Green Synthesis of Natural Plant Extracts And Its Potential Activites

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ABSTRACT

Due to plant distinct qualities and many surface capabilities for diagnosis and targeted cell therapies, gold nanoparticles (AuNPs) have been widely used in medical applications. Current nanotechnology research has a fresh focus on environmentally safe green production of metallic nanoparticles using renewable bioresources. Aqueous extracts of Bay leaf, Cassia bark, and Cardamom were used in the current study to create gold nanoparticles (AuNPs) with outstanding stability in a cost-effective and environmentally friendly manner. Spectroscopic analysis was used to assess how reaction variables like temperature and pH affected the biogenesis of nanoparticles. The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide reduction assay (MTT Assay) was used to determine the in vitro cytotoxicity of these gold nanoparticles.

Keywords: Gold nanoparticles (AuNPs); MTT Assay; Bay leaf; Cassia bark; Cardamom;

1. INTRODUCTION

The gold nanoparticles are among these nanoparticles, and they are well known for their promising uses in sensing, optics, electronics, catalysis, medicine, and catalysis. The usage of gold nanoparticles is expanding as a result of their unique physiochemical characteristics, such as size and charge, and biological characteristics, such as antibacterial activity, which may be tailored to the situation[1,2]. Gold nanoparticles are a unique ingredient that have been used to treat a variety of pathophysiological disorders, including anti-HIV, anti-malarial, and anti-arthritic agents. Physicochemical processes or physical processes can both be used to create nanoparticles. Recent research has focused heavily on the use of biologic synthesis techniques without the need for risky chemicals in synthesis processes in order to prevent adverse effects on biomedical applications to synthesise biocompatible metal.Utilizing biological components like plant biomass or extracts as an alternative to chemical and physical processes to produce nanoparticles in a sustainable manner is an option[3]. Additionally, biologically produced nanoparticles are useful for biomedical applications. The process of synthesis is currently referred to as "Green synthesis[4]".Improved knowledge of the toxicity of nanoparticles will aid in preventing negative consequences and could be useful

when creating nanoparticles. Many times, an appropriate surface modification can make harmful nanoparticles less harmful, enabling their use in therapeutic procedures. Unquestionably, cell-based tests are the screening procedure that are used the most frequently. The dose of nanoparticles that inhibits cell development by 50% (IC50) is a typical way to express the toxicity of nanoparticles. To determine a reasonable dose for the initial animal research, results from cell testing are employed[5]. As far as we are aware, relatively few studies have examined the toxicity of AgNPs made from plant extracts on adult zebrafish.Traditional in vivo research is time- and money-consuming, even if studies on the toxicity of nanoparticles in mammalian cell lines have been regarded as a standard tool for risk prediction in humans.. The cell-based test also has limitations in terms of accurately simulating the metabolism of the entire animal body. As an alternative to typical mammalian cell lines and mouse models, researchers are interested in employing zebrafish and their embryos as the paradigm for in vivo studies. They have been used in studies on vertebral genetics, developmental biology, and toxicity due to their great genetic and physiological similarities to mammals[6]. A growing area of the material sciences is nanotechnology. High surface-to-volume ratios, quantum size effects, and other characteristics distinguish nanomaterials from their bulk counterparts[7]. Nanoscale materials' advantageous optical, mechanical, and electrical capabilities enable innovative uses in high-tech and biological research[5]. In the past few decades, applications of nanotechnology have expanded in numerous fields. Every element of contemporary life now utilises nanoparticles (NP) and nanocomposites, including biomedicine, foodstuff storage, packaging, and other sectors. Researchers and industry are always searching for NP, including various metal NP such as copper oxide (CuO), silver (Ag), and gold (Au)[6] with more adaptable and usable properties.

