Avens Publishing Group
J Chem Applications
July 2026 Volume:6, Issue:1

Open Access **Research Article**

Journal of
Chemistry & Applications

Sustainable Fabrication of Gold Nanoparticles Using Catunaregam spinosa Fruit Extract for Antioxidant Applications

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Submission: 20 June, 2026
Accepted: 28 July, 2026
Published: 30 July, 2026

Keywords: Gold Nanoparticles; *Catunaregam Spinosa*; Green Synthesis; Antioxidant Activity; DPPH Assay.

Abstract

The green synthesis of gold nanoparticles (AuNPs) has emerged as a promising and sustainable approach in nanotechnology owing to its environmental friendliness, cost-effectiveness, and simplicity. In the present study, a rapid and eco-friendly method was developed for the synthesis of AuNPs using *Catunaregam spinosa* fruit extract as a natural reducing, capping, and stabilizing agent. The biosynthesized AuNPs exhibited an average particle size of 10 ± 2 nm. Comprehensive characterization of the synthesized nanoparticles was carried out using UV–Visible spectroscopy, Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), and transmission electron microscopy (TEM). The formation of AuNPs was confirmed by the characteristic surface plasmon resonance (SPR) absorption band in the UV–Visible spectrum, while XRD analysis revealed their crystalline nature with a face-centered cubic (FCC) structure. TEM analysis demonstrated that the nanoparticles were predominantly spherical and well dispersed. The antioxidant potential of the synthesized AuNPs was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, which demonstrated significant concentration-dependent antioxidant activity. These findings indicate that *Catunaregam spinosa* fruit extract-mediated AuNPs represent an environmentally sustainable nanomaterial with promising potential for biomedical and antioxidant applications.

Introduction

Nanoscience and nanotechnology have emerged as rapidly advancing multidisciplinary fields that integrate principles from chemistry, biology, physics, and engineering to design, synthesize, and manipulate materials at the nanoscale. Owing to their unique physicochemical properties, nanomaterials have attracted considerable attention in both academic research and industrial applications. Among them, metallic nanoparticles (MNPs) have gained particular interest because their optical, electrical, catalytic, and biological properties are strongly dependent on particle size and morphology, distinguishing them from their bulk counterparts [1].

The unique physicochemical properties of MNPs, including their high surface-to-volume ratio, enhanced surface reactivity, and tunable optical characteristics, have enabled their application in diverse fields such as antimicrobial agents, biosensors, catalysis, drug delivery, water purification, and biomedical imaging [2]. Among the various noble metal nanoparticles, gold nanoparticles (AuNPs) have attracted significant attention owing to their excellent biocompatibility, chemical stability, and remarkable optical properties. These characteristics make AuNPs highly suitable for applications in catalysis, sensing, photonics, biomedical diagnostics, targeted drug delivery, imaging, and antimicrobial therapy.

Several methods have been developed for the synthesis of AuNPs, including chemical, physical, and biological approaches. Conventional chemical methods generally involve the reduction of gold salts using reducing agents such as sodium borohydride, hydrazine, citrate, and other synthetic chemicals [3]. Although these methods efficiently produce nanoparticles with well-controlled size and morphology, they often require hazardous reagents, consume substantial energy, and generate toxic by-products, thereby limiting their environmental sustainability and biomedical applicability.

In recent years, green synthesis has emerged as an environmentally benign and sustainable alternative for nanoparticle production. Biological resources such as microorganisms, fungi, algae, and plant extracts have been extensively explored as eco-friendly reducing and stabilizing agents for the synthesis of metal nanoparticles [4]. Among these biological resources, plant-mediated synthesis is particularly advantageous because plant extracts are rich in naturally occurring phytochemicals that simultaneously function as reducing, capping, and stabilizing agents. This eliminates the need for toxic chemicals and provides a simple, rapid, cost-effective, and environmentally friendly route for nanoparticle synthesis.

More recently, biomass-derived materials have attracted considerable interest as renewable resources for sustainable nanotechnology. Biomass materials, including medicinal plants, agricultural residues, natural polymers, and other renewable biological resources, contain abundant bioactive compounds such as polyphenols, flavonoids, alkaloids, proteins, carbohydrates, and organic acids, which facilitate the reduction of metal ions and stabilize the resulting nanoparticles. Compared with conventional chemical synthesis, biomass-assisted approaches offer several advantages, including reduced environmental impact, lower production costs, enhanced biocompatibility, improved nanoparticle stability, and greater sustainability. Consequently, biomass-mediated nanomaterials have found promising applications in catalysis, environmental remediation, sensing, energy storage, antimicrobial therapy, antioxidant activity, and drug delivery [5-9].

Catunaregam spinosa is a medicinal plant widely used in traditional Ayurvedic medicine because of its diverse therapeutic

ISSN: 2380-5021

properties. The fruits of *C. spinosa* are rich in phytochemicals, including flavonoids, alkaloids, phenolic compounds, coumarins, steroids, carbohydrates, and other bioactive constituents. These naturally occurring compounds not only contribute to the medicinal value of the fruit but also serve as effective reducing, capping, and stabilizing agents during the green synