

# "A Comparative Investigation into the Biocompatibility and Cytotoxicity of Phytofabricated Gold and Titanium Dioxide Nanoparticles for Biomedical Applications"

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## **Abstract:**

Titanium Nanoparticles from *Peltophorum petrocarpum* and Gold Nanoparticles from *M. elengi* fabricated via eco-friendly ("green") chemical pathways have attracted considerable interest because their distinctive physicochemical features and inherent biocompatibility enable a broad spectrum of applications from biomedicine to food-packaging technologies and environmental remediation. In this study, Gold (Au) and titanium dioxide (TiO<sub>2</sub>) nanoparticles were synthesized using plant-mediated green chemistry and subsequently characterized by Fourier-transform infrared spectroscopy (FTIR) and high-resolution transmission electron microscopy (HRTEM). Biocompatibility was assessed on a liver cancer cell (HEPG2) cell line employing the MTT assay. Both Au and TiO<sub>2</sub> nanoparticles demonstrated pronounced, dose-dependent compatibility with the cells, exhibiting minimal cytotoxic effects across the tested concentration range. These encouraging in-vitro findings lay the groundwork for extended biological evaluations and potential translational applications of the green-synthesized Au and TiO<sub>2</sub> nanomaterial.

## **1.Introduction**

Nanotechnology has made significant advancement in the field of functional nanomaterial development due to their unique physical and chemical properties including size dependent properties [1]–[4], which make them several applications including sensors, cancer cell diagnostics and energy storage [5]–[8]. Among them Au and TiO<sub>2</sub>nanoparticles (NPs) have attracted great attention for their potential applications.

Au nanoparticles have been extensively used in various fields due to their unique properties such as size, shape, high stability, non-toxicity, biocompatibility and target cells [9]. Au Nps are easy to synthesis and functionalize with targeting moieties [10], [11]. Method of selection is crucial to prepare Au NPs with reproducibility, stability, size and shapes. Top down and bottom up approach are the method for preparation of Au nanoparticles with high quality.

Numerous research articles have been reported on synthesis of gold NPs as well as functionalized Au NPs [12]–[15]. One of the challenging aspects while preparation of Au NPs is to prepare Au NPs free from aggregation, to avoid this, capping agent such as citrate, polymers, surfactants, chelating ligands have been added during synthesis. However, the synthesis of gold NPs involves lot of chemicals and requires harsh environmental conditions. Alternatively, ecofriendly green chemical route has been developed to prepare Au NPs with improved physicochemical properties. For instance, OM El-Borady et al. prepared Au NPs by green chemical route using common reed leaf extracts. The prepared NPs exhibited notable anticancer, anti-oxidant, and photocatalytic activities [16]. Au NPs prepared through bio-inspired pathway using thyme plant extract was examined for catalytic properties on reduction of 4-nitrophenol [17]. Au NPs prepared using vitex negundo plant extract has shown significant antibacterial activity where the plant extract acts as capping and stabilizing agent. It was reported that the polyphenols, flavonoids, terpenoids, biological active compounds present in plant extracts were responsible for reduction of Au NPs [18]. Another research article authored by Scholastica O. Anadozie et al. highlights the size of Au NPs produced was about 2-17 nm and exhibit anti-cancer activity in dose dependent manner [19].

