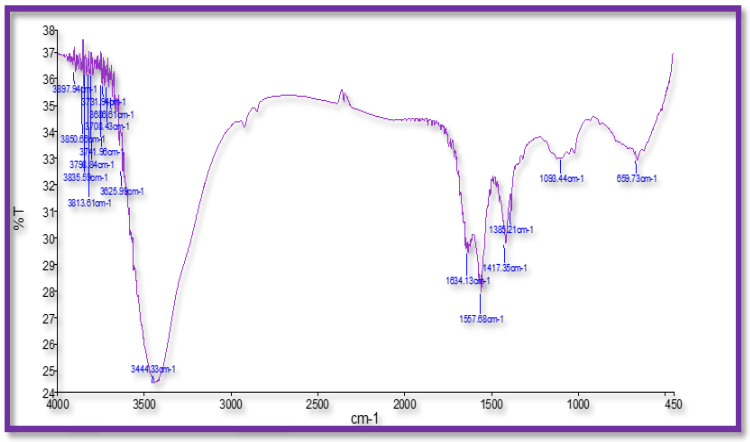


Fig 4.2: FTIR spectra of synthesised gold nanoparticles

3.5. SEM and TEM analysis

TEM and SEM images of the gold nanoparticles showed that they are clearly dispersed. They reflect the size and shapes of nanoparticles (Fig. 5a, b, c, d). It was discovered that the surface study of gold nanoparticles extracted from Laetiporus versisporus represents that they are in different shapes. TEM images exhibited illustrates that some particles are clumped and some are spherical that show an average size of 10 nm.

