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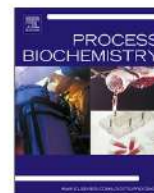

Unveiling the anticancer and antimycobacterial potentials of bioengineered gold nanoparticles

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ABSTRACT

Synthesis of gold nanoparticles was carried out using *Pongammia pinnata* (pongam) leaf extract and their anticancer and antimycobacterial activities were studied. Gold nanoparticle formation was confirmed by UV–vis, XRD and HR-TEM. The anticancer efficacies of the biogenic gold nanoparticles were analyzed using cytotoxicity, cell morphology analysis, oxidative DNA damage, apoptosis detection and toxicity studies. Biogenic gold nanoparticles inhibited breast cancer cell line (MCF-7) proliferation with an efficacy of IC_{50} of 1.85 μg/mL. The antimycobacterial potential of the biogenic gold nanoparticles was screened against *M. tuberculosis* by Luciferase Reporter Phage (LRP) assay. The gold nanoparticles showed inhibition against sensitive *M. tuberculosis* with the minimum inhibitory concentration (MIC) of 10 μg/mL whereas no inhibition was found against the rifampicin resistant *M. tuberculosis.*

1. Introduction

Cancer occurrence and mortality are fast rising concerns worldwide. The reasons are very complex in nature but reflect both the aging and increasing population, as well as the changes in the occurrence of the major risk factors for cancer are associated with socioeconomic development [1]. GLOBOCAN 2018 (International Agency for Research on Cancer) estimates cancer incidence and mortality across 20 world regions have reported about 18.1 million new cancer cases and cancer deaths was reported to be 9.6 million in 2018 [2]. There is an elevated demand for the development of novel strategies towards competent diagnostics and treatment of cancer. During the recent past, the exceptional growth in research and applications in the area of nanoscience and technology has brought hope for the development of classical cancer therapies [3].

Tuberculosis is an airborne communicable disease in humans caused by *Mycobacterium tuberculosis* (MTB). MTB infects individuals in two ways that it may develop either active tuberculosis or latent tuberculosis infection (LTBI), which may result in reactivation tuberculosis in future. One third of the world's population is estimated to have LTBI, which may develop into an active TB disease within a few years or decades after the primary infection with MTB [4]. Tuberculosis (TB) and malignancy signify global threats and inflict formidable suffering worldwide. There are major connections that have been observed between tuberculosis and cancer risks. Initially, tuberculosis increases the lung cancer risk and further, promotes the reactivation of latent *M tuberculosis* infection and also, it attributes to immunosuppression to cancer treatment and can reactivate the latent tuberculosis infection. A recent study with tuberculosis patients of around 15,000 people reported that, 1.29 % of people had cancer outside the lungs and 3.4 % have been reported to have lung cancer in the tested population [5–7].

Gold colloids have been studied for potential applications in the field of medicine for centuries. Nonetheless synthesis and various applications of gold nanoparticles have only recently acquired the wide interests of scientists [3]. Gold nanoparticles have numerous advantages over different nanomaterials, primarily due to their various sizes, shapes and their unique physical and chemical properties. The various approaches of physical, chemical and mechanical synthesis have raised the concerns for environment and human health due to the hazardous and effective cytotoxicity of the produced nanomaterials [8].

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In this context, development of efficient and ecofriendly benign synthesis methods is necessary for the synthesis of greener nanomaterials with high reproducibility and purity [9–11]. The biogenic methods have recently drawn remarkable interest for large scale production of metal and metal oxide nanomaterials with reduced environmental issues [12] as well as remarkable biological properties, leading to an increased attention to this biogenic approach [13–18]. In this study, the medicinal plant *Pongammia pinnata* classified under Fabaceae family, has been used for the biosynthesis of gold nanoparticles. The presence of phytochemical constituents such as flavonoids, glycosides, furane flavones, furanodiketones, offers potential therapeutic values [19]. *Pongammia pinnata* (*P. pinnata*) has potent antiplasmodial [20], antioxidant and anti-hyperammonemi [21], antidiarrhoeal [22], antiulcer [23], antihyperglycaemic and anti-lipidperoxidative activities [24]. Owing to the medicinal properties of *Pongammia pinnata* extract, this study performed one pot green synthesis of gold nanoparticles using the *P. pinnata* leaf extract and characterized using different spectroscopic and microscopic methods. In addition, the current study has evaluated the antimycobacterial and anticancer activities of the synthesized gold nanoparticles and the possible mechanism of action was also investigated.

