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RESEARCH PAPER

# Exploring the Antimicrobial Potential and Anticancer Activity on MCF-7 as a Human Cancer Cell Line with Gold Nanoparticles Derived from Yapa Tangerine Leaves Extract

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## ABSTRACT

In this study, gold nanoparticles were synthesized using a simple, cost-effective, and environmentally friendly method. *Yapa tangerine* tree leaf extract was employed as both a reducing and capping agent to reduce gold ions and facilitate nanoparticle formation. The morphology, size, and purity of the gold nanoparticles were analyzed using TEM, XRD, UV-Vis, and FT-IR techniques. The surface plasmon resonance peak of the gold nanoparticles was identified at 520 nm via UV-Vis analysis. TEM and XRD confirmed that the nanoparticles had an average size of 25-35 nm and exhibited uniformity. After characterization, the anticancer activity of the synthesized gold nanoparticles was tested against the MCF-7 cell line, showing significant cytotoxicity at low concentrations. The antibacterial activity of the gold nanoparticles was also evaluated against six Gram-positive and Gram-negative bacterial strains. The MIC values were found to be 9.37 µg/mL for *Escherichia coli*, 18.75 µg/mL for *Klebsiella pneumoniae*, 150 µg/mL for *Staphylococcus aureus*, 37.5 µg/mL for *Streptococcus mutans*, 37.5 µg/mL for *Streptococcus mitis*, and 75 µg/mL for *Enterococcus faecalis*. These findings suggest that gold nanoparticles have strong potential for future biological and biotechnological applications.

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## INTRODUCTION

Nanotechnology, centered on the creation and application of nanoparticles, has achieved remarkable progress across many scientific fields, offering innovative solutions to complex challenges. Defined by their small size ranging from 1 to 100 nm in at least one-dimension nanoparticles have paved the way for nanomedicine in the medical field. This breakthrough enhances our ability to diagnose, monitor, and treat diseases, while also addressing a variety of pharmacological challenges with novel approaches to managing diverse medical conditions [1-3].

The green synthesis of nanoparticles has

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emerged as a highly respected method in materials science and nanotechnology, owing to its multiple advantages. This environmentally friendly approach is recognized for its ability to produce nanoparticles without relying on hazardous substances or toxic chemicals, thus aligning with the growing demand for sustainable practices in research and industry [4-6].By adhering to strict safety standards, green synthesis minimizes environmental impact and offers practical benefits, such as simpler processes and milder reaction conditions. These factors can lead to lower production costs and reduced by-products. Moreover, nanoparticles produced through this method often demonstrate enhanced stability and biocompatibility, making them suitable for diverse applications in medical and environmental fields [7]. The rising interest in plant-based green synthesis for nanoparticle production is largely due to the wide variety of bioactive phytochemicals present in plants, including phenolics, alkaloids, steroids, curcumins, and flavonoids [8].

These natural substances, though chemically complex, are environmentally friendly and fulfill several essential functions in nanoparticle synthesis. They serve as reducing agents that facilitate the transformation of metal ions into nanoparticles, as capping agents that stabilize these particles and prevent aggregation, and as stabilizing agents that promote long-term stability [9, 10]. By using plant extracts, the synthesis process becomes simpler and more cost-effective, reducing reliance on harmful chemicals and enhancing safety, particularly in medical and biotechnological applications. Furthermore, the resulting nanoparticles typically possess well-defined sizes and shapes, which are vital for achieving reliable performance in various applications. The phytochemicals involved can also cause the nanoparticles with additional properties, such as antimicrobial and antioxidant capabilities, further extending their potential uses [11, 12].

In the field of nanotechnology, metallic nanoparticles have demonstrated a range of unique properties, opening up new possibilities, particularly in the area of targeted drug delivery systems [13]. Metallic nanoparticles (MNPs) are widely used as carriers for delivering various therapeutic agents. Many MNPs, including silver and gold have adjustable optical properties that can be finely tuned for specific applications [14].

