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Biosynthesis of gold nanoparticles from biological source and its effect on *in vitro* anti-cancer activity

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Cancer is usually defined as a state, where in the cells, it tends to divide and grow uncontrollably and has the ability to destroy normal healthy cells. Thanks to the advancements in nanotechnology as it has paved its way and has found numerous applications in the field of cancer diagnosis and treatment. Nanoparticles, which are usually (1–100 nm), are widely used in the treatment of cancer. One of the major problems encountered in treating cancer patients is that they exhibit multiple drug resistance, and hence, it becomes tedious to treat them. Therefore, in these scenarios, nanoparticles are employed as they can serve as drug-targeting molecules. In particular, AuNPs (gold nanoparticles) are employed in cancer treatment due to their ability to reverse multidrug resistance. AuNPs have the potential to exhibit minimum cytotoxicity and also have the ability to serve as an antioxidant. Various spices (i.e., clove, cinnamon, turmeric, and cardamom) are said to have high antioxidant content, and they are also beneficial in cancer treatment. Moreover, it is proven that AuNPs, which have been synthesized from biological sources, have higher efficacy in cancer cells and less toxicity toward normal cells. Thus, the main aim of this approach was to synthesize gold nanoparticles from spices, which will exhibit higher efficacy in destroying cancer cells and inducing apoptosis while offering less toxicity toward normal cells. This study is an attempt to understand the study of the biosynthesized AuNPs which will help in destroying cancer cells.

Keywords: biosynthesis, *in vitro* anti-cancer, gold nanoparticles

Introduction

The term "Nanotechnology" was first coined by the great scientist Norio Taniguchi in 1974. Studying and manipulating data under incredibly micro sizes is called nanotechnology. Nanotechnology is part of innovation in science and engineering that will transform many sectors such as medicine, biomedical, and biomaterial. Particles that are ranging between 1 and 100 nanometers in size are called nanoparticles. Nanoparticles due to their specific advantages such as biocompatibility, reduced toxicity, stability, permeability, and retention effect are widely used in cancer treatment and diagnosis. The biosynthesis of metal nanoparticles, such as gold and silver, has recently hooked the attention of various biomed and biotech fields.

The development of abnormal cells and their ability to divide uncontrollably often leads to the condition known as cancer. Cancer is a leading cause of death worldwide and accounts for nearly one in six deaths across the globe. Gold nanoparticles have attracted wide applications due to their electrical conductivity and high chemical and thermal stability. AuNPs are set to possess minimum cytotoxicity and also have the potential ability to serve as an antioxidant. It is also emerging as an important drug delivery vector due to minimal cytotoxicity when placed in an *in vivo* condition.

Effects of AuNP's

The monolayer culture is made with 10% medium, dimethyl ethanol amine (DMEA), and fetal bovine serum (FBS) along with the antimicrobes. Then, the culture medium is incubated in a CO_2 incubator at 37°C for growth. The dead cells are removed the next day by phosphate-buffered saline (PBS), and the live cells are detached using trypsin, which is a proteolytic enzyme. Now the cells are seeded in a 96-well plate along with the culture medium and allowed for growth. Now different concentrations of AuNPs are added to individual wells, and a calorimetric assay for accessing cell metabolic activity (MTT assay) is performed. MTT assay is a method used to detect cell toxicity against cancer and non-cancer cells.

- Cell viability refers to the proportion of healthy cells in a sample population.

$$\{Cell\ viability\ (\%)\ =\ (sample\ A/Control\ A)\ \times\ 100\}$$

Sample collection

Spices have high antioxidant content and are highly beneficial for human health. Spices such as clove, cinnamon, turmeric, and cardamom are rich sources of powerful antioxidants. Turmeric has antibacterial, antiviral, antifungal, antioxidant, and anticancer activities and can reduce the risk of various malignant diseases. Turmeric extract is highly cytotoxic to mammalian cells, and it is effective in reducing animal tumors. Curcumin from turmeric has been tried to treat cancer lesions in human participants.

Synthesis of nanoparticles

Biosynthesized nanoparticles from curcumin have higher efficacy in cancer cells and less toxicity toward normal cells. The biosynthesis of gold nanoparticles is considered to be more eco-friendly than the physical and chemical methods which are expensive. The nanoparticle size should range from 70 nm to 200 nm for efficient drug delivery. Gold nanoparticles synthesized from plant extracts have a particle size ranging from 15 to 80 nm and also show that the activity becomes quicker as they get smaller in size.