Utilizing materials at the level of atoms, molecules, supramolecular structures, and their unique characteristics at the nanoscale, nanoscience has focused on the subject of drug delivery. Due to their invaluable contributions to numerous sectors, including biomedicine, sensors, optical, electronic, catalytic use, and cancer therapies, nanoparticles (NPs) have attracted tremendous attention in the current environment[9]. The effects of NPs are especially prone to harm creatures living in water. A large number of NPs float in the water as colloidal particles. column, making it easier for aquatic organisms to absorb them through their skin, gills, and gut. There have been reports of harmful biological impacts on aquatic life at many levels, including biochemical, behavioral, and ecosystem effectsNPs have been observed to change fish hatch in several different NP formulations, according to periodical reports[10].

As a result of its use in studying[11-16] the nanotoxicity of various nanomaterials, the Zebrafish (Danio rerio) has recently become quite well-liked in the scientific community. Due to its unique characteristics, which include a quick life cycle, clear larvae and embryos, inexpensive costs, and easy lab upkeep, it is the greatest in vivo model for all types of research.Numerous Nanotoxicological tests have been performed on a variety of nanomaterials, which zebrafish models, including titanium oxide and zinc oxide nanoparticles. Metals like cadmium, silver, and zinc can interfere with ion regulatory processes, bind sulphur groups to proteins, changing their structure and activity, create reactive oxygen species (ROS), which interfere with endocrine functioning and prevent DNA and protein synthesis. Morphological defects, as well as changes to neurodevelopment and mobility, might result from disturbances in these processes[17-19]. With the help of bay leaf, cardamom, and cassia bark, we were able to create gold nanoparticles for this investigation and detected cytotoxicity.

2. Materials and Methods

All chemicals were purchased from Sigma-Aldrich and applied exactly as they were. All tests' water was prepared in the lab using double distillation. The National Centre for Cell Science (NCCS) provided the cell lines.

2.1 Bay leaf, cassia bark, and cardamom

A local market in Chennai was used to get samples, which were mashed up using a mortar and pestle and a powder mill.1g of leaf extract is diluted in 30 ml of double-distilled water. It is then cooked for a further 20 minutes at 60 to 65°C in a water bath. The extract was obtained through filtering.

3 Gold Nanoparticles Made Through Green Synthesis

By using green extracts of Bay leaf, Cassia bark, and cardamom extracted in an aqueous solution, gold nanoparticle (AUNP) was produced using a green synthesis process by reducing gold chloride (AuCl3). Six test tubes were used, three of which were labelled as controls (C) and the remaining three as test tubes (T). Each test tube received 0.5 ml of the extract and 4.5 ml of double-distilled water. Gold chloride (Au(III)Cl3) (10 l) was added to the test tube with the letter T. For 24 hours, both test tubes were incubated in the dark. The following day, Test tubes with the designation T showed a colour change.

4 Stability Testing of Gold Nanoparticles

With the use of a UV-visible spectrophotometer, the stability of gold nanoparticles is assessed after 24 hours, 48 hours, the third day, the fifth day, the tenth day, the twentieth day, the thirty-first day, the forty-first day, the fifty-first day, and the sixty-first day. While the surface plasmon resonance phenomenon, which is the collective oscillation of electrons in the conduction band, absorbs light in the blue-green region of the spectrum, red light is reflected, creating rich red color, and both types of light are therefore affected. The strong absorbance band that results from the LSPR of gold nanoparticles in the visible spectrum (500nm-600nm) can be measured using UV-Vis Spectroscopy.

5. Optimization of Gold nanoparticles

5.1 Temperature-based adjustments for optimisation

Experiments were conducted at 4, 27, 37, and 60°C to determine how temperature affected the shape of gold nanoparticles. Only the Bay leaf demonstrated good stability values on the UV-Vis Spectroscopy examination of the bio synthesised Gold Nanoparticles. Therefore, in this case, we are simply optimising nanoparticles made from extracts of bay leaves. I took four test tubes and wrote 4, 27, 37, and 60 degrees on each. 4.5 millilitres of twice-distilled water and 0.5 ml of leaf extract are added to each test tube.Each test tube gets 10 microliters of gold chloride poured to it. In the refrigerator was a test bottle marked 4°C. For 27°C, a different test tube was held at room temperature.Next, a test tube labelled 60°C was placed in a hot air oven, while a test tube marked 37°C was kept in an incubator.