Similarly, TiO<sub>2</sub> has also been used for several applications including drug delivery, sensors, energy storage and photocatalytic applications [20]–[23]. TiO<sub>2</sub> NPs have been synthesized through various chemical routes such as hydrothermal, sol-gel, solvothermal, and precipitation technique. Synthesis of TiO<sub>2</sub> NPs through chemical routes produced larger particles and consumes high energy; moreover, the chemical synthesis route is not ecofriendly and produce secondary waste. Like gold NPs, TiO<sub>2</sub> has also been prepared through green chemical approach. Medicinal plants such as Trigonell foenum-graecum, Aloe Vera, Jatropha, Acorus Calamus leaf, Moringa oleifera, Hibiscus, and Azadirachta indica have been utilized for the preparation of TiO<sub>2</sub> NPs [24]–[31]. Jyoti Fulekar et al. prepared TiO<sub>2</sub> nanoparticles using rhizospheric microorganisms and roots as natural source for methyl orange dye degradation [32]. Syzygium cumini extract has been used for preparation of TiO<sub>2</sub> NPs [33]. The TiO<sub>2</sub> has been used to remove Pb<sup>2+</sup> (82.53%) from industrial waste water. TiO<sub>2</sub> NPs has been prepared using phyllanthus niruri extract exhibited significant methyl orange dye adsorption (273.37mg g<sup>-1</sup>) capacity [34]. Citrus lemon is well known source of vitamin-C, which is used to prepare TiO<sub>2</sub> NPs and successfully applied for dye-sensitized solar cells [35]. Biological fouling caused due to

undesired accumulation of microorganisms, bio waste, and diatoms on submerged artificial surface. Green synthesized  $\text{TiO}_2$  using algal extracts showed excellent antifouling characteristics [36].  $\text{TiO}_2$  NPs has been prepared by both conventional chemical route as well as green chemical approach and compared the efficacy against methylene blue dye degradation [37]. The results showed that 89% dye degradation efficacy was achieved by biomediated synthesis of  $\text{TiO}_2$  which is high when compared to chemically prepared  $\text{TiO}_2$ . Bixa orellana seed extract is possessing excellent health benefits which is also used for the preparation of  $\text{TiO}_2$  NPs and applied to dye sensitized solar cells. The results showed that current conversion efficiency was found to be 3%, Bixa orellana seed extracts acts as stabilizing and capping agent. Significant antibacterial and antioxidant properties were achieved by  $\text{TiO}_2$  NPs prepared via psidium guajava mediated biosynthesis. The authors suggested that psidium guajava mediated biosynthesis can satisfy  $\text{TiO}_2$  production in large-scale [38]. Plant extracts such as ranunculus muricatus, cinnamomum tamala leaves, averrhoa bilimbi fruit and pandanus amaryllifolius leaves extracts have been used for the preparation of Au/ $\text{TiO}_2$  nanocomposite [39]–[41]. The composite consist of Au- $\text{TiO}_2$  improves the photocatalytic performance.

Literatures evidenced that Au and  $\text{TiO}_2$  NPs have emerged as promising tool for various biomedical applications, their widespread use is depends on their biocompatibility. Biocompatibility is described the material should be compatible with living organism/tissue when administered. Green synthesis of gold as well as  $\text{TiO}_2$  nanoparticles was prepared using plant extracts. The plant extracts acts as both stabilizing and reducing agent. However, limited number of research articles has been reported on the biocompatibility of green synthesized Au and  $\text{TiO}_2$ . The biocompatibility assay needs to be documented in order use the NPs for various applications. This research article report the preparation of Au and  $\text{TiO}_2$  NPs and comparative study of biocompatibility [45]- [46].

## **2. Materials and Methods**

Gold chloride ( $\text{HAuCl}_4$ ), Glycerin and Sodium citrate, Titanium Nitrate, Ethyl alcohol, All chemicals and solvents used were of analytical grade are used.

## **2.1 Preparation of Au NPs using M. Elengi fruit extracts**

Aliquots of Au (III) chloride solution (15mM) was added drop wise to 50 ml of freshly prepared M. Elengi fruits extracts. The reaction mixture was stirred to 10 minutes and the appearance of wine red colour was observed. The color change was due to Au NPs formation. The UV-Vis spectrophotometric measurement of the prepared NPs was carried out in different time period to confirm the stability.

