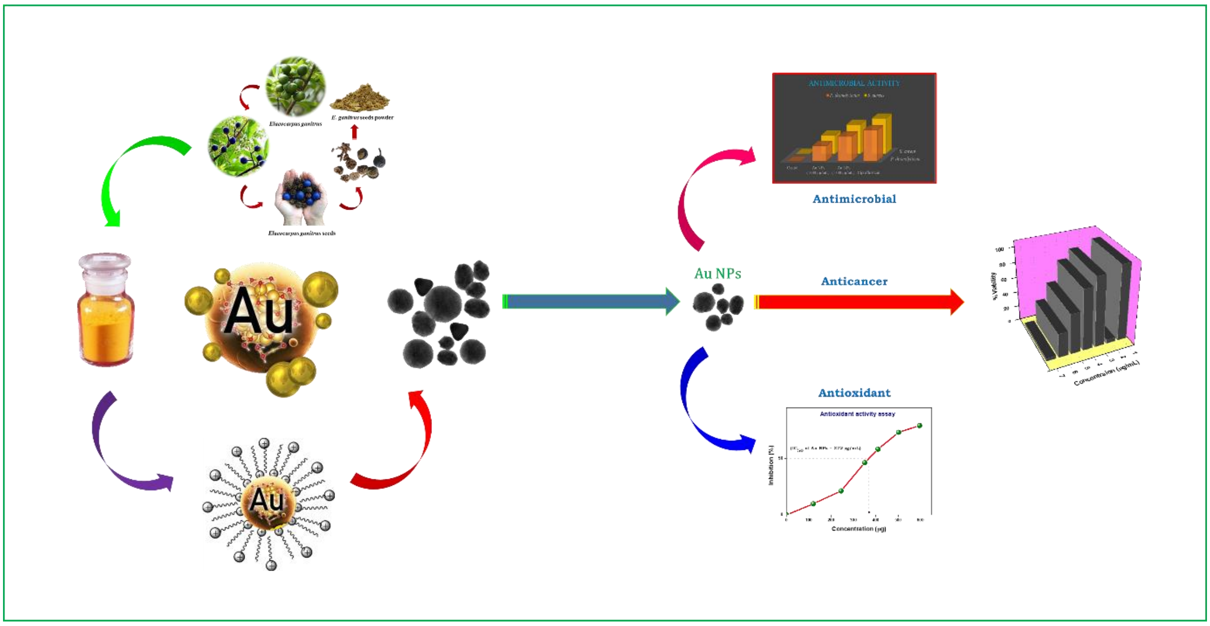
# *Elaeocarpus ganitrus* Assisted Hydrothermal Synthesis of Gold Nanoparticles: Application to Antibacterial, Antioxidant and Anticancer Activities

**Vinay S P[a]*, Udayabhanu[b], Nagaraju G[b], Chandrasekhar N[a]**

[a]*Research and Development Center, Department of Chemistry, Shridevi Institute of Engineering and Technology, Tumakuru, Karnataka, India- 572106.*
[b]*Energy Materials Research Laboratory, Department of Chemistry, Siddaganga Institute of Technology, Tumakuru - 572103, Karnataka, India- 572103.*

**Corresponding author*: vinaysp.chem@shrideviengineering.org

## Graphical abstract



## Abstract

In the present study, we have followed the hydrothermal path for the synthesis of Gold nanoparticles (Au NPs) from the biomaterial *Elaeocarpus ganitrus* seeds extract, which is a rapid, eco-friendly, non-chemical way. The prepared NPs were thoroughly analysed by PXRD & HR-TEM studies and also tested for photocatalytic dye degradation and anticancer studies. Besides, antioxidant, antibacterial and anticancer properties of Au NPs were studied. *In vitro* studies revealed the dose-dependent cytotoxic effect of Au NPs. The prepared nanoparticles showed good cytotoxic impact against Prostate cancer (PC-3) cells line. The results of the present study could contribute to synthesize new and cost-effective drugs from *Elaeocarpus ganitrus* seeds extract by using bio approach.