Among MNP, gold nanoparticle (Au-NPs) has been interested because of their unique properties that are valuable in various biomedical applications. Their chemical inertness and resistance to surface oxidation make Au-NPs an ideal choice for nanoformulation [15]. It is well-known that gold nanoparticles (Au-NPs) combined with phytochemicals have been widely used for their antitumor properties. In addition to these benefits, Au-NPs also serve as excellent drug carriers. They can enhance the antibacterial effects of drugs and play a vital role in developing effective strategies against resistant bacteria [16]. In this framework, Aljarba et al. reported the article about the increasing concern over antibiotic resistance and the limitations of current anticancer and antimicrobial therapies, such as poor solubility, stability, and side effects, which have driven research into alternative strategies. Gold nanoparticles (Au-NPs), synthesized from natural and chemical sources, have shown significant promise due to their biocompatibility, ease of surface modification, and optical properties. Studies indicate that Au-NPs exhibit strong anticancer and antimicrobial activities by inducing oxidative stress, membrane damage, and DNA disruption in both cancer cells and drug-resistant bacteria. The review underscores the potential of Au-NPs as effective therapeutic agents in biomedical applications and suggests their future role in developing nanotechnology-based treatments for infectious diseases and cancer [17]. Rajan & co-worker synthesized gold nanoparticles using phytochemicals from *Areca catechu* nuts at varying temperatures and through microwave irradiation, resulting in monodispersed spherical nanoparticles averaging 13.7 nm in size. Characterization confirmed their crystalline nature, and FTIR identified the biomolecules responsible for their stabilization. The gold nanoparticles demonstrated effective scavenging of harmful radicals (NO and DPPH), cytotoxic effects on HeLa cell lines, and enhanced antibacterial activity against various bacterial pathogens [18]. In another study, El-Borady et al, reported the biosynthesized gold nanoparticles (AuNPs) using *Presley* leaf extract, resulting in various shapes, including spherical and semi-rod forms. Characterization confirmed their formation, with AuNPs exhibiting the highest antioxidant activity among the four formulations. The AuNPs demonstrated significant antimicrobial activity

against two gram-negative bacteria but were ineffective against gram-positive strains. Notably, AuNPs showed the best anticancer activity against human colorectal cancer cells in MTT assays. These findings highlight *Presley* leaf as an effective and economical source for multifunctional AuNPs [19].

## MATERIALS AND METHODS

### *Materials*

Sodium hydroxide (NaOH) and Gold (III) chloride trihydrate were sourced from Sigma, while methanol was also acquired from the same supplier. Double-distilled water was used throughout all experimental procedures. The MCF-7 cell line was purchased from the Pasteur Institute of Iran. For antibacterial testing, Mueller-Hinton broth and Mueller-Hinton agar were provided by Merck, Germany. The bacterial strains used for the antibacterial assessment included *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus mitis*, and *Enterococcus faecalis*, all of which were obtained from the Pasteur Institute of Iran.

### *Extraction Process of Yapa tangerine tree leaf*

The *Yapa tangerine* tree leaf extract, sourced from the forests of Mazandaran in northern Iran, underwent a cleaning process to remove any dust or debris before being dried at room temperature. After drying, the plant material was finely ground using a grinder. To obtain the methanolic extract, 250 gr of the powdered plant were soaked in 300 ml of methanol and continuously shaken for 72 h. The methanol solvent was subsequently evaporated using a rotary evaporator, yielding a reddish-colored extract from the *Yapa tangerine* tree leaf.

### *Eco-friendly synthesis of gold nanoparticles using Yapa tangerine tree leaf extract*

In recent years, the focus on sustainable and low-cost methods for synthesizing nanoparticles has become increasingly crucial. This study aimed to synthesize gold nanoparticles using *Yapa tangerine* tree leaf extract based on the procedure outlined by Ebrahimzadeh and colleagues [20], with slight modifications. Initially, 39 mg of gold salt was dissolved in 5 mL of deionized water (20 mM) under continuous stirring. Subsequently, 10 mL of *Yapa tangerine* tree leaf extract was added to the gold solution. The color of the solution shifted from yellow to red brown, indicating the successful synthesis of gold nanoparticles. After 2 h, the nanoparticles were isolated by centrifugation at 12,000 rpm for 1 h and washed three times using double-distilled water.