## **2.2 Preparation of TiO<sub>2</sub> NPs using Peltophorum pterocarpum extract**

Freshly picked Peltophorum Pterocarpum flowers were cleaned with distilled water and then dried in the dark to preserve their delicate scent. Distilled water and 5 grams of desiccated Peltophorum Pterocarpum flowers were mixed then heated to 80 °C for 30 minutes. Before being stored at 4°C for the experiment, this extracts were filtered. Known quantity of the freshly prepared Peltophorum Pterocarpum flower extracts were gently added to a titanium trichloride (TiCl<sub>3</sub>) solution was stirred continually to produce the TiO<sub>2</sub> NPs. As TiO<sub>2</sub> nanoparticles formed, the fluid's colour changed from pink to brown. Further, the obtained TiO<sub>2</sub> was dried at 50°C and sintered at 600 °C.

## **2.3 Characterization**

The green synthesized nanoparticles were characterized using FTIR, SEM, and HR-TEM (200 KV acceleration voltages). FTIR spectroscopy was performed to identify the functional groups NPs.

## **3. Biocompatibility and Cytotoxicity Assessment**

**Cytotoxicity of Titanium and Gold Nanoparticals in HEPG2 (Liver Cancer Cell) using MTT Assay.**

### **3.1 Preparation of Test Material**

All Test Samples were filter sterilized using 0.22µ filters and diluted by double dilutionmethod in MEM with FBS.

### 3.2 Chemicals and Materials-

Sr No.	Chemicals	Materials
1.	Cell Culture Plates	96 well microtiter plates (Himedia)
2.	Cell culture flasks	T25 Flasks (Himedia)
3.	Trypsin/EDTA	0.25% Trypsin and 0.02% EDTA in Dulbecco's Phosphate Buffered Saline (Himedia)
4.	DMSO	Dimethyl sulfoxide (Himedia)
5.	Cell culture Medium	Eagle's Minimum Essential Medium (EMEM)10% (v/v) Fetal Bovine Serum
6.	Cell Line	(Human embryo kidney Cancer Cell)
7.	Culture Conditions	37°C with 5% CO <sub>2</sub>

### 3.3 Preparation of Cells

HEK293 (Human embryo kidney Cancer Cell) were cultured in MEM with NEAA media supplemented with 10% (v/v) fetal bovine serum. Cells were cultured at 37°C and 5% CO<sub>2</sub>; complete medium was changed every 2 to 3 days.

### 3.4 MTT Assay Procedure

- Cells were seeded in 96 well plates at a concentration of 1,00,000 cells per well (100ul). The plates were incubated at 37<sup>0</sup>C at 5% CO<sub>2</sub> for 24hrs.
- After the incubation period cells were observed for half confluence monolayer.
- Culture medium was removed and the cells were treated with 7 different concentration of test item. Cisplatin used as standard.
- Cells in cell culture medium without any test item incubated and under the same conditions served as control.
- Plates were incubated 37<sup>0</sup>C and 5% CO<sub>2</sub> for 24 hrs.
- After 24hrs, cells were observed under inverted microscope for changes in morphology or death if any.
- After observation, culture medium were removed and 100 ul fresh medium added with 10 ul mg/ml MTT reagent in each well.

- Plated were incubated for 4hrs at 37<sup>0</sup>C in 5% CO<sub>2</sub> incubator.
- 100ul solubilization(DMSO) added into each well.
- 10. Plates were allowed to stand for 1hr at 37<sup>0</sup>C in 5% CO<sub>2</sub>.
- 11. After checking for complete solubilization of the purple formazan crystal absorbance was measured at 570nm using microplate reader.
- IC<sub>50</sub> values were calculated by plotting a log graph for the concentration of the test items vs %cell survival.
- Percentage of cell survival was calculated using the formula:

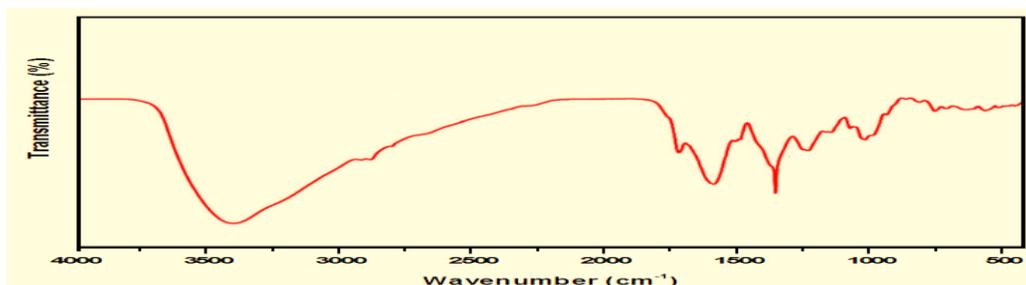
$$\text{Percentage cell survival(\%)} = \frac{\text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