5.2 Gold Nanoparticles at Various pH Optimization

Using four test tubes, four distinct pH levels were optimised for Gold nanoparticles0.5 millilitres of Bay leaf extract and 4.5 millilitres of distilled water are added to each test tube.Each test tube gets 10 microliters of gold chloride poured to it. Using pH paper, the liquids' pH was evaluated. The pH was set to 5,6,7,8,9. Drop by drop additions of HCL were used to lower the pH and NaOH to raise it. All test tubes had markings and were kept in the dark for a full day. The following day, absorbance was measured with the aid of a spectrophotometer.

6. Study of In Vitro Cytotoxicity

6.1 preparation of the cell culture medium

For the purpose of cultivating C2C12 cells, DMEM (Dulbeco's Modified Eagle's Medium) is used. Before using the chamber, laminar airflow was exposed to UV light to sterilise it. Items were cleaned with a 70% ethanol wipe. DMEM liquid media, 44 ml, was placed in a conical flask.A serological pipette was used to add 5ml of foetal bovine serum to the conical flask. Penicillin/Streptomycin and 500 l of L-Glutamine were added to this. pipette to thoroughly blend.

6.2 Culture of Cells

In DMEM supplemented with streptomycin, penicillin, and 10%FBS, the C2C12 cell lines were kept alive.The cells were then at a temperature of 37°C and 5% CO2 in a humidified cell incubator. The haemocytometer is used to count the cells, and the data is utilised to calculate the dilutions needed for the experiment. The well plate was filled with pre-diluted cell dilutions and a control row of medium.The cells per well were used to plate myoblasts in 96-well plates. To verify that there are cells in each well, a phase contrast microscope was used to see the cells. the cells for 24 hours at 37 °C. In 96-well plates, the cells were planted and given the following day to adhere to the walls. The confluency of the plate was examined under a microscope after 24 hours. The cells were then subjected to a range of AuNP concentrations for roughly 24 hours under 5% CO2 ambient conditions (100,50,25,12.5,6.25,3.125 g/ml). MTT solution was finalised at a concentration of 0.5 mg/mLthen applied to the cells; make sure to use clean tips to prevent contamination. MTT is applied to each well after that. For an additional four hours, cells were incubated at 37°C. View the cells once more under a contrast microscope after 4 hours. MTT and media were carefully taken out of the wells. 0.1% NP-40 in isopropanol and 4 mM HCl were used to lyse the cells. after the MTT solution was removed. Cells reexamined under a microscope. Using an Elisa reader, 560 nM was found to be the optical absorbance of the cell suspensions.

