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Original Research Article

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Biological synthesis of gold nano particle from *Chryseobacterium* spp and its medical application in the cancer diagnosis

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ABSTRACT

Keywords

Gold nanoparticle, Biological synthesis, *Chryseobacterium*, Medical application, Cancer diagnosis Nanocrystalline silver and gold nanoparticles produced by ecofriently plant materials are widely used to control many plant, animal and human pathogenic microorganisms. To keep in mind, an attempt was made to evaluate the potential of suppressive activity of nanoparticles produced by *Chryseobacterium spp*. The gold nanoparticles produced by the plant in TSM medium amended with an aqueous solution @ $2x10^{-3}$ M chloroauric acid and $3x10^{-4}$ M silver nitrate; respectively were quantified by spectroscopically at different wavelengths in the range of 300-700nm. The readings of UV-VIS spectra showed that there were two prominent peaks at 410nm and 450nm for silver and gold nanoparticles production respectively. These peaks were found to be quiet stable even after two months which coincided with the stability of nanoparticle production. The results revealed that they were able to suppress the growth of cancer cells significantly.

Introduction

Nanotechnology is a emerging field that is developing in many areas, including the field of medicine, lifescience and in new technologies. Due to their unique properties they are the leading phenomenon in present world. The metal gold has some distinctive features like less oxidation compared to other metals. Gold nanoparticles possesses number of potential applications for instance, an effective drug delivery against microorganism. Recent studies had showed that the synthesis of nanoparticles using microorganisms had led to exciting area of research in the cancer diagnosis. Cancer is a disorder of cells and although it usually appears as a tumour (a swelling) made up of a

mass of cells, the visible tumour is the end result of a whose series of changes which may have taken many years to develop [1]. Cancer is a important cause of mortality worldwide people and the number of people who are affecting is increasing [7]. The origin of cancer can be related to metabolic alteration, such as mitochondrial increase of glycolysis, which largely depends on this metabolic pathway needed to convert glucose to pyruvate for the generation of ATP to meet the cancer cell energy need. The present study demonstrate the efficacy of biologically synthesised silver nanoparticles (AgNPs) as a anti-tumour agent using lung cancer cell lines invitro and invivo.

Gold nanoparticles (AuNPs) are currently playing a significant role for human welfare in the field of clinical diagnosis as well as several biomedical applications. More and more research shows that AuNPs-based technologies are becoming promising approaches in cancer research and AIDS treatment. Gold (Au) is unique compared to other metals because of its resistance to tarnishing. According to the earliest records use of Au for medical purposes can be traced back to the Chinese civilization in 2500 BC, and after that, several ancient cultures have utilized Au-based materials for medicinal purpose for the treatment of a variety of diseases such as smallpox, skin ulcers, measles, and syphilis [6]. In today's era of nanotechnology, gold nanoparticles (AuNPs) have been used for the treatment of diseases like rheumatoid arthritis, and so forth, while considerable research is currently going on for unveiling potential anticancer and antimicrobial and bio diagnostic applications of Au-based materials and compounds for clinical applications [5].

Diagnosis and treatment of cancer in nascent stages are of great importance because of the widespread occurrence of the disease, high death rate, and the frequency of reoccurrence even after treatment. According to cancer statistics 2010 done by American Cancer Society, estimated new cancer cases were 1,529,560 and 569,490 death in both males and females in the US.

The gold nanoparticles was produced by both chemical and biological synthesis using tri sodum citrate as reducing agent and *using chryseobacterium* in biological synthesis. GNPs are easy of synthesis, functionalization and biocompatibility [Shree R. singh *et al.*, 2011].

Actinomycetes have the ability to produce antibiotics and *chryseobacterium* which comes under actinomycetes are gram negative and widely distributed in soil has eminent properties in the synthesis of gold nanoparticles.the synthesized GNPs for drug delivery is the preliminary application in a particular target. The main objective of this study is that synthesizing the gold nano particle and with that nanoparticle the cancer will be diagnoised by the targeted drug delivery system. The cancer activity will be tested using the animal cell line and further implemented on the animal model (mouse) for checking the activity of gold nanoparticle having the activity against the cancer.