2. Materials and methods

2.1. Materials

Fresh *P. pinnata* leaves (Fig. 1a) were collected from the Sathyabama Institute of Science and Technology (12.8713°N, 80.2224°E), Chennai, India. Chloroauric acid ($HAuCl_4$) was procured from Loba-Chemie, Mumbai, India.

2.2. Synthesis and characterization of gold nanoparticles

One gram of *P. pinnata* leaves was thoroughly washed and smashed into tiny pieces and boiled in 100 mL Millipore water for 10 min at 65 °C. In this experiment, 10 mL of *P. pinnata* leaf extract was added to 90 mL of 1 mM aqueous chloroauric acid solution. The gold nanoparticle formation was confirmed using the UV–vis absorption spectra (Shimadzu UV-1800, Japan). XRD pattern of gold nanoparticles were recorded using BRUKER AXS, D8 Discover, Germany and High resolution Transmission electron microscopy (HR-TEM) was performed with a JEOL 3010, Japan with a UHR pole piece.

2.3. In vitro anticancer activity

2.3.1. Cell culture

The human breast cancer cell line (MCF-7) was procured from National Centre for Cell Sciences (NCCS), Pune, India. The cancer cells were grown in Dulbecco's Modified Eagle Medium supplemented with 2 mM L-glutamine (Himedia, India), antibiotics [100 U of penicillin/mL; 100 g/mL gentamicin] and 10 % FBS (Sigma-Aldrich, India) and maintained in CO_2 incubator (Lark Innovation Fine Technology, Chennai, India) [10].

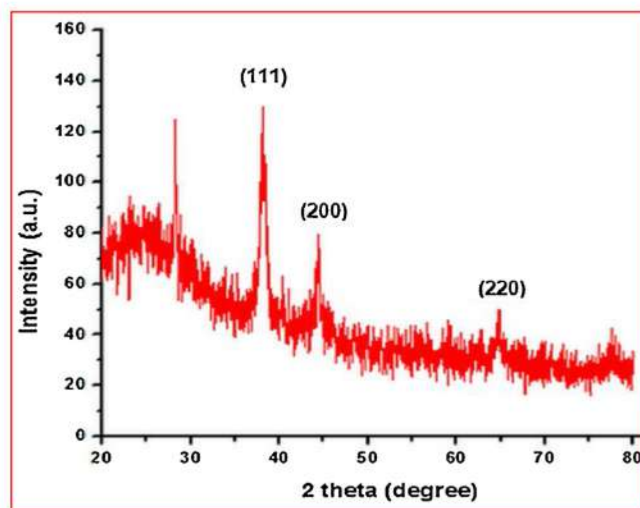

Fig. 2. XRD patterns of biogenic gold nanoparticles.

2.3.2. Analysis of cytotoxicity using MTT assay

The cytotoxicity of cells exposed to gold nanoparticles was performed according to our previous report [11]. The MCF-7 cancerous cells were treated with varying concentrations of biogenic gold nanoparticles (2, 4, 6, 8 and 10 μg/mL) and incubated for 24 h and 48 h along with the experimental control. The cytotoxicity assay was carried out on MCF-7 cells according to the previous study and cell viability was calculated using the formula given below [25].

$$\%ofcellviability = \frac{ODofthecellstreatedwithbiogenicgoldnanoparticlesat570nm}{ODofthecellscontrolcellsat570nm} \times 100$$

2.3.3. Analysis of cell morphology

After cytotoxicity assessment, the cell morphology was observed under inverted microscope (Leica microsystems, Germany) after the treatment with IC_{50} concentration of the gold nanoparticles. The cancer cells without gold nanoparticle treatment were maintained as the control group.