7. Results and discussion

7.1 Extraction of Cardamom, Cassia Bark, and Bay Leaf

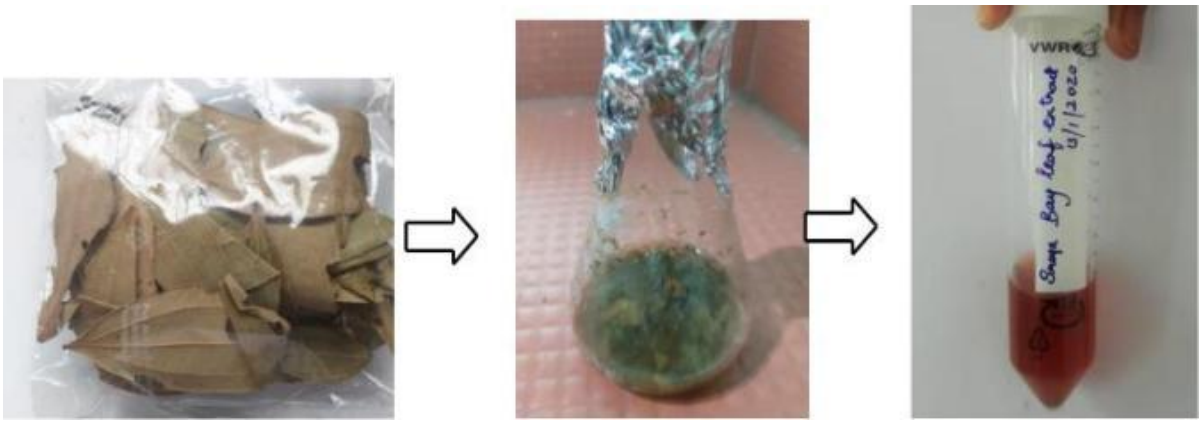

Fig. 1. (a) collected Bay leaf samples (b) boiled powder of Bay leaf (c) Filtered extract of Bay Leaf

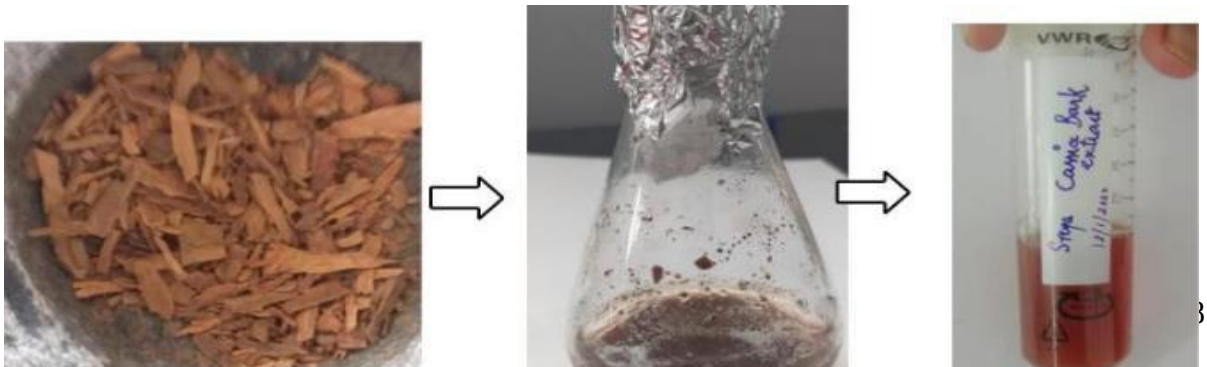

Fig. 2. (a) collected Cassia Bark samples (b) After boiling powdered extract of cassia Bark (c) Filtered extract of Cassia Bark

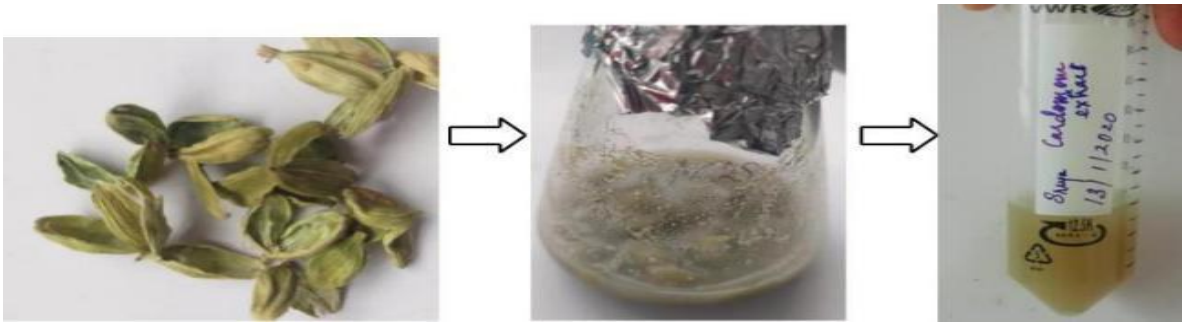

Fig. 3. collected Cardamom samples after removing fruit (b) After boiling powdered extract of Cardamom (c) Filtered extract of Cardamom