### 3.5 Comparative IC<sub>50</sub> values of MTT Assay of Standard with Titanium and Gold Nps.

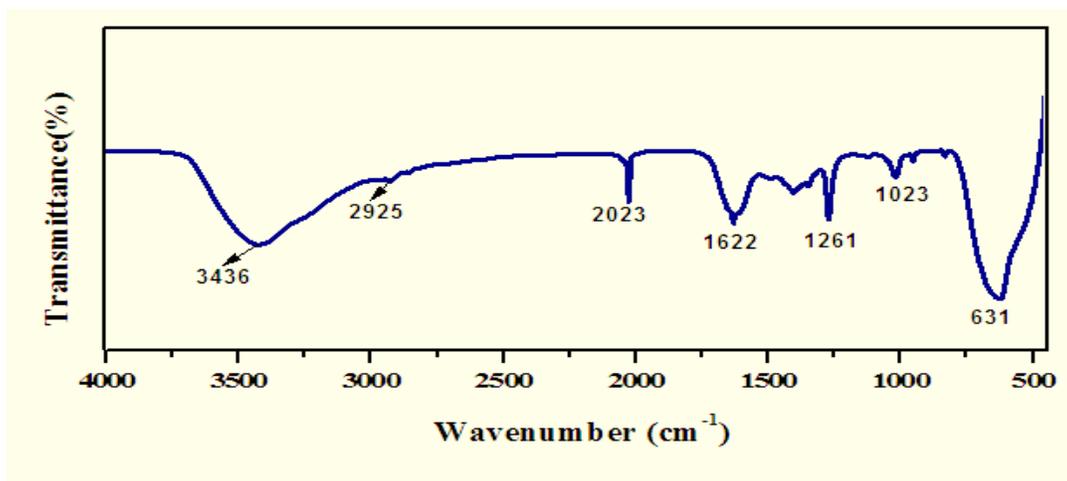
Sr. No.	Test Item	Log IC <sub>50</sub> Value	IC <sub>50</sub> Value
1.	Standard Cisplatin	0.756	2.1309
2.	TiNps	1.313	3.721
3.	AuNps	0.545	1.7204

The biocompatibility and cytotoxicity of the synthesized Au NPs and TiO<sub>2</sub> NPs were evaluated using cell viability assays (e.g., MTT assay)

### 4. Results and Discussion:



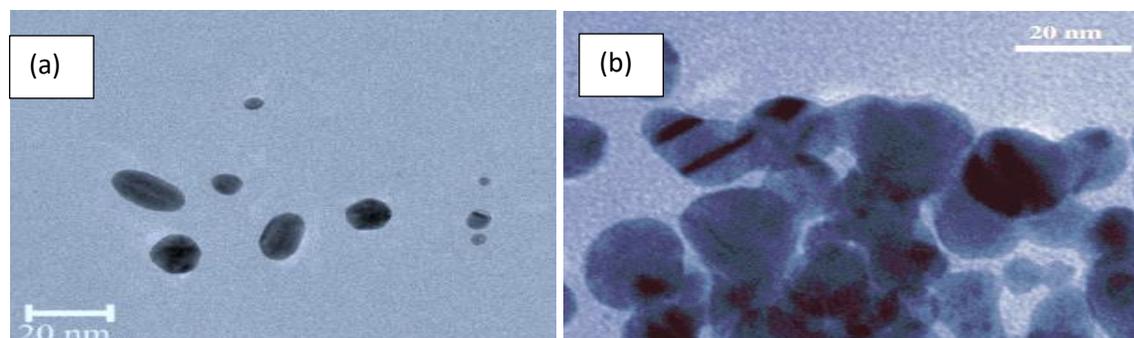
**Figure 1: FTIR spectra of (a) Au NPs prepared by M.Elengi**