### *Antibacterial activity*

The antibacterial properties of gold nanoparticles and *Yapa tangerine* tree leaf extract were evaluated against six strains of Gram-positive and Gram-negative bacteria through the broth microdilution technique. The strains included *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus mitis*, and *Enterococcus faecalis*. To conduct the experiment, varying concentrations of nanoparticles and plant extract were introduced into individual wells. Subsequently, 100 µL of the bacterial suspensions at a concentration of 0.5 McFarland standards were added to each well. The plates were then agitated for 1 min on a shaker to homogenize the mixtures. After mixing, the plates were incubated at 37°C for 24 h. The lowest concentration at which no visible turbidity, and hence no bacterial growth, was detected was determined as the minimum inhibitory concentration (MIC).

### *Cytotoxicity of synthesized gold nanoparticles using Yapa tangerine tree leaf extract*

The anticancer potential of gold nanoparticles synthesized with *Yapa tangerine* tree leaf extract was investigated against the MCF-7 cell line using the MTT assay. The procedure was based on the methodology reported by Kiani et al. [21] MCF-7 cells were initially cultured in DMEM medium, supplemented with 1% penicillin-streptomycin and 10% FBS. The cells were incubated under controlled conditions in flasks. After incubation, trypsin was used to detach the cells, which were then collected through centrifugation. The cells were incubated for 24 h. Afterward, the medium was removed, and various concentrations of the gold nanoparticles were introduced to the cells. In the next step, the culture medium was replaced with fresh MTT solution at a concentration of 0.5 mg/mL. After 4 h of incubation, the medium was discarded, and 100 µL of DMSO was added to each well. After 15 min, absorbance was measured at a wavelength of 570 nm. Cell survival at each concentration was determined by comparing the absorbance values between the treated and control groups.

## RESULTS AND DISCUSSION

### *UV-VIS Spectroscopy*

One of the initial indicators for the successful synthesis of gold nanoparticles is the presence of a characteristic absorption peak around 520 nm, observed via UV-Vis spectroscopy. To monitor the production of gold nanoparticles facilitated by *Yapa tangerine* tree leaf extract, UV-Vis spectra were examined over a range from 400 to 800 nm. Gold nanoparticles are known for their optical property of surface plasmon resonance (SPR), which is visually identified by the darkening of the solution to a red brown color, and its intensity can be measured using UV-Vis spectroscopy. Previous research has established that a more pronounced absorption peak is indicative of a higher yield of gold nanoparticles [22]. As shown in Fig. 1, the gold nanoparticles formed exhibit a strong peak at 528 nm, which confirms the reduction of gold ions ($Au^{3+}$) to elemental gold ($Au^{0}$), validating the