Objectives

- To synthesize the metal nanoparticle (gold) using cinnamon, clove, turmeric, and cardamom extracts.
- Screening of gold nanoparticles synthesized.
- To check the stability of AuNPs.
- Studying antioxidant activity.
- To characterize the synthesized nanoparticles (UV, TEM, EDX, XRD, and FTIR).
- *In vitro* cytotoxicity of AuNPs using normal cell line.
- *In vitro* anti-cancer activity of AuNPs against breast cancer.

Apoptosis analysis of AuNPs using a fluorescence microscope.

Experiment

Aim: To synthesize the gold nanoparticles from turmeric and to study their effects against the colon cancer cell lines (**Figure 1**).

(I) Sample Collection

Materials required:

- Fresh samples of cinnamon, clove, turmeric, and cardamom are collected and ground up to 70% fine powder.

Procedure: (Extraction)
2° g of powder is dissolved in 30 ml of distilled water and well mixed. The mixture is then boiled for 20 min at 60°C. The solution is filtered using filter paper.

1. Cardamom
2. Turmeric
3. Clove
4. Cinnamon

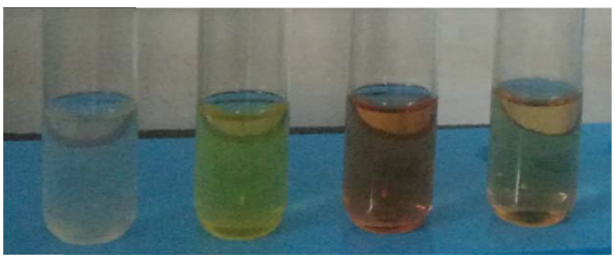


FIGURE 1 | The solution of cardamom, turmeric, clove, and cinnamon.

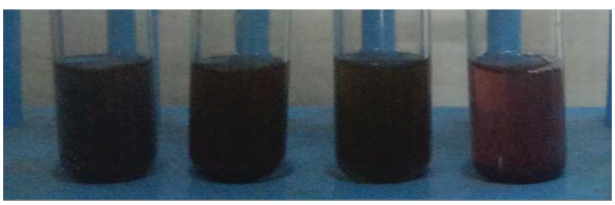


FIGURE 2 | Solution for synthesis of gold nanoparticles.

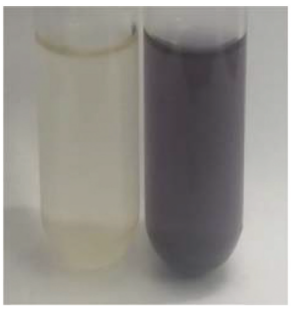
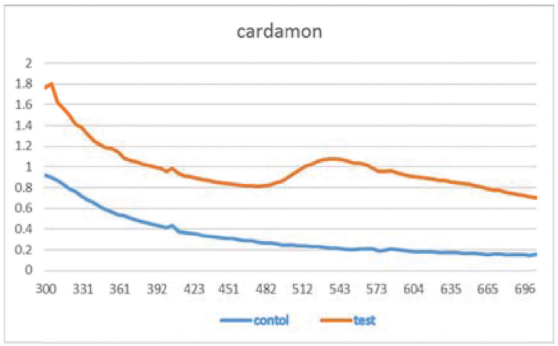
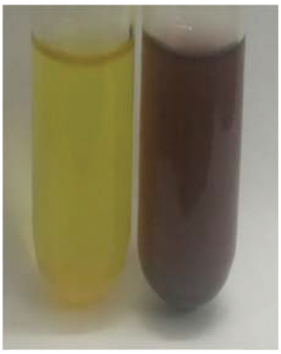
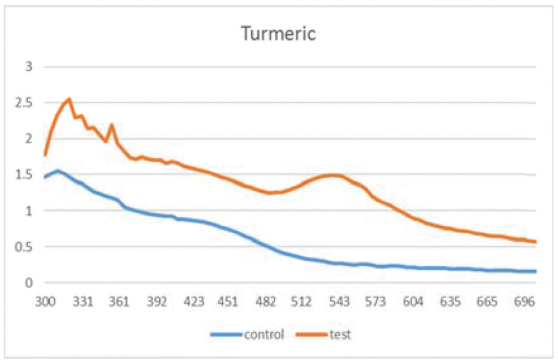
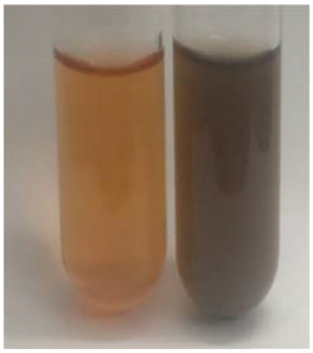
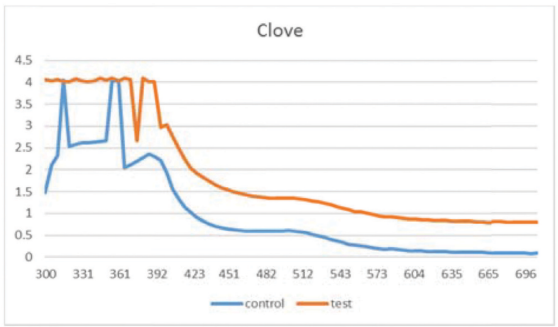
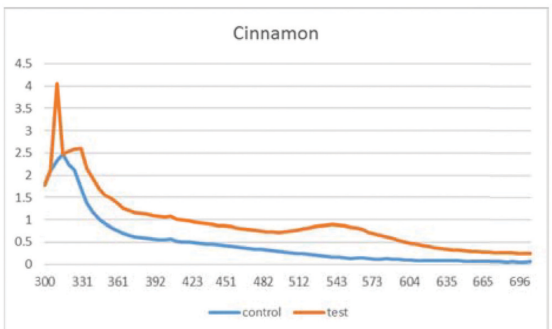
Samples	AuNPs synthesized	UV visible spectroscopy of AuNPs
Cardamom 		
Turmeric 		
Clove 		
Cinnamon 		