**Keywords:** *Elaeocarpus ganitrus*, Hydrothermal synthesis, Gold nanoparticles, Prostate cancer, Cancer nanomedicine.

## 1. Introduction

Nano based research is a promising area in biomedical approach especially in malignant therapy and diagnostics. Cancer is a multifaceted disease, extremely variable in its presentation, development and outcome, which is life threatening disease in world and increased the morbidity and mortality of human life. Despite of aggressive research efforts, cancer treatment is still limited to extensive therapies including chemotherapy, radiotherapy and surgery but some cases these therapies fail or cause the reoccurrence of tumor cells [1]. Also majority of cancer drugs kill both cancerous and normal cells at random [2]. Most of the oxidative diseases are due to free radicals resulting in oxidative stress. Many degenerative diseases of aging such as cataracts, cardiovascular disease cancer, brain dysfunction and weak immune system are produced by excess free radicals generated in body. Reactive oxygen species are to be deactivated before they damage cells. Further, the abrupt increase in the bacterial resistance against many antibiotics [3], scientists have researched methods to develop new effective antimicrobial agents that overcome resistances of these microorganisms and are also cost-effective [4]. Therefore, alternative drugs, which are less toxic, eco-friendly and inexpensive are need to be explored. The present work, Au NPs shows effective cytotoxicity against prostate cancer (PC-3) cells, and the percentage of cell viability was significantly reduced by the increasing concentration at 20 to 100 µg/mL.

*Elaeocarpus ganitrus*, is a large evergreen broad-leaved tree whose seed is traditionally used for prayer beads in Hinduism. The seeds are known as Rudraksha, or Rudraksh [5]. *E. ganitrus* seeds are usually found in the foothills of icy Himalayan Mountains to South-East Asia, New Guinea to Australia, Indonesia, Nepal, Hawaii, and Guam. *E. ganitrus* trees are rarely found in South India [6]. Even if found, they usually do not yield *E. ganitrus* seeds. However, two *E. ganitrus* trees in Udupi Kakkunje Garodi yield *E. ganitrus* seeds the whole year. The seeds are covered by an outer husk of blue color when fully ripe, and for this reason, are also known as blueberry beads [7,8]. Phytochemical constituents present in *E. ganitrus* are isoelaeocarpine, rudrakine, elaeocarpidine, epiisoelaeocarpiline, alkaloids, flavonoids, carbohydrates, tannins, quercetin, proteins, ethanol, ellagic acid, fat and gallic acid [9,10].

Au is an important and distinctive inorganic substance, because of its distinctive characteristics and novel applications in various fields of science and technology [11]. Au can exhibit piezoelectric, pyroelectric, optoelectronics, catalysis and semiconducting properties [12]. Due to this, Au is a multifunctional compound which is used in the field of light-emitting diodes, biosensors, diluted and ferromagnetic materials for spintronics solar cells, photo-catalysis, transistors and also acts as anti-oxidants and anti-bacterial agent [13].

In general, Au NPs can be synthesized by numerous ways, like biosynthesis [14-21], combustion route [22], and microwave irradiation [23], c-irradiation [24,25], hydrothermal synthesis [26], ultrasonic irradiation [27,28].

Herein, we report upon the synthesis of gold nanoparticles using biomaterial *Elaeocarpus ganitrus* seeds extract under the hydrothermal synthesis method. The *Elaeocarpus ganitrus* seeds extract act both as a reducing agent and as a capping agent in the reaction, reducing the Chloroauric acid ($HAuCl_4$) to the Au NPs. The Au NPs were characterized using XRD and TEM analysis. Furthermore, the characterized material was examined for Antibacterial, Antioxidant and Anticancer activity against the Human Prostate Cancer (PC-3) cell line.

## 2. Experimental

### 2.1. Preparation of Au nanoparticles

The synthesis of gold nanoparticles using *Elaeocarpus ganitrus* seeds extract under the hydrothermal synthesis method [29]. The *Elaeocarpus ganitrus* seeds extract was used to synthesize Au NPs as described in the previous lines [30].