7.2 Synthesis of Gold Nanoparticles

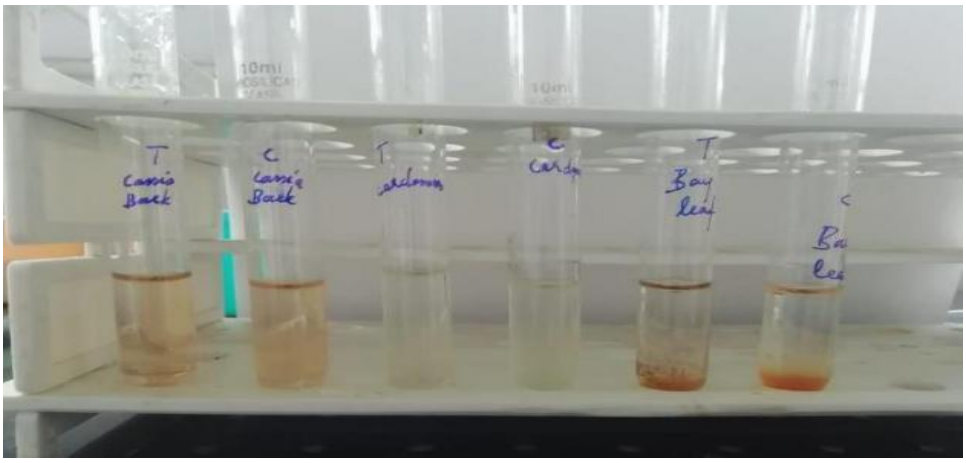

Fig. 4. Test tubes containing 4.5 ml of double distilled water and 0.5ml of plant extract

Fig. 5. Colour change after adding AuCl3 to the Test tubes.

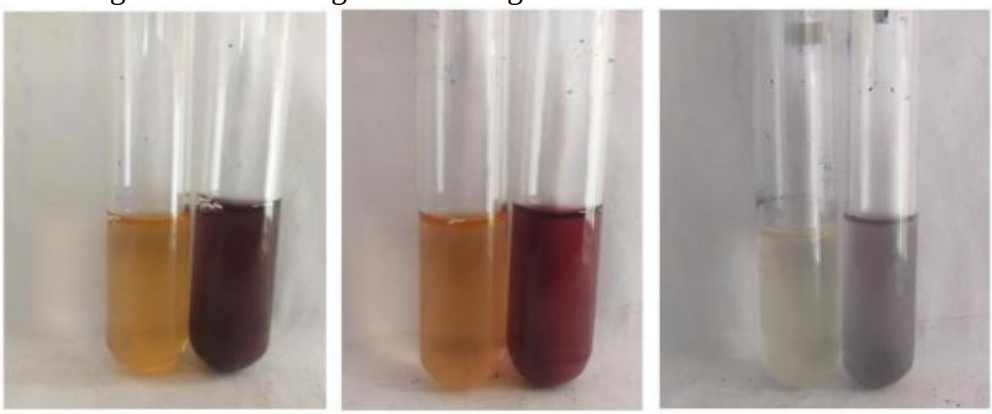

Fig.6. (a) Control (C) and Test (T) of Bay leaf, (b) Control (C) and Test (T) of Cassia Bark, (c) Control (C) and Test (T) of Cardamom.

7.3 Stability of Gold Nanoparticles

The UV-Visible Spectrophotometer investigation only revealed good stability values for Bay Leaf. While Cassia Bark likewise exhibited a high absorbance value, it was not long-lasting. Cardamom lacked a high absorption value.