FIGURE 3 | Synthesis of gold nanoparticle confirmed via UV-visible spectrometer.

(II) Synthesis of Gold Nanoparticles

Materials required:
10° ml of different sample extracts.
Gold chloride (Aucl4) (**Figures 2**, **3**).
Procedure:

(1) 8 ml of double distilled water is mixed with 2 ml of extract.

(2) To this 40 microliter of gold chloride solution is added.

(3) Test tubes are incubated for 24 h.

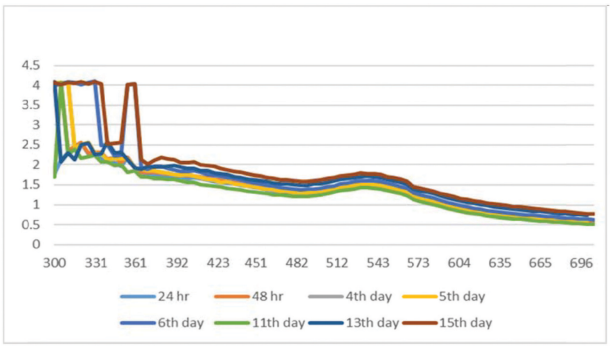


FIGURE 4 | Stability check of AuNPs synthesized from turmeric at different time antioxidants by DPPH scavenging activity.

Stability analysis of AuNP's using turmeric extract

- Antiviral. A recently published study in the Journal of General Virology describes how curcumin helps prevent transmissible gastroenteritis virus (TGEV).
- Antioxidant. When the body is exposed to free radicals or unstable molecules reacting to the environment and other triggers, cells become damaged.
- Anti-inflammatory. Help to reduce inflammation in the body.
- The antioxidant activity of synthesized stable gold nanoparticles is studied by DPPH scavenging activity and phosphomolybdenum assay.
- The DPPH scavenging activity of the sample was measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method.

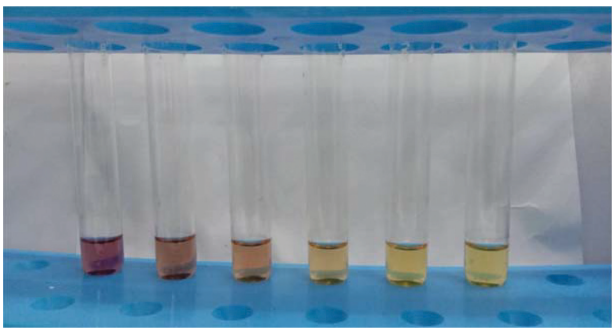


FIGURE 5 | DPPH scavenging activity of synthesized stable AuNPs.

TABLE 1 | Scavenging activity of gold nanoparticles.

Concentration	Absorbance	Scavenging activity (%)
Control	0.162	
5	0.117	27.7
10	0.096	40.7
15	0.088	45.6
20	0.030	81.5
25	0.026	84

Procedure

- 0.4 mM solution of DPPH in methanol was prepared, and 2 ml of this solution was added to different concentrations of the sample and was allowed to stand at room temperature for 20 min (**Figures 4**, **5**).
- Absorbance was read at 517 nm against blank samples.
- The lower absorbance of the reaction mixture indicated higher free radical scavenging activity.
- The percentage of the DPPH radical scavenging is calculated.
- Radical scavenging activity (%) = (Abs control - *Abs sample/Abs control)* × 100 (**Table 1**).