Chloroauric acid ($HAuCl_4$) was used as a precursor and *Elaeocarpus ganitrus* seeds extract was used as a reducing agent for NPs synthesis. Hydrothermal synthesis, 33 mL *Elaeocarpus ganitrus* seeds extract was taken in a beaker with constant stirring. Add 330 mg of $HAuCl_4$ into a beaker then the solution mixture is stirred for 30 min to obtain a homogeneous solution. This mixture is taken into a 50 mL Teflon vessel. Then, the resulting mixture was sealed in a stainless steel autoclave and maintained at 180 °C for 3 hr. After the reaction was completed, the autoclave was cooled down to room temperature naturally. The products were collected by centrifugation and washed with water and ethanol five times, respectively. Further, the synthesized Au NPs were dried and stored for further examination [31].

### 2.2. Characterization

X-ray diffraction (XRD) data were recorded in Rigaku Smart Lab. The shape and size of the nanoparticles were determined by Transmission Electron Microscopy (TEM) (JEOL 3010).

## 3. Results and discussion

### 3.1. XRD Study

X-ray diffraction measurement (XRD) provides information about geometric arrangements and inter atomic distance. Results represents that crystalline planes are characteristics of monoclinic and cubic nanoparticles. XRD patterns (Fig. 1a.). shows four main characteristic diffraction peaks at $2\theta$=38°, 46°, 64°, and 77°, confirms that Au NPs are face centered cubic (fcc) structure with lattice planes (111), (200), (220) and (311) respectively, average crystallite size was found to be 18 nm determined by Debye-Scherrer's equation. Fig. 1b illustrates the lattice parameter of Au NPs was calculated by utilizing the Rietveld refinement examination. The Refined Parameters, for example, occupancy, atomic functional positions for Au NPs were summarized in Table 1. The structure of the resultant data is according to the JCPDS card number 65-2870. XRD plot clearly indicates formation highly pure Au NPs as there is no impurity peaks were observed [33].

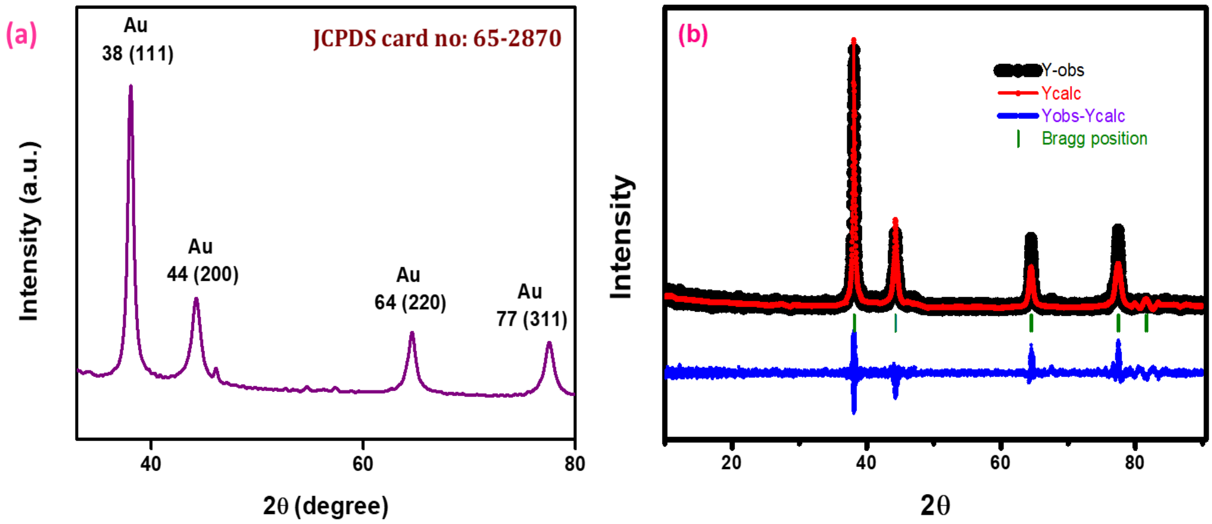



Fig. 1 (a). PXRD pattern of the Au NPs. (b). Rietveld refined pattern.