Materials and Methods

Collection of the sample

The soil sample was collected from the costal region of Rameshwaram, Ramanathapuram district, Tamilnadu. From that soil sample the chryseobacterium spp was isolated.

Synthesis of Gold Nanoparticles

Chemical reduction method

The chloroauric acid [0.02M] was reduced to gold nanoparticles by using trisodium citrate [0.01M] which acts as reducing agent when boiled at 80 degree Celsius for 30 minutes at water bath where a color change occurred to purple. This method is also called as citrare synthesis method. This shows that chloroauric acid is reduced and gold ions were produced.

The solution was filtered with the wattman filter paper and lyophilized at a temperature of-70 degree celcius for 48 hours and further it was characterized at various methods in order to confirm that synthesized particle is nanoparticle or not and its size was characterized.

Biological synthesis

The biological synthesis method was done by

a actionomycete spp called *chryseobacterium* which produce enzymes namely reeducates and dehydrogenase which has the ability to reduce chloroauric acid. The synthesis was done with an ISP2 media, 0.1M stock solution prepared and taken at different concentrations (0mM, 0.5mM, 0.8mM, 1mM, 1.5mM, 1.8mM and 2mM).two control were taken with and without culture. The samples were made up to 1ml using ISP2 media, and then Chryseobacterium was inoculated in all the test tubes and incubated for two days at 37 degree Celsius.

After incubation the color change was obtained from green to yellow. Then this medium was inoculated to 100ml of ISP2 medium with different concentration (0.2M, 1.5M and 2M). The wavelength obtained for concentrations was 533nm, 528nm, 541nm and 325nm respectively by using UV-Spectrophotometer. There was no color change in both the controls. Chryseobacterium spp is a gram negative bacterium. It is marine bacteria which was collected from Rameshwaram marine soil. The chryseobacterium has the ability to reduce the chloroauric acid into gold nanoparticle.

Characterization

The synthesized gold nanoparticles were lyophilized at temperature of-70 degree celcius for 2 days.

UV-Spectro photometer

Initially the gold nanoparticle was confirmed by UV-Spec and the wavelength obtained was at 525nm with absorbance of 0.488.

SEM analysis

The synthesized nanoparticles were lyophilized for 3 days at and the sample was

conformed as nanoparticles.

X-Ray diffraction

The presence nanoparticles was conformed in X-Ray diffraction by calculating the devdy scherr equation.

Flurosence analysis

The flurosence was measured and a nanoparticle was comformed.

Results and Discussion

UV-Spectrophotometer is used to find out the simple molecules in a reaction mixtures. UV visible spec for GNPs from Chryseobacterium spp is 525nm. The UV Spectrophotometer is used for the initial conformation of the synthesized gold nanoparticle. For the GNPs the wavelength was observed at 528.0nm and the absorbance range about 0.361. This wave length initially confirm the produced particle was gold naoparticle. After the UV Spec readings the nanoparticle was confirmed with other characterization method which include Scanning Electron Microscopy, Flourimetry, and FTIR methods. The Scanning Electron Microscopy is used to find out the size of the gold nanoparticle produced.

The flourimetry is used to find the fluorescence capacity of the produced gold nanoparticle.

The fluorescence was calculated with the fluorescence emission and fluorescence expiation. For the good fluorescence capacity the particle should have the fluorescence value of 1 the FTIR (Fourier Transform Infra-Red) method is used to characterize the produced gold nanoparticle.