(I) Phytochemical analysis confirmed the presence of constituents such as alkaloids, carbohydrates, glycosides, saponins, proteins, phenol, flavonoids, and terpenoids.

- Carbohydrates:
  - Fehling's test: One ml of filtrate was boiled in the water bath with 1 ml each of Fehling solutions I and II. A red precipitate indicates the presence of sugar.
  - Fehling's solution I: Copper sulfate (34.66 g) was dissolved in distilled water and made up to 500 ml with distilled water.
  - Fehling's solution II: Potassium sodium tartrate (173 g) and sodium hydroxide (50 g) were dissolved in water and made up to 500 ml.
- Proteins:
  - Biuret test–1 ml of the filtrate was treated with 2% of copper sulfate solution. To this

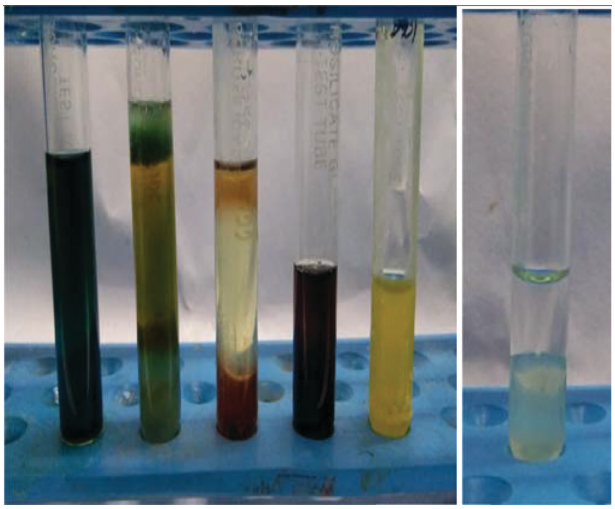


FIGURE 6 | Phytochemical analysis.

1 ml of ethanol (95%) was added followed by excess potassium hydroxide pellets. The pink color in the ethanolic layer indicates the presence of proteins.

- Terpenoids:
  - Salkowski test–1 ml of extract and 2 ml of chloroform were added and mixed well. A little concentrated H2SO4 was carefully added to form a reddish-brown layer.
- Steroids:
  - To 1 ml of extract, 2–3 drops of sulfuric acid are added. The blue color indicates its presence.
- Flavonoids:
  - 3 ml of dilute ammonia was added to 1 ml of filtrate followed by the addition of 1 ml of concentration of sulfuric acid. The yellow color shows the presence of flavonoids.
- Glycosides:
  - Borntrager's test–To 1 ml of filtrate, 3 ml of chloroform was added and shaken. The chloroform layer was separated, and 10% ammonia solution was added to it. The pink color indicates the presence of glycosides (**Figure 6**).

Carbohydrates — — —
Proteins — — —
Terpenoids + + +
Steroids — — —
Flavonoids + + +
Glycosides — — —

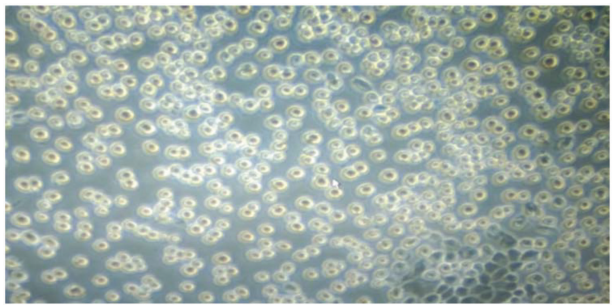


FIGURE 7 | Detachment of cells after trypsin treatment.

Cell toxicity

Toxicants that can cause cell death and serious organ dysfunction.

- Cells are seeded in a 96-well plate with an appropriate medium for growth.
- Different concentrations of gold nanoparticles are added to each well.
- Plate is incubated at 37°C for 24–36 h.
- Medium is removed, and plates are viewed under a microscope.
- PBS wash is done.
- 20 ml of MTT assay solution is added to each well.
- Viability of the cells is checked.

Cell line

Permanently established cell culture that can proliferate indefinitely given appropriate fresh medium and space.

- Check cells in a T flask under a microscope for their growth.
- Remove the culture media carefully.
- Add PBS for the removal of dead cells.
- Trypsin is added for the detachment of cells in the flask.
- Flask is kept undisturbed for 5 min.
- Check cells under the microscope to confirm the detachment of cells from the surface.
- Fresh medium is added for growth (usually 10% medium is used along with DMEA, FBS, and antimicrobes) (**Figure 7**).

Result and conclusion

Synthesized gold nanoparticles from spices exhibit higher efficacy in destroying the cancer cells and inducing apoptosis while offering less toxicity toward normal cells is observed.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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