**Table 1:** Rietveld refined structural parameters for Au NPs

| **Au NPs** | **Au (JCPDS No.:65-2870)** |
|---|---|
| Crystal system | Cubic |
| Space group | Pm-3m (221) |
| **Lattice parameters (A°)** | |
| a =b=c | 4.0272 |
| α= β =λ | $90^{O}$ |
| Unit cell volume ($A°^{3}$) | 67.365 |
| **Atomic coordinates** | |
| x | 0.0000 |
| y | 0.0000 |
| z | 0.0000 |
| **Refinement parameters** | |
| $R_{P}$ | 43.3 |
| $R_{WP}$ | 45.7 |
| $R_{Exp}$ | 32.3 |
| $\chi^{2}$ | 2.0 |
| S | 1.4136 |
| $R_{Bragg}$ | 0.984 |
| $R_{F}$ | 0.459 |
| $Q_{D}$ | 1.7382 |

### 3.2. Transmission Electron Microscopy (TEM) studies:

TEM analysis of Au NPs in optimal conditions is presented in Fig. 2. TEM image of Au NPs shot from different magnifications shows that the particles are in nanoscale and uniformity. Shape of formed nanoparticles are quasi-spherical in nature and size found to be in the range of 24 to 30 nm. HR-TEM images give clear information about the d-spacing value of Au material. For Au, the d-spacing value is 0.14 nm, which belongs to the (111) plane [34].