Fig.1 Chryseobacterium



Fig.2 UV Spectrophotometer

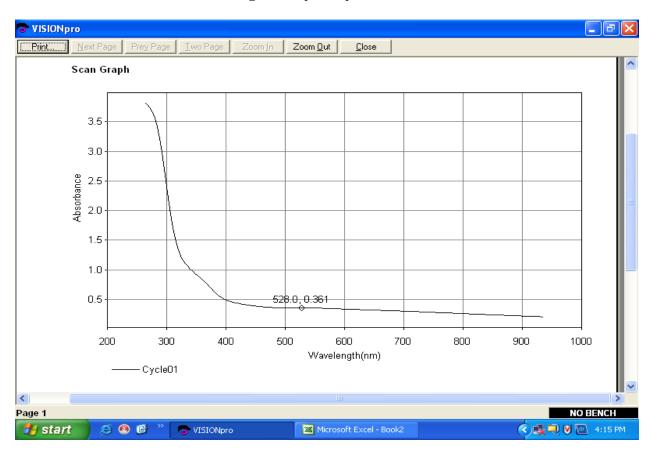


Fig.3 SEM analysis

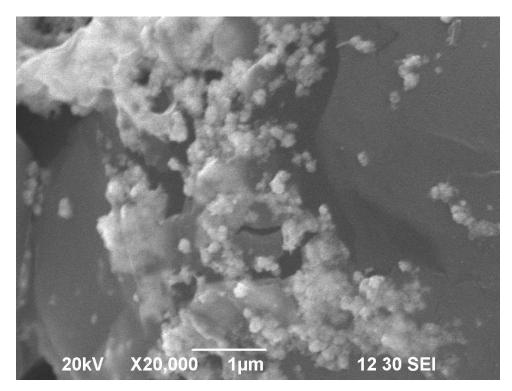
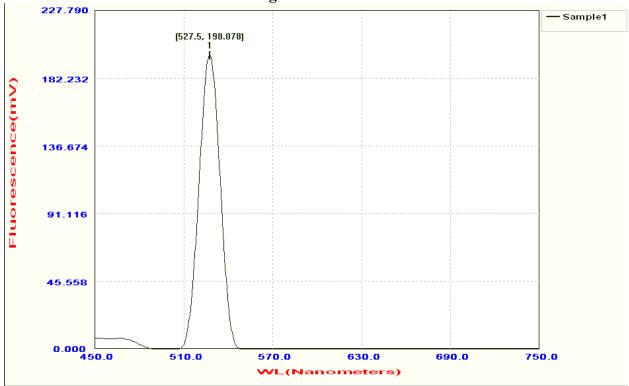


Fig.4 Fluorescence



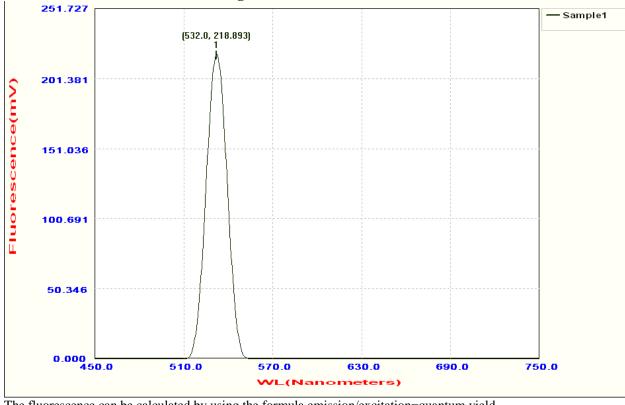


Fig.5 Fluorescence Emission

The fluorescence can be calculated by using the formula emission/excitation=quantum yield.

In the FTIR the chemical bonds present in the synthesized particle was identified. Thus with these information the produced particle was confirmed as gold nanoparticle.

The produced gold nanoparticle was applied in the human non-small cell lung carcinoma cell line for the identification of the activity of the nanoparticle. The gold nanoparticle was bounded with the drug molecule which was targeted at the site of the cancer.

Thus this is technically called as targeted drug delivery system. The gold nanoparticle kills the cancer cells in the cell line studies thus my further work was studying the activity of the gold nanoparticle in the animal model studies.

UV Spectrophotometer

This graph shows that the nano particles was produced at the nanometer of 528 and absorbance was found at 0.0361. This is the prilimanary stage to confirm the nano particles by its wavelength.

Flourimetry

This is used to find out the emission and excitation of the GNPs. The result showed by this

SEM analysis

The morphology (size and shape) of GNPs was amalysed using SEM. the size of the GNPs may be elliptical or spherical.

Fluroscence

The value obtained by using this formula should be less than 1.

The fluorescence can be used to estimate fluorescence capacity of gold nano particle the value of fluorescence is less than 1.

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