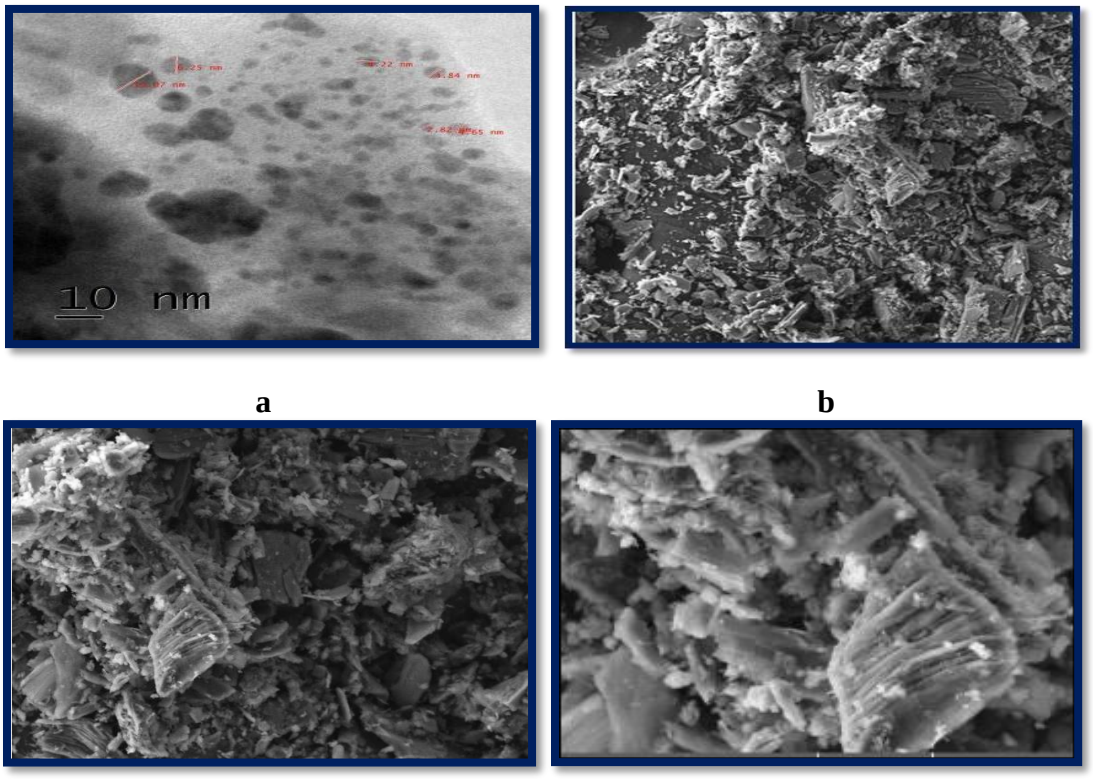


Fig 5: Images of gold nanoparticles observed under (a) TEM, (b), (c) and (d) SEM

3.6. Anticancer activity

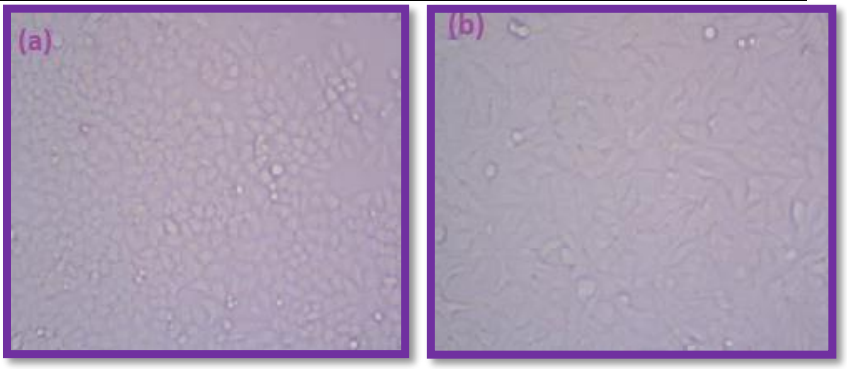
MTT assay is a calorimetric assay which predicts the proliferation of cells, cell cytotoxicity and enumeration of viable cells in a sample. NADP dependent oxidoreductase enzyme which is present in mitochondria of viable cells plays a key role in MTT assay. The principle behind the assay is that MTT is reduced to water insoluble Formazan crystals by mitochondrial dehydrogenase present in the live cells. Nonviable cells do not produce mitochondrial dehydrogenase. Thus dead cells do not cause this change. So finally the amount of Formazan crystals produced is directly proportional to the number of viable cells in the sample.

Gold nanoparticles synthesized from mushroom *Laetiporus versisporus* was tested for anticancer activity against human breast cancer cell lines- MCF-7. Different concentrations (6.5, 12.5, 25, 50 and 100 µg/ml) of gold nanoparticles were treated with MCF-7 cells which showed increasing percentage of inhibitory activity as 9.28, 21.38, 38.87, 52.05 and 58.53 respectively Table 1. This was revealed by MTT assay by the color formation. MTT or (3-(4, 5Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide) is a light sensitive, yellow colored tetrazolium salt. It was treated with nanoparticles treated MCF-7 cells and then with DMSO,

which was then incubated. MCF-7 cells without any treatment acted as a control Fig 6a. After incubation, the cells treated with 6.5 and 12.5 of gold nanoparticles showed 9.28% and 21.38% of cell inhibition (Fig 6b, c) and it showed maximum resemblance with the control whereas the MCF-7 cells treated with maximum concentration of gold nanoparticles showed maximum percentage of cell inhibition (Fig 6d, e, f). The increase in concentrations of nanoparticles is directly proportional to the percentage of cell inhibition (Fig. 7).

Table1: percentage of MCF-7 cell lines inhibited by the action of Gold nanoparticles (AuNps) synthesized from mushroom *Laetiporus versisporus*

Concentration of AuNps (µg/ml)	% of Cell inhibition
6.5	9.28
12.5	21.38
25	38.87
50	52.05
100	58.53