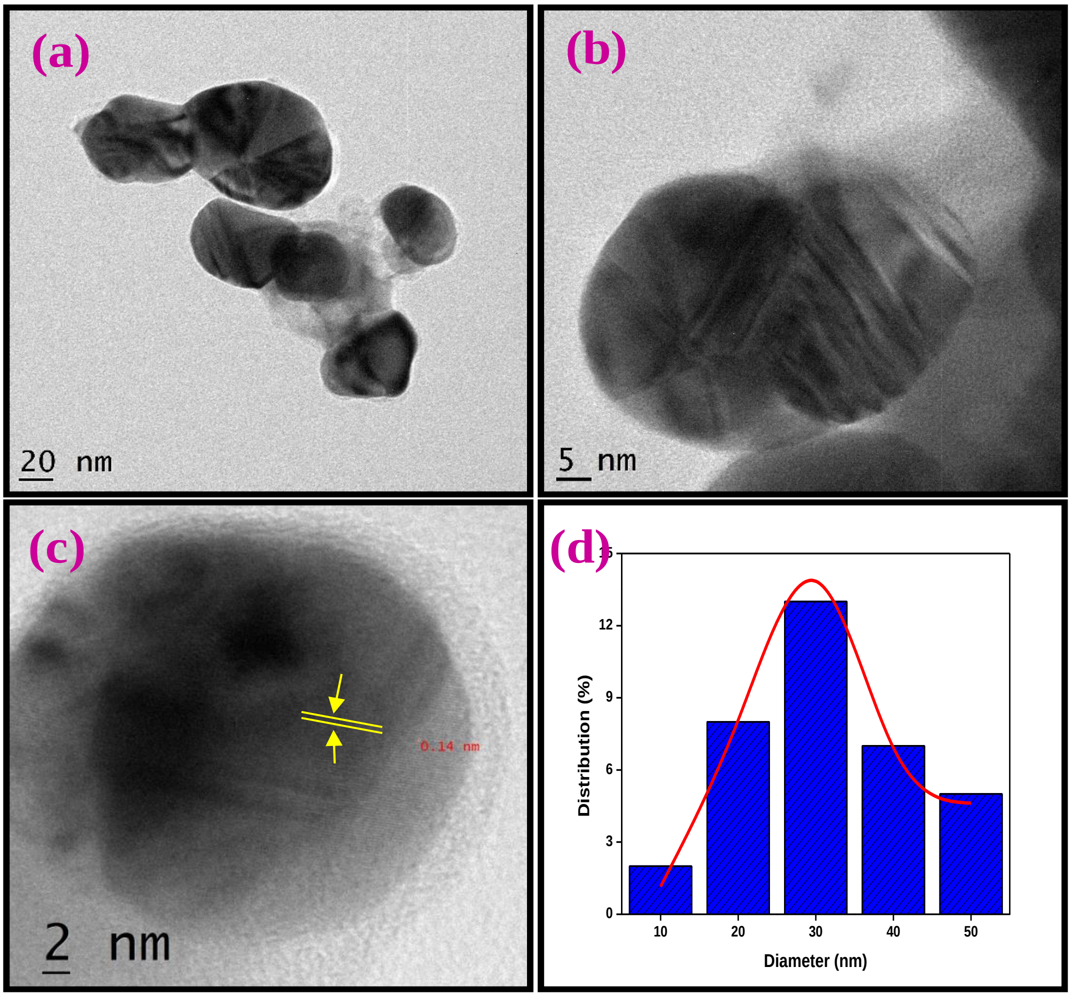



Fig. 2 (a), (b) TEM images, (c) HRTEM image, (d) Histogram show the range of Particle size distribution.

**4. Antibacterial activity**

The antibacterial activity of Au nanoparticles showed significant antibacterial efficiency against Gram-negative (*P. desmolyticum*) and Gram-positive (*S. aureus*) bacteria, as shown in fig. 3. The sizes of the zones of inhibition obtained with the pathogens are presented in Table 2.

**Table 2:** Antibacterial activity of Au NPs against pathogenic bacteria

| Sl. No | Treatment | ***Pseudomonas desmolyticum*** **(mean ± SE)** | ***Staphylococcus aureus*** **(mean ± SE)** |
|---|---|---|---|
| **1** | Control | NA | NA |
| **2** | Au NPs (1000 µg/mL) | 9.47 ± 0.30 | 11.50 ± 0.29 |
| **3** | Au NPs (1500 µg/mL) | 15.50 ± 0.30 | 18.50 ± 0.29 |
| **4** | Ciprofloxacin (5 µg/mL) | 19.63 ± 0.17 | 22.17 ± 0.44 |

Values are the mean ± SE of inhibition zone in mm. NA Symbols represent no antibacterial activity was found in this work.

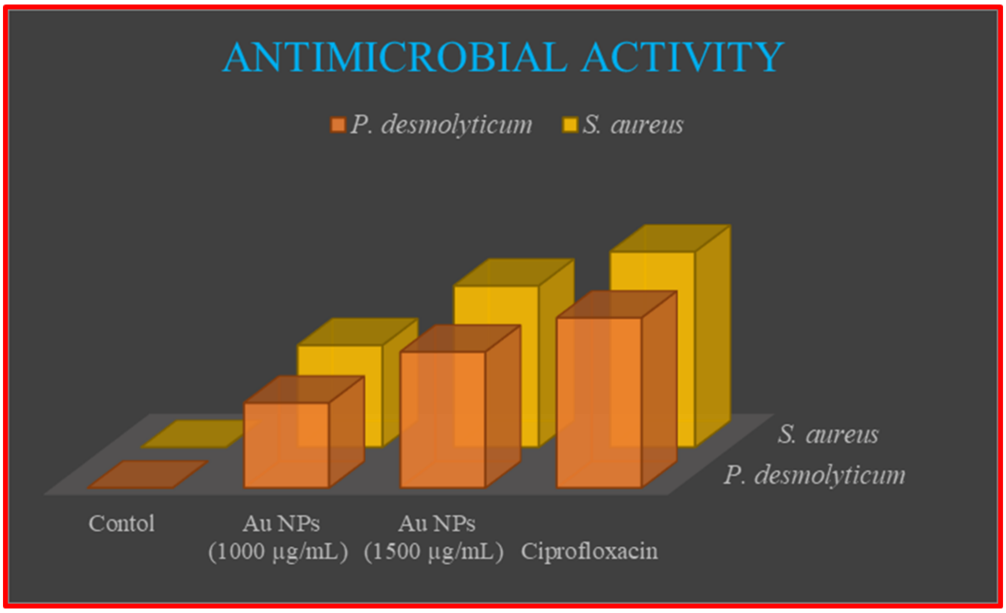



Fig. 3. Bar diagram of inhibition zone of antibacterial activity of Au NPs.