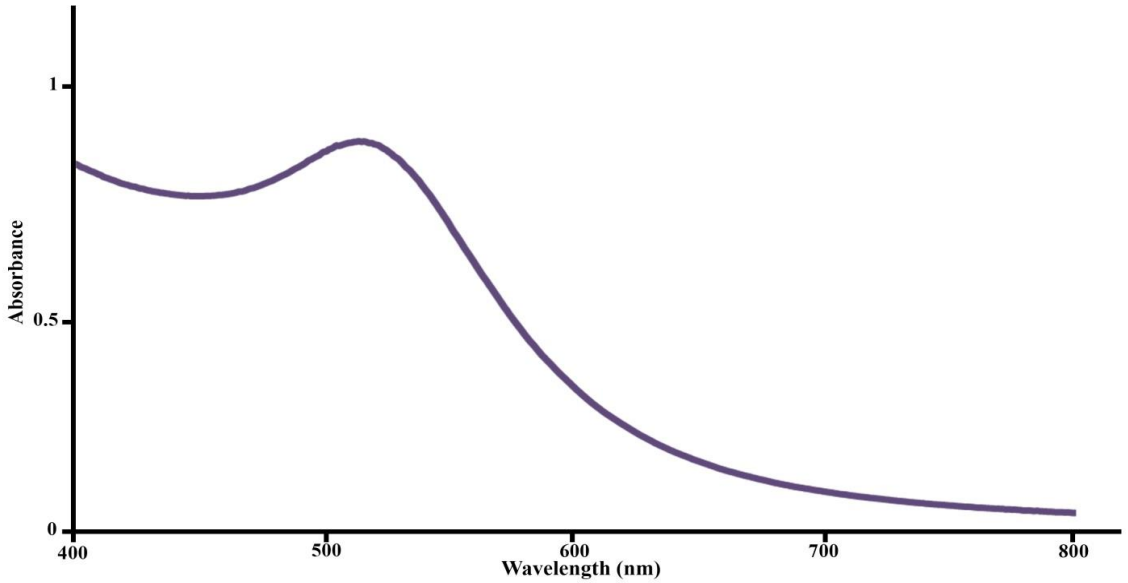


Fig. 1. UV–Vis spectra of gold nanoparticles synthesized using *Yapa tangerine* tree leaf extract.

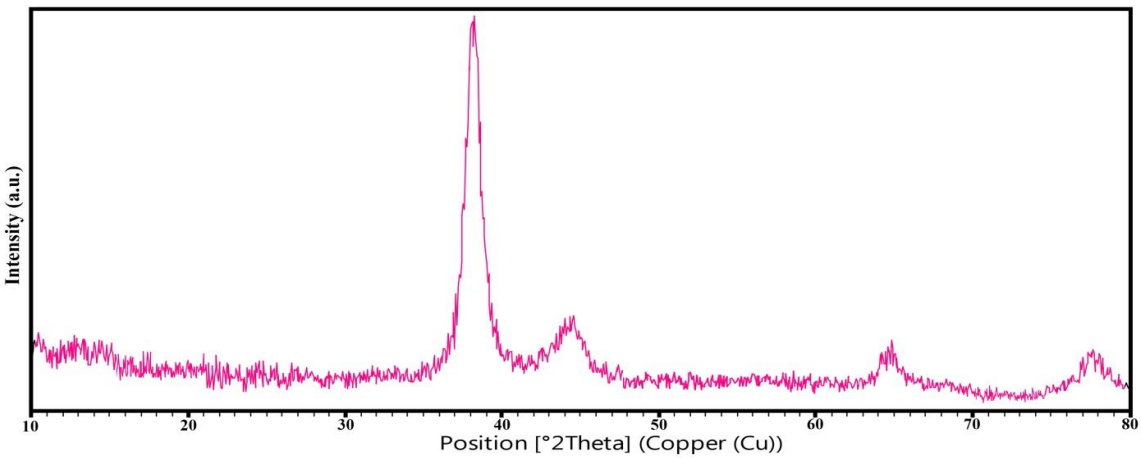


Fig. 2. XRD pattern of gold nanoparticles synthesized using *Yapa tangerine* tree leaf extract.

nanoparticle formation process.

*XRD characterization*

The crystalline structure and purity of the gold nanoparticles synthesized with *Yapa tangerine* tree leaf extract were assessed using X-ray diffraction (XRD). The results, as depicted in Fig. 1, display four dominant peaks at diffraction angles of 38.23°, 44.56°, 64.12°, and 77.26°, which correspond to the (111), (200), (220), and (311) crystal planes, respectively· These results align with the standard reference JCPDS card No· 00-004-0784. The analysis confirms that the gold nanoparticles were successfully synthesized without any detectable impurities. Al-Radadi et al. also reported [23] similar XRD patterns for gold nanoparticles synthesized with *Passiflora ligularis* extract, showing identical peak positions. Furthermore, using the Debye-Scherrer equation, the particle size was estimated to be around 29.3 nm for the synthesized nanoparticles.

*FT-IR spectroscopy*

*Yapa tangerine* tree leaf extract was employed in this research to synthesize gold nanoparticles, making it important to identify the functional groups in the plant extract responsible for nanoparticle formation through FT-IR spectroscopy. Fig. 3 presents the FT-IR spectra of the *Yapa tangerine* tree leaf extract and the gold nanoparticles synthesized using the extract. The *Yapa tangerine* tree leaf extract spectrum reveals six notable peaks at 3427, 2935, 1711, 1279, 1056, and 827.13 $cm^{-1}$, which are associated with stretching vibrations of the hydroxyl bond, stretching of the C-H group, stretching or bending of the C=O group, C-O stretching of the ester group, C=O stretching of the alcoholic group, and C-H bending of the alkynes group [24]. Upon examining the FT-IR spectrum of the synthesized gold nanoparticles, it is evident that the peaks are consistent with those of the extract, with variations only in peak intensity. This is likely due to the interaction of the extract with the surface of the nanoparticles. These functional groups play a crucial role in reducing gold ions to form gold nanoparticles. Isa et al. similarly found that the FT-IR peaks of *Artocarpus odoratissimus* peel extract were similar to those of the gold nanoparticles, with only slight shifts in peak intensity and wavelength [25].