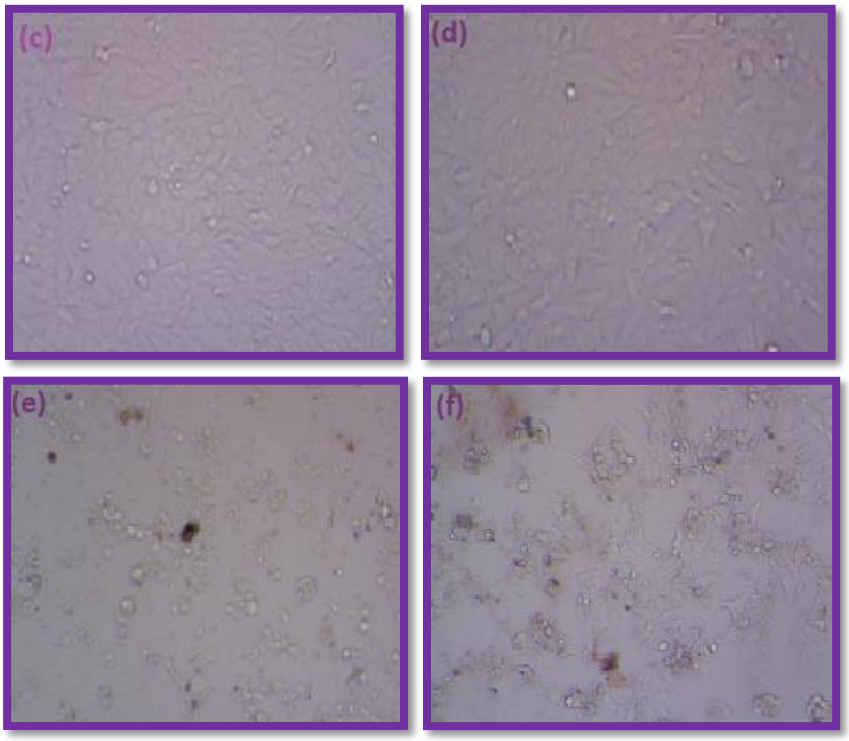


Fig 6 (a) Control cells - MCF-7 cells, (b) cells treated with 6.5 µg of AuNps, (c) cells treated with 12.5 µg of AuNps (d) cells treated with 25 µg of AuNps (e) cells treated with 50 µg of AuNps (f) cells treated with 100 µg of AuNps

A similar work with marine bacterium *Streptomyces griseus* against breast cancer cells and colon cancer cells was done by [11] [17] and [19] executed the same concept with lung and liver cancer cell lines by silver and gold nanoparticles. Fig. 6a shows the MTT assay done with the control against the breast cancer cell lines and (b) gold nanoparticles (100 µg) against human breast cancer cell lines.

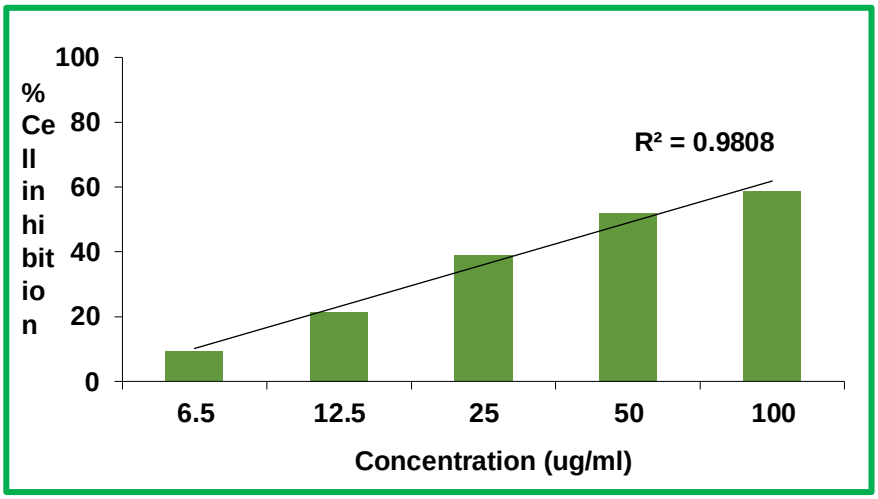


Fig 7: Correlation of concentration of gold nanoparticles used against MCF-7 cells with the percentage of cell inhibition

4. Conclusion

The current study evaluates the biosynthesis of gold nanoparticles from wild edible mushroom *Laetiporus versisporus*. Characterization techniques like UV Visible Spectroscopy, X ray diffraction studies, FTIR, SEM and TEM were done to confirm the presence of crystalline gold nanoparticles, reduction of gold ions to gold nanoparticles by the biomolecules in the mushroom and shape and size of the newly synthesized gold nanoparticles. Those synthesized nanoparticles were tested for anticancer activity against human breast cancer cell lines (MCF-7). Different concentrations of the nanoparticles (6.5 µg, 12.5 µg, 25 µg, 50 µg and 100 µg) and the control cell lines were taken for the MTT assay and it was found that at lower concentration of treatment of nanoparticles on MCF 7 cells could inhibit only a mere percentage of cell lines and increasing the concentration of gold nanoparticles increases the inhibition of MCF-7 cells. A linear correlation of increase in concentration with increase in percentage of cell inhibition was understood. This study would be taken in advance with the development of anticancer drugs against human breast cancer cell lines.

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