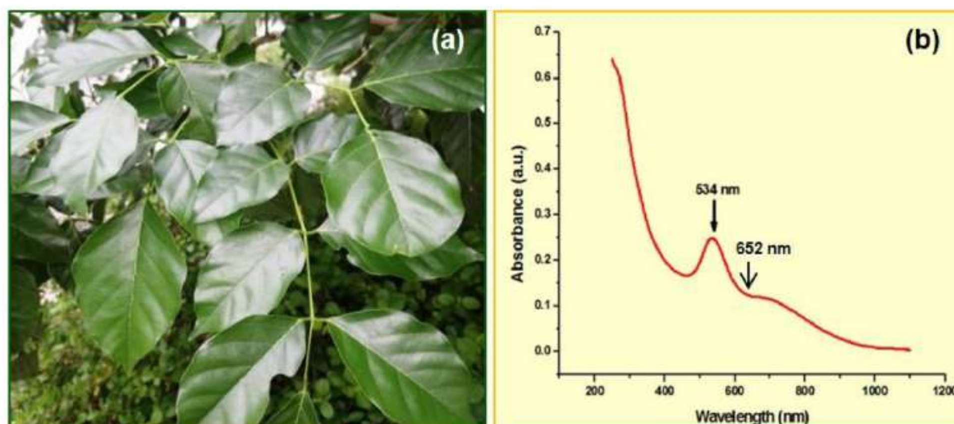

Fig. 1. (a) *Pongamia pinnata* leaves; (b) UV–vis spectra of *Pongamia pinnata* leaves mediated synthesis of biogenic gold nanoparticles.

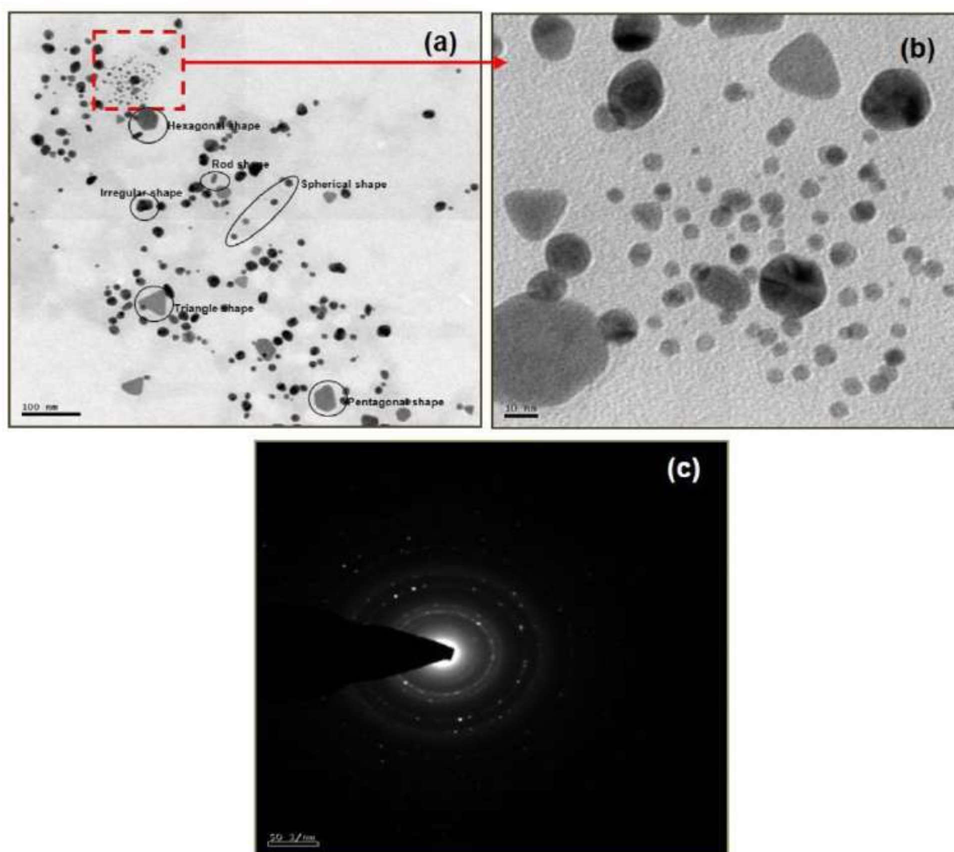

Fig. 3. Transmission electron microscopy (TEM) images. (a, b) Biogenic gold nanoparticles; (c) SAED pattern of biogenic gold nanoparticles.