## 5. Antioxidant activity

DPPH, a stable free radical with a characteristic absorption at 517 to 520 nm, was used to study the radical scavenging activity of Au NPs. The decrease in absorption is considered as a measure of the extent of radical scavenging. The percentage of inhibition or scavenging of free radicals was determined. The Au NPs were inhibiting the DPPH free radical scavenging activity with $IC_{50}$ value of 372 µg/ mL (Fig. 4).

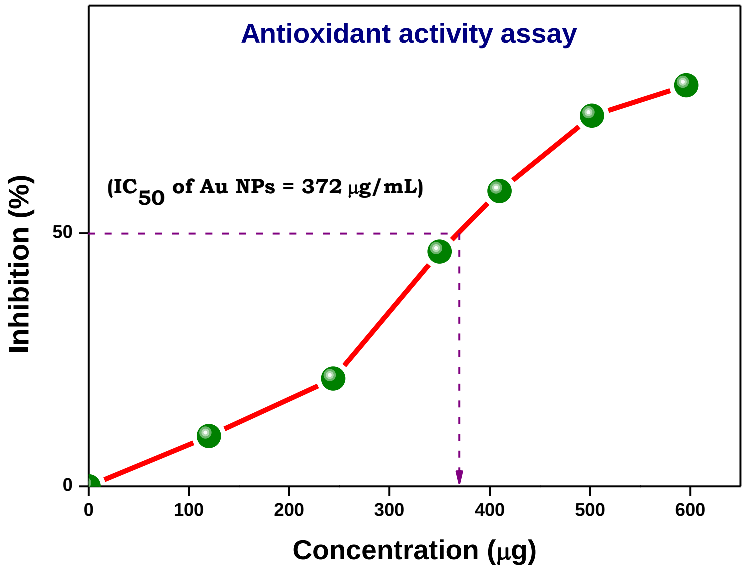
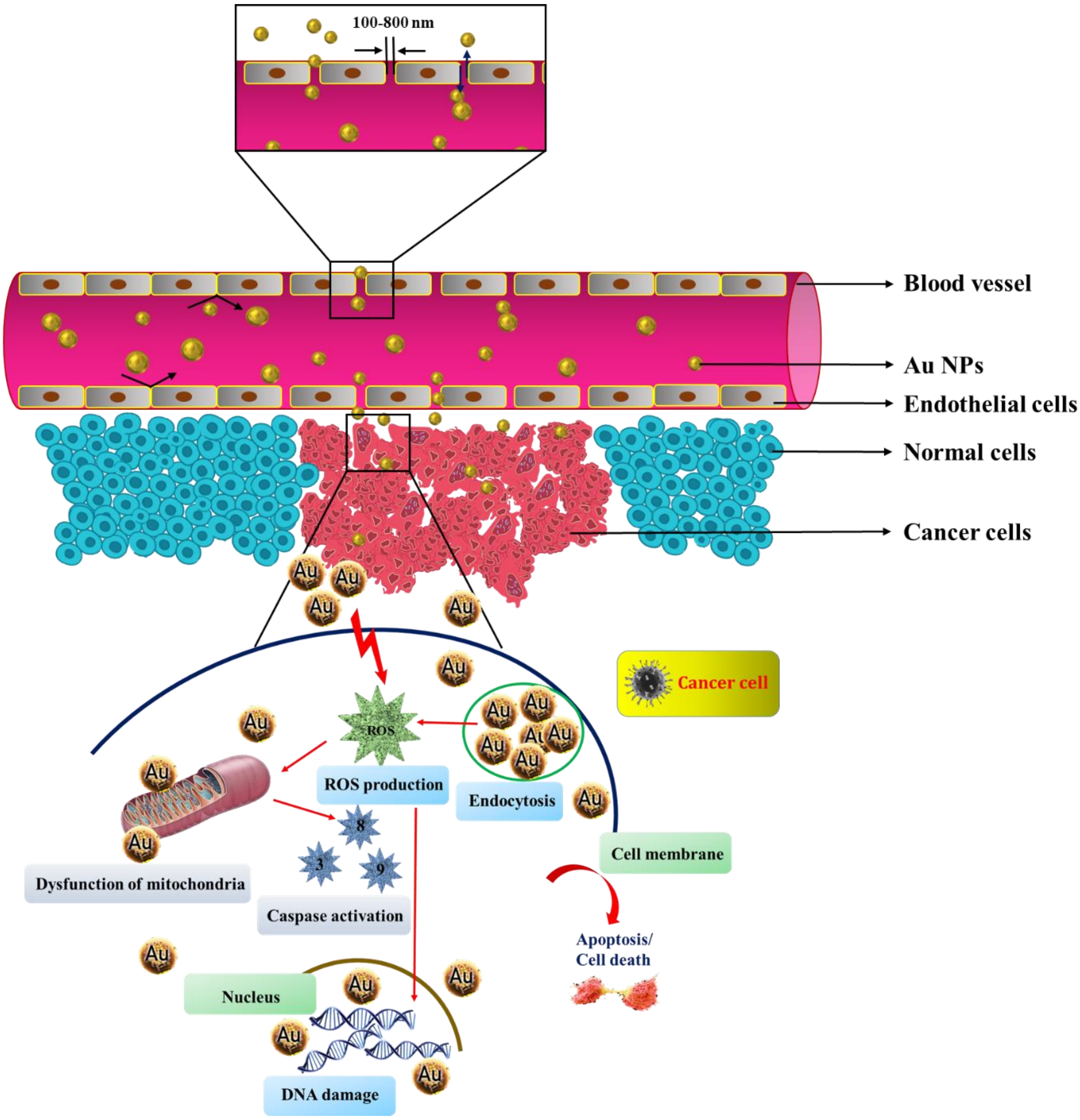



Fig. 4. Percentage inhibition of DPPH radical.

**6. Anticancer activity**

The present work agreement with the desired application for cancer treatment. Anticancer activity in terms of the cell viability against the PC-3 cell line is presented in fig. 5 and 6. MTT assay was performed to assess the effect of Au NPs concentration (20-100 µg/mL) on cell viability of PC-3 cell line. The percentage of cell death in PC-3 cells gradually increases with increasing the concentration of Au NPs concentration (Scheme. 1) [35,36]. The PC-3 cells are exposed to Au NPs at the concentration of 20, 40, 60, 80 and 100 µg/mL. Curve software has been utilized to evaluate the IC50 values (Table. 3) [37]. The results clearly illustrate that the Au notably inhibits the cancer cells with a moderate concentration. The percentage of live cells was reduced as the concentration of Au NPs against PC-3 cell line [38,39].




Scheme. 1. Schematic mechanism of anticancer activity for Au NPs.

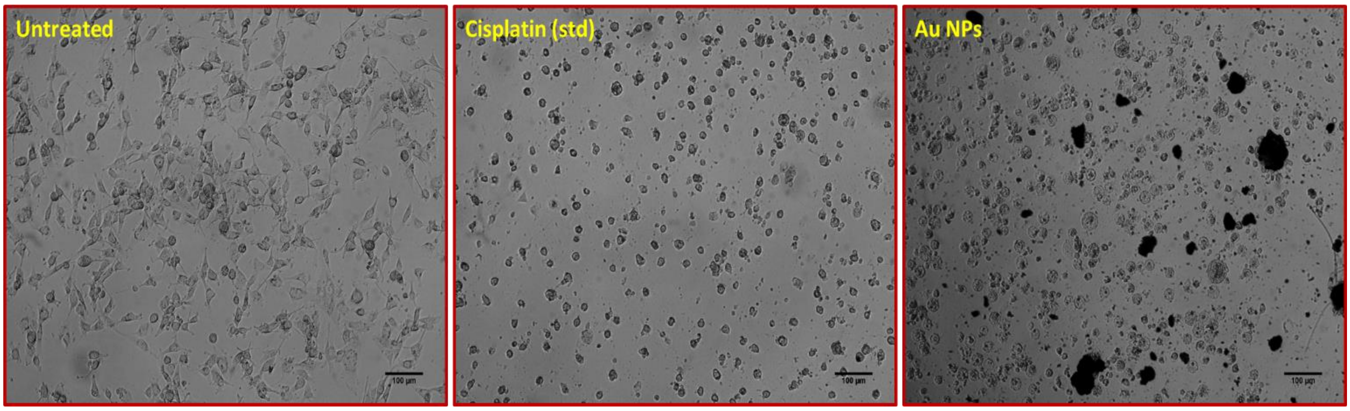



Fig. 5. Shows the cytotoxicity of Au NPs against PC-3 cancer cell line (a = Untreated, b = Treated with standard and c = Treated with nanoparticles).