*TEM images*

The structural and morphological characteristics of gold nanoparticles synthesized with *Yapa tangerine* tree leaf extract are closely tied to their physical and chemical attributes. Transmission Electron Microscopy (TEM) serves as an essential tool for analyzing the structure and morphology of synthesized materials. Fig. 4 presents TEM images of gold nanoparticles produced using *Yapa tangerine* tree leaf extract. It is evident that these

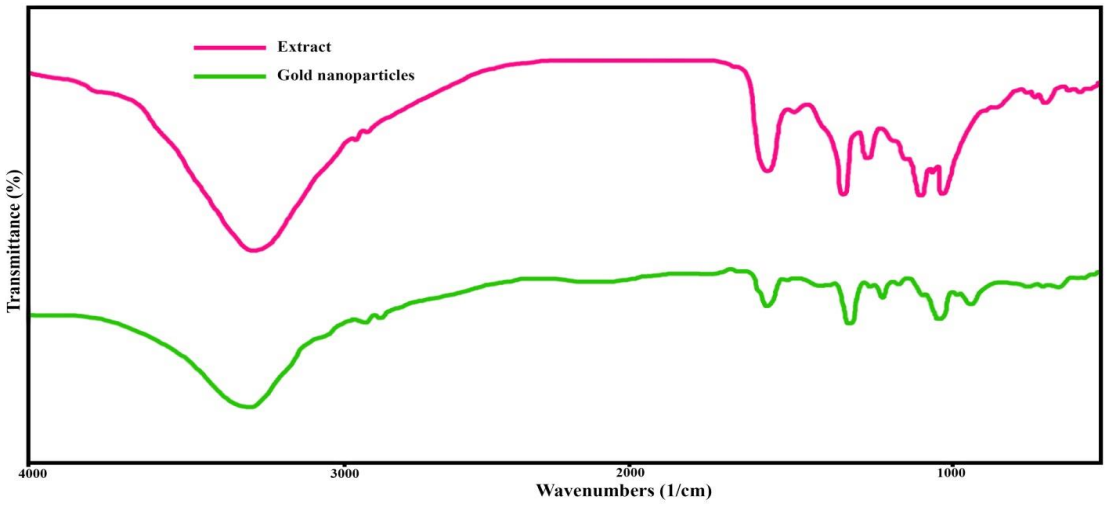


Fig. 3. FT-IR spectra of *Yapa tangerine* tree leaf extract and gold nanoparticles synthesized using natural extract.

nanoparticles are spherical in shape and evenly dispersed throughout the plant extract. The plant extracts appear as a grayish coating covering the nanoparticle surfaces. The synthesized nanoparticles showed exceptional uniformity and homogeneity. A closer inspection revealed that their sizes ranged between 25-35 nm.

*Antibacterial activity*

The present study examines the antibacterial efficacy of *Yapa tangerine* tree leaf extract and gold nanoparticles against selected gram-positive and gram-negative bacterial strains. The bacterial strains employed in this investigation include *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus mitis*, and *Enterococcus faecalis*. Table 1 summarizes the findings regarding antibacterial activity. The results clearly indicate that the *Yapa tangerine* tree leaf extract exhibited low antibacterial activity against all tested bacterial strains. Conversely, gold nanoparticles