2.3.4. Analysis of oxidative DNA damage

The comet assay was performed according to the protocol described elsewhere [10,26]. Briefly, the MCF-7 cancer cells were washed with phosphate buffer solution and the cells were collected by centrifugation. The collected cancer cells were treated with gold nanoparticles (IC_{50} concentration) for 48 h and washed with phosphate buffer solution. Briefly, the low melting agarose (0.5 %) along with cell suspension (100 μL) was spread on microscopic slide contains melting agarose (1%) and then exposed to the lysis buffer solution for 1 h at 4 °C. After incubation, single gel electrophoresis was carried out according to our previous report [10].

2.3.5. Detection of apoptotic cells

The cancer cells (MCF-7) at > 80 % confluence were then treated with the gold nanoparticles (IC_{50} concentration) and then apoptotic staining was performed according to our previous report [10]. The cells stained were observed under a fluorescence microscope (EVOS® FLoid® Cell Imaging).

2.3.6. Toxicity by DAPI staining

Toxicity assessment was performed with IC_{50} concentration of gold nanoparticles and DAPI staining was performed according to our previous study [11]. After the experimental procedure, the cells were observed under a fluorescent microscope (EVOS®FLoid®Cell Imaging).

2.4. Anti-mycobacterial activity

The antimycobacterial potential of the biogenic gold nanoparticles was screened against *M. tuberculosis* using Luciferase Reporter Phage (LRP) assay. Briefly, the suspension of *M. tuberculosis* was prepared by inoculating a loopful of colonies into 0.3 mL of sterile distilled water, vortexed thoroughly and then added with 3.7 mL of sterile distilled water. The turbidity of suspension has been adjusted to Mcfarland standard 2. About 100 μL of *M. tuberculosis* suspension was added to the vial containing 350 μL of 7H9 broth plus 50 μL of nanoparticles and the vial containing 400 μL of 7H9 broth, marked as the test and control, respectively. Then, the vials were incubated at 37 °C for 72 h. Subsequently, 40 μL of 0.1 M $CaCl_2$ and 50 μL of mycobacteriophage were added into all the vials and further incubated for 4 h. After incubation, 100 μL of aliquot from each vial was added with 100 μL of D-Luciferin in a RIA vial and RLU was measured using the Luminometer (Berthold). The % of RLU reduction was calculated based on the following formula.

% of RLU reduction = (Control RLU - Test RLU)/Control RLU × 100 [27].

3. Results and discussion

3.1. Synthesis and characterization of gold nanoparticles using P. pinnata

On mixing the *P. pinnata* leaf broth with 1 mM aqueous chloroauric acid, the solution changed color from pale green to ruby red, representing the formation of gold nanoparticles by visual observation. UV–vis spectra recorded for the reaction solution showed the presence of the surface plasmon resonance bands at 534 nm and 652 nm (Fig. 1b). The gold nanoparticle absorption band was slightly asymmetrical with indications of an additional weaker component at 652 nm. The presence of this shoulder indicated either formation of the gold nanoparticles with different shapes (triangle, rod, hexagonal, pentagonal) or anisotropy in the gold nanoparticles. The crystalline nature of the gold nanoparticles was confirmed by the XRD measurement, as shown in Fig. 2. XRD pattern shows the main characteristic patterns of the gold nanoparticles at 2θ values of 38.4°, 44.6°, 64.7°, and