Table 1. Absorbance value of Cassia Bark, Bay leaf and Cardamom from UV visible Spectrophotometer

Time		Absorbance Value (nm)	
	Cassia Bark	Bay Leaf	Cardamom
24 hrs	536.2	540	-
48 hrs	529.2	534.8	316.6
3rd day	529.2	539.6	319.2
5th day	529.2	539.8	-
10th day	529	539.2	-
20th day	529.2	534.8	-
30th day	529.2	539.4	-
40th day	529.2	540	-

50th day	529.0	540	
60th day	528.6	540	--

Optimization of Gold Nanoparticles.(8.4)

Table 2. Absorbance value at different temperature

Temperature	Absorbance value (nm)
4℃	529.2nm
27℃	529.2 nm
37℃	539.8nm
60℃	540 nm

With the rise in temperature from RT to 60°C, the absorption bands of AuNPs underwent a red shift from 539.8 to 540nm.

7.5 Optimization of Gold Nanoparticles at different pH

Table 3.Temperature vs absorbance

Temperature	Absorbance value (nm)
5	540.4nm
6	526.4 nm
7	526.4nm
8	518.0 nm

7.6 Cell Structures

Only cells that were still alive after 24 hours of exposure to the sample were able to successfully metabolise the MTT dye, 3-(4,5-dimethylthiozol-2-yl)-2,5-diphenyl tetrazolium bromide. The detergent-dissolved purple precipitate was then subjected to spectrophotometric analysis.. Figures demonstrate that AuNps at concentrations of 3.125, 6.25, 12.5 and 25 g/ml had no impact on the C2C12 cell lines' viability, while 50 and 100 g/ml exhibit a mild toxicity after prolonged exposure. The findings of this study indicate that as nanoparticle concentration increased, so did the cytotoxicity of gold nanoparticles produced biologically.

8. Summary and Conclusions

The toxicity of green synthesised AuNP was evaluated through investigation of morphological and physiological alterations in Zebrafish. As a comparison to the untreated Zebrafish employed as the experiment's negative control, the zebrafish were to be exposed to a range of doses of synthetic AuNP. It is anticipated that the concentration and exposure time of AuNP will affect its cytotoxicity towards adult zebrafish. At a temperature of 37 °C and a pH of 5, the steady absorbance value was attained. The value of absorbance decreased as a result of the drop in temperature. The morphological properties of the nanoparticles were strongly influenced by temperature.. In order to look at in vitro cytotoxicity, the C2C12 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) under typical growth

circumstances. A microscope was used to examine cell development. In a 96-well plate, the MTT assay was used to assess cell viability. At doses of 100, 50, 25, 12, 5, 6, and 3.125 g/ml, gold nanoparticles were added.. The viability of the C2C12 cell lines was unaffected by AuNps at concentrations of 3.125, 6.25, 12.5 and 25 g/ml, while 50 and 100 g/ml are somewhat hazardous when used long-term. The findings of this study indicate that as nanoparticle concentration increased, so did the cytotoxicity of gold nanoparticles produced biologically. This work taught us that, when exposed in low concentrations, green-synthesised gold nanoparticles do not harm normal cells. Given that it displayed stronger stability than AuNPs generated from cardamom and cassia bark, bay leaf can be used as a cheap source for the manufacture of gold nanoparticles. 37°C and a pH of 5 are the ideal conditions for the biosynthesis of gold nanoparticles. Only cells that are still alive after being exposed to the substance for 24 hours were able to metabolise a dye effectively in the MTT assay, which was analysed spectrophotometrically. According to the study's findings, there are certain circumstances under which biologically produced gold nanoparticles are harmful to cells. With an increase in nanoparticle concentration, cell growth increased. Gold nanoparticles exhibit a negligible harmful effect at greater concentrations, according to the cell viability test. This leads us to the conclusion that stem cell therapies employing the Myoblast cell line may make use of gold nanoparticles.

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