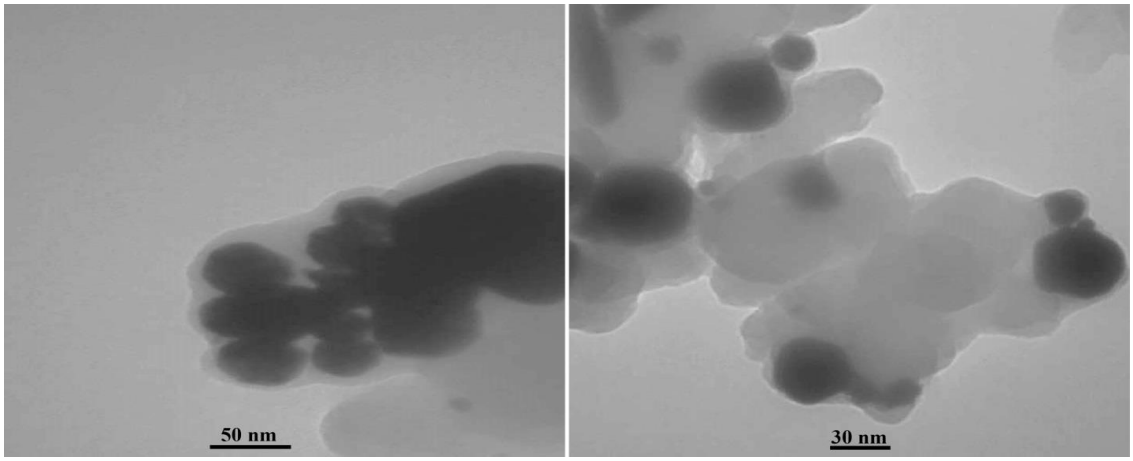


Fig. 4. TEM images of gold nanoparticles synthesized using *Yapa tangerine* tree leaf extract.

Table 1. Antibacterial properties of *Yapa tangerine* tree leaf extract and green synthesized gold nanoparticles against Gram-positive and Gram-negative bacteria.

| Strain | Extract | | Gold nanoparticles | |
|---|---|---|---|---|
| | MIC (µg/ml) | MBC (µg/ml) | MIC (µg/ml) | MBC (µg/ml) |
| *Escherichia coli* | >4000 | >4000 | 9.37 | 37.5 |
| *Streptococcus mutans* | >4000 | >4000 | 37.5 | 150 |
| *Klebsiella pneumoniae* | >4000 | >4000 | 18.75 | 37.5 |
| *Streptococcus mitis* | >4000 | >4000 | 37.5 | 75 |
| *Enterococcus faecalis* | >4000 | >4000 | 75 | 75 |
| *Staphylococcus aureus* | >4000 | >4000 | 150 | 300 |