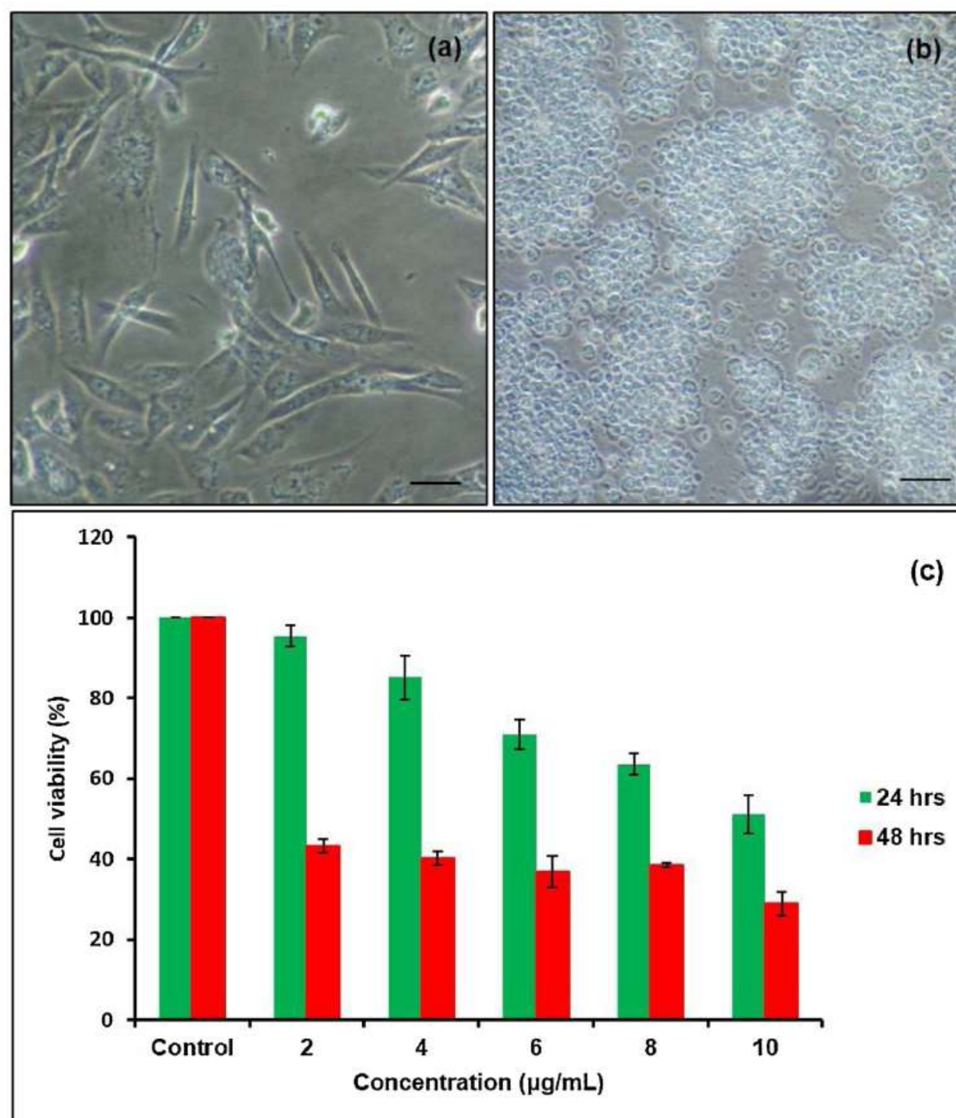

Fig. 4. MCF-7 cell morphology assessment. (a) Control; (b) Gold nanoparticles treated cells; (c) Cytotoxicity of biogenic gold nanoparticles against MCF-7 cancer cells (Magnification 40 ×).

77.8° corresponding to the reflections from the planes (111), (200), (220), and (311) of fcc of gold (JCPDS 04-0784) [28]. The width of the (111) peak was referred for calculating the size of the crystallite employing Scherrer equation. The representative TEM images of the *P. pinnata* leaf broth mediated gold nanoparticles showed spherical shaped particles and some of the particles were irregular, triangle, hexagonal, pentagonal and rod in shapes (Fig. 3a& b). The SAED (Fig. 3c) of the gold nanoparticles and the spots could be indexed based on the fcc structure of gold. The ability to grow gold nanoparticles of rod, hexagonal, pentagonal, and triangle like morphologies by biogenic techniques are tremendously exciting given that the catalytic, electronic, optical and biological properties of metal nanoparticles are strong functions of not only nanoparticle size but shape as well [29–31].