**Figure 1: (b) FTIR obtained for TiO<sub>2</sub> NPs prepared by *Peltophorum Pterocarpum* Flower extracts**

The presence of biomolecules on Au NPs was assessed by FTIR spectroscopy. As shown in figure 1(a), the peak at 1065 cm<sup>-1</sup>, due to C–O bond derived from protein of fruit extract. The peaks appeared at 1233 cm<sup>-1</sup> and 1384 cm<sup>-1</sup> are attributed to the following functional groups like ester, amine and carboxylic group. The peak observed at 3422 cm<sup>-1</sup> is due to stretching of N-H and O-H molecules. The absorption band at 1381 cm<sup>-1</sup> is due to flavonoids present in fruit extract. The observed FTIR characteristic bands confirmed that biomolecules such as flavonoids behind the reason for reducing Au<sup>3+</sup> ions to form Au NPs.

FTIR was performed to confirm the functional groups of green synthesized TiO<sub>2</sub> nanoparticles. Figure 1(b) shows the FTIR spectra of TiO<sub>2</sub> NPs. The peak appeared at 631 cm<sup>-1</sup> indicates the O-Ti-O lattice vibration of rutile TiO<sub>2</sub>. The peaks at 1023 cm<sup>-1</sup>, 1261 cm<sup>-1</sup>, 1622 cm<sup>-1</sup> and 2023 cm<sup>-1</sup> are corresponds to -C-O-C stretching vibrations of alcoholic group, C=C stretching vibration, C-H stretching vibration, –C=O bond in Quinone structure respectively. The peaks at 2925 cm<sup>-1</sup> and 3436 cm<sup>-1</sup> are attributed to the C-H extended vibration for CH<sub>3</sub> and –OH stretching vibration of weakly bound water molecules respectively.



**Figure 2: (a) HRTEM images of Gold nanoparticles synthesized using M. Elengi extract and (b) TiO<sub>2</sub> nanoparticles synthesized by Peltophorum Pterocarpum flower extracts**

High resolution transmission electron microscope (HRTEM) was done to elucidate the structure and morphology of prepared samples. Figure 2 shows the HRTEM images of Gold nanoparticles obtained by M. Elengi extract with different magnification. As observed from figure 2(a), the mean particles size of Au NPs was observed as 14.5nm. Figure 2 (b) shows the HRTEM image of TiO<sub>2</sub> NPs, in which the particles size is observed as ranging from 18nm to 50 nm, also the spherical morphology is observed for both the samples.

## 5. Conclusion

The green synthesis of gold and titanium dioxide nanoparticles offers a sustainable and environmentally friendly approach to nanoparticle production. This study highlights the importance of biocompatibility and cytotoxicity in determining the potential applications of nanoparticles in biomedicine and other fields. The IC<sub>50</sub> values, which represent the concentration of the sample required to inhibit cancer cell growth by 50%, were calculated for Cisplatin and both the samples. The IC<sub>50</sub> value for cisplatin was found to be 2.1309 µg/ml, for Green synthesized TiNps was 3.721 µg/ml while for Green synthesized AuNps was 1.7204 µg/ml.

1. The results of this study demonstrate that both Green synthesized TiNps and Green synthesized AuNps exhibit significant inhibitory effects on cancer cell growth, with Green synthesized AuNps showing a higher percentage inhibition (93.64%) compared Green synthesized TiNps (87.26%).

2. The IC<sub>50</sub> values further support these findings, with AuNps requiring a lower concentration (1.7204 μg/ml) to achieve 50% inhibition compared to TiNps (3.721 μg/ml).
3. Thus the result indicates that AuNps is more effective in inhibiting the cancer cell growth compared to TiNps, with higher percentage inhibition and lower IC<sub>50</sub> value.

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