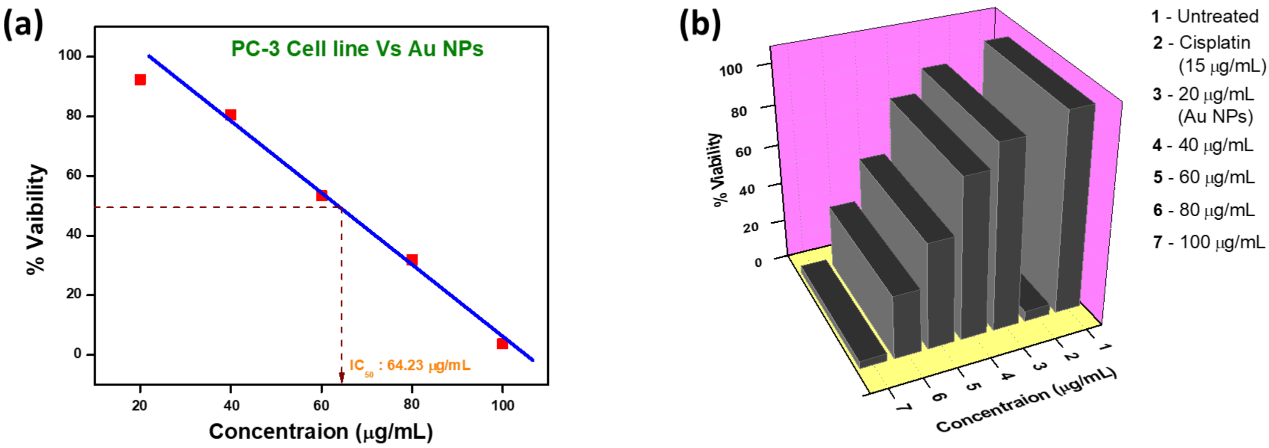



Fig. 6 (a). Percentage of viability in PC-3 cell line with different concentrations of Au NPs. (b). Percentage of cell inhibition in PC-3 cell line.

**Table 3:** Cytotoxicity of Hydrothermal synthesis of Au NPs against PC-3 Cancer cell line

| Cell line | Standard Camptothecin | Concentration of Au NPs sample (µg/mL) | | | | | | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|---|---|---|---|
| | | Control | 20 | 40 | 60 | 80 | 100 | |
| PC-3 | 5.14 ± 0.32 | 100 | 92.83 ± 0.33 | 80.00 ± 0.29 | 53.50 ± 0.29 | 31.83 ± 0.17 | 3.33 ± 0.17 | 64.23 ± 0.33 |

## 5. Conclusion

This study has led to the synthesis of Au NPs using the extract of *Elaeocarpus ganitrus* seeds under the hydrothermal synthesis method. The prepared Au NPs was confirmed by XRD study. The poly-dispersed Au nanoparticles were spherical in shape with an average size range of 30 nm. The NPs were crystalline in nature, showing the characteristic f.c.c (face-centered cubic) structure. Au NPs exhibited significant antibacterial activity against gram +ve and gram -ve bacteria. Prepared NPs exhibited outstanding antioxidant activity. The cytotoxic activity of the Au nanoparticles exhibited strong cytotoxicity and improve the anticancer activity against the PC-3 cancer cell line. Consequently, this work has established the relevance of novel biomaterial *Elaeocarpus ganitrus* seeds extract in nanotechnology, through the rapid and eco-friendly synthesis of Au NPs with potent antibacterial, antioxidant and anticancer activities.

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**Compliance with ethical standards**

Conflict of interest: The authors declare that they have no conflict of interest.

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