3.2. In vitro anticancer studies

3.2.1. Cell morphology and cytotoxicity studies

MCF-7 cancer cells and the gold nanoparticle treated MCF-7 cancer cells were examined for their morphology using an automated inverted microscope (Leica microsystems, Germany). The structural changes of the normal and gold nanoparticle treated cells are shown in in Fig. 4a& b. The gold nanoparticle treated cells have shown cell shrinkage, changes in membrane integrity, nuclear condensation, membrane damage, and finally, inhibition of cellular growth. The cytotoxic study carried out using MTT assay was carried out with standard protocols [10]. The cytotoxicity of the gold nanoparticles on MCF-7 cells with varying concentrations (0 – 10 µg/mL) with two time intervals (24 and 48 h) was studied. MCF-7 cancer cells treated with gold nanoparticles of dosage 1.85 µg/mL resulted in 50 % cell inhibition after 48 h incubation (Fig. 4c). However, the inhibition (50 %) representing cell toxicity was not observed for all the dosages (0 – 10 µg/mL) after 24 h incubation. Hence, the cytotoxicity of the gold nanoparticles on MCF-7 cancer cells was based on time and dose dependent inhibitory activity. Similarly, various research studies on the biogenic gold nanoparticles showed cytotoxic effects against various cancer cell lines [32–34].

3.2.2. DNA damage by comet assay and DAPI staining

Genotoxicity represents DNA strand breaks was evaluated by comet assay was carried out on MCF-7 cancer cells after exposure to the biogenic gold nanoparticles (IC_{50} of 1.85 µg/mL) for 48 h. The results showed that no damage was observed in the control MCF-7 cells whereas in MCF-7 cells treated with biogenic gold nanoparticles, significant DNA damage was observed as evidenced from the appearance of a longer tail of DNA in the condensed form (Fig. 5b). The fluorescent

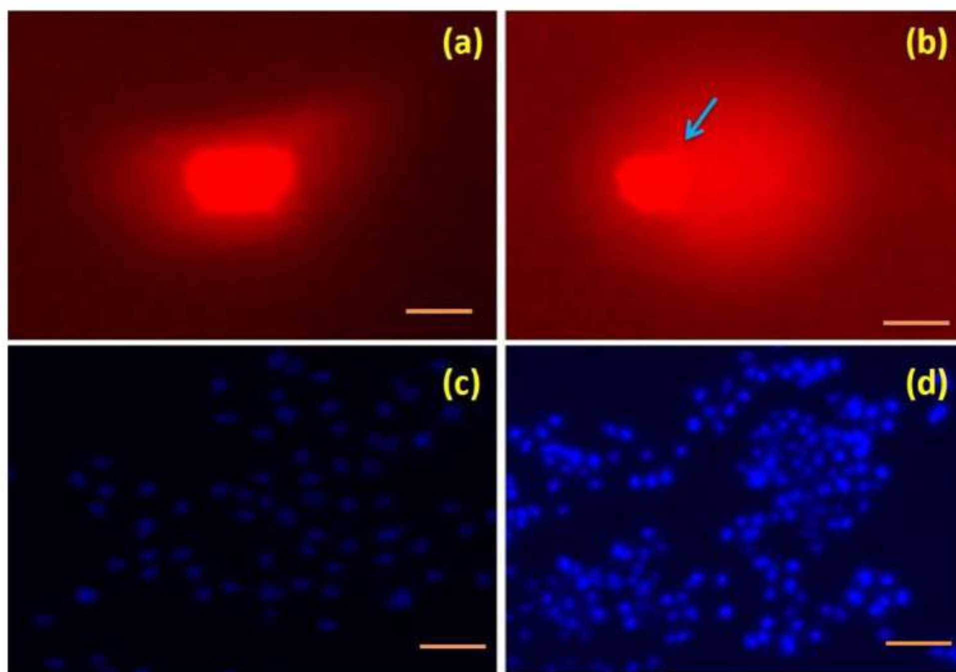

Fig. 5. Apoptosis induction of biogenic gold nanoparticles by comet assay. (a) Control; (b) Gold nanoparticles treated MCF-7 cells (IC_{50} concentration); DAPI staining of MCF-7 cells (c) Control; (d) Gold nanoparticles treated MCF-7 cells (IC_{50} concentration) (Magnification 40 ×).