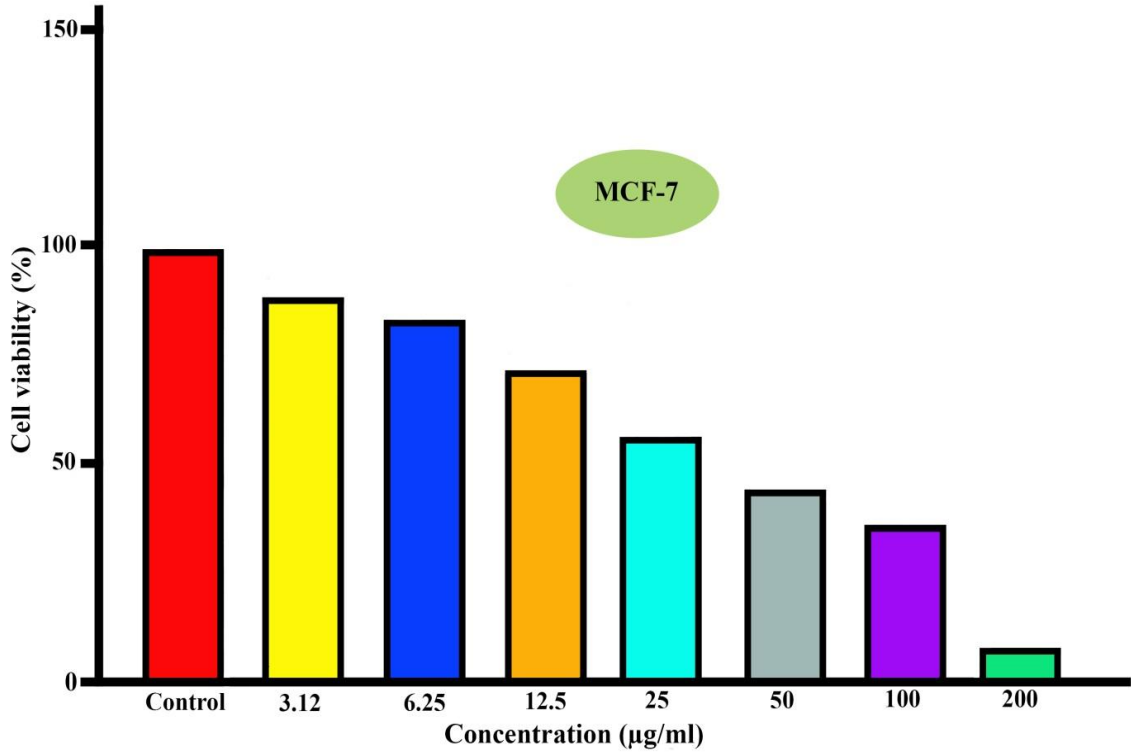


Fig. 5. Cytotoxicity analysis of biosynthesized gold nanoparticles using *Yapa tangerine* tree leaf extract on MCF-7 cancer cell line.

demonstrated substantial antibacterial effects across various bacterial strains. Specifically, the minimum inhibitory concentration (MIC) of gold nanoparticles were 9.37 µg/mL for *Escherichia coli*, 18.75 µg/mL for *Klebsiella pneumoniae*, 150 µg/mL for *Staphylococcus aureus*, 37.5 µg/mL for *Streptococcus mutans*, 37.5 µg/mL for *Streptococcus mitis*, and 75 µg/mL for *Enterococcus faecalis*, respectively. It is noteworthy that the antibacterial efficacy was significantly higher against gram-negative strains than against gram-positive bacteria. This discrepancy may be attributed to the thinner structure of the cell wall in gram-negative bacteria, which facilitates easier penetration. Although several mechanisms have been suggested to elucidate the antibacterial action, the exact mechanism remains unidentified. One proposed mechanism is as follows: Initially, nanoparticles bind to the bacterial cell wall, leading to the release of metal ions. Then, the nanoparticles then infiltrate the bacterial cell. Subsequent damage occurs to the DNA, RNA, and proteins. Finally, this results in the generation of oxidative stress, ultimately causing bacterial cell death.

*Cytotoxic effect on cancer cells*

The anticancer effect of the synthesized gold nanoparticles was tested against the MCF-7 breast cancer cell line by evaluating the viability of cancer cells using the MTT assay. Concentrations of 3.12, 6.25, 12.5, 25, 50, 100, and 200 µg/mL of gold nanoparticles synthesized from *Yapa tangerine* tree leaf extract were selected for testing (Fig. 5). The results showed a dose-dependent decrease in cancer cell viability, with survival dropping from 92.3% at 3.12 µg/mL to 15.6% at 200 µg/mL. The IC50 value of the gold nanoparticles for the MCF-7 cell line was calculated to be 31.4 µg/mL. These results highlight the significant anticancer potential of the synthesized nanoparticles. Several mechanisms have been suggested for this effect, one of which is the interaction of nanoparticles with cancer cells, leading to the release of gold ions. These ions induce oxidative stress by damaging the cells, which subsequently activates pathways responsible for cancer cell death through apoptosis.

## CONCLUSION

This study successfully synthesized gold

nanoparticles using a green, cost-effective method with *Yapa tangerine* tree leaf extract as the reducing and capping agent. The nanoparticles were thoroughly characterized, revealing a uniform size of approximately 25-35 nm and significant optical properties. Biological assessments demonstrated the nanoparticles' potential, showing strong anticancer and antibacterial activities. These findings suggest that the synthesized gold nanoparticles hold considerable promise for future applications in the fields of biomedicine and biotechnology. Further exploration and optimization could expand their utility in various therapeutic and industrial settings.

## ACKNOWLEDGMENT

In this study, all procedures were carried out in compliance with the Helsinki Declaration and the ethical standards set forth by the institutional research committee. The ethical approval code was obtained from Birjand University of Medical Sciences (IR.BUMS.REC.1398.346).

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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