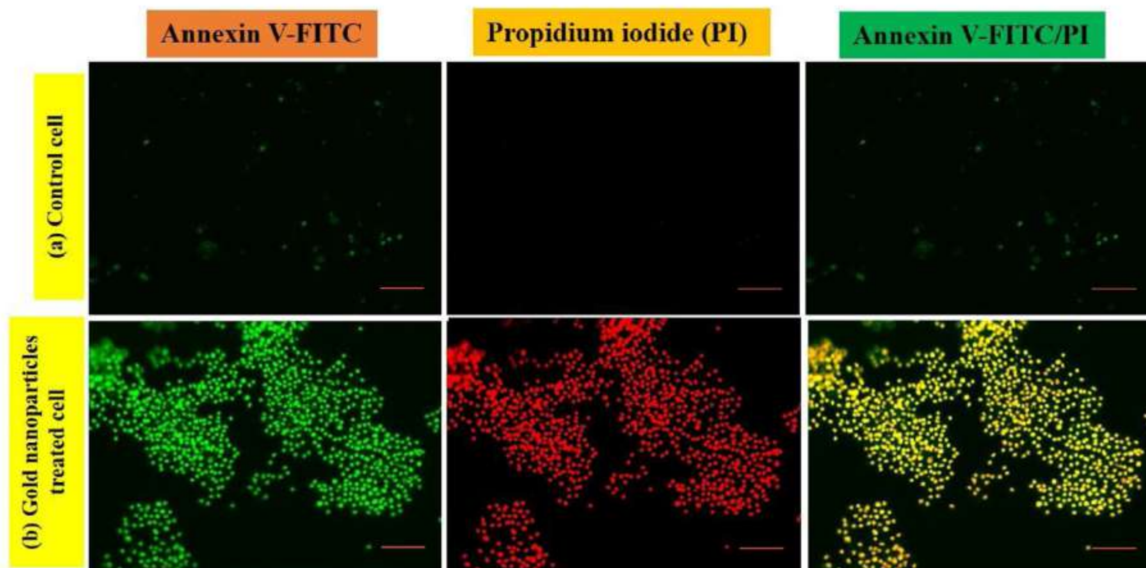

Fig. 6. Morphology of apoptotic cells staining with Annexin V-FITC and PI. (a) Control MCF-7 cells; (b) Gold nanoparticles treated MCF-7 cells (Magnification 40 ×).

Table 1
Antimycobacterial activity of biogenic gold nanoparticles.

Strains	Biogenic AuNPs			Rifampicin
	50 μg/mL	25 μg/mL	10 μg/mL	2 μg/mL
Drug sensitive *M. tuberculosis*	Inhibition (77.23 %)	Inhibition (68.66 %)	Inhibition (65.93 %)	Inhibition (96.85 %)
Rifampicin resistant *M. tuberculosis*	No Inhibition (46.81 %)	No Inhibition (43.19 %)	No Inhibition (37.73 %)	No Inhibition (0 %)

microscopic images of the control cells showed the absence of DNA damage confirmed by presence of spherical nucleoids and a lower comet score of 5.5 % (Fig. 5a). On contrary, the MCF-7 cells treated with biogenic gold nanoparticles (IC_{50}) for 48 h showed larger tails of nucleoids, which indicated increased levels of DNA damage with comet scores of DNA tail being 30.26 % (Fig. 5b). Similarly, commercial size dependent (5 and 50 nm) silver, gold and platinum nanoparticles showed genotoxicity on human bronchial epithelial cells [35,36]. In our previous studies, the green synthesized gold nanoparticles showed a potent genotoxic effect against the two cancer cell lines, MDA-MB-231 & MCF-7 [10].

DAPI staining of MCF-7 cancer cells treated with biogenic gold nanoparticles clearly indicated the induction of apoptosis. Fig. 5c shows that the control cells were found to be negatively stained with DAPI, whereas the cells exposed to the biogenic gold nanoparticles emitted bright blue fluorescence (Fig. 5d) due to condensed nucleus and fragmented chromatin formation. A similar study has reported that the medicinal plant mediated synthesized gold nanoparticle treated various

cancer cells such as MCF-7, MDA-MB-231 and gastric carcinoma cell lines [10,37], clearly indicated the induction of apoptosis, condensed nucleus and damage in cell structure.

3.2.3. Detection of apoptotic cells by dual staining

The control MCF-7 cells showed less intensity for Annexin V/PI double staining. The biogenic gold nanoparticle treated MCF-7 cells showed an intense positive staining in the MCF-7 cells. This result showed the early event of apoptosis, due to the plasma membrane asymmetry [38,39]. The plasma membrane with phosphatidylserine residues in the non-viable cells were detected using the binding of high affinity Annexin V-FITC in cancer cells. On this basis, it has been speculated that the biogenic gold nanoparticle treated MCF-7 cancer cells induced apoptosis in the present study. Furthermore, Fig. 6b confirms that the MCF-7 cells exposed with the biogenic gold nanoparticles were stained intensively to double staining showing the intensive binding of Annexin V-FITC and PI onto the phosphatidylserine residues in the MCF-7 cancer cells. On contrary, the control cells were stained negative for double staining, revealing the viability of the cells (Fig. 6a).

3.3. *In vitro antimycobacterial activity using LRP assay*

The antimycobacterial activity of *P. pinnata* mediated synthesized gold nanoparticles was tested against drug sensitive and drug resistant *M. tuberculosis* (Table 1). The biogenic gold nanoparticles showed inhibition against the drug sensitive *M. tuberculosis* with the minimum inhibitory concentration (MIC) of 10 μg/mL, whereas, no inhibition was found against the rifampicin resistant *M. tuberculosis* at different concentrations ranging from 10 μg/mL to 50 μg/mL. *P. pinnata* seed extract mediated zinc oxide nanoparticles were synthesized and their anticancer (MCF-7), antibacterial (Gram negative and Gram positive) and antifungal activities were carried out [40]. Further, zinc oxide nanoparticles have shown potent antibacterial activity at 25 μg/mL, anticancer and antifungal activities at 50 μg/mL concentration. The silver nanoparticles synthesized using the seed extract of *P. pinnata* was carried out and the antibacterial activity was studied against the Gram negative bacterium, *E. coli* and the synergistic effect with ampicillin antibiotic was also studied [41]. However, our study with gold nanoparticles synthesized using the leaf extract of *P. pinnata* showed potent antimycobacterial activity against the drug sensitive *M. tuberculosis.*

4. Conclusion

Biosynthesis of gold nanoparticles was carried out using a medicinal plant (*P. pinnata)* and the potential antimicrobial and anticancer efficacies of the synthesized gold nanoparticles were investigated. TEM characterization showed that the synthesized gold nanoparticles were largely spherical in shape with an average size of 16 nm. The synthesized gold nanoparticles were found to have an inhibitory potential against the cancer cell line (MCF-7) based on the cytotoxicity assay with the IC_{50} value of 1.85 μg/mL. Furthermore, gold nanoparticle treatment inhibited the cancer cell proliferation through induction of apoptosis and DNA damage in cancer cells. The results showed that significant amounts of apoptotic cells were observed upon exposure to biogenic gold nanoparticles. In addition, antimycobacterial activity of the biogenic gold nanoparticles was tested against both drug resistant and drug sensitive *M. tuberculosis.* The results revealed that the gold nanoparticle treatment was effective against the drug sensitive *M. tuberculosis* with the MIC of 10 μg/mL. Overall, these findings suggested that the biogenic gold nanoparticles synthesized using *P. pinnata* can be utilized as potent anticancer and antimycobacterial agent for pharmaceutical applications.

CRediT authorship contribution statement

K. Govindaraju: Conceptualization, Funding acquisition, Writing - original draft. **R. Vasantharaja:** Conceptualization, Investigation. **K.S. Uma Suganya:** Investigation, Methodology. **S. Anbarasu:** Investigation, Resources. **K. Revathy:** Investigation, Visualization. **A. Pugazhendhi:** Supervision, Writing - review & editing. **D. Karthickeyan:** Resources, Formal analysis. **G. Singaravelu:** Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.procbio.2020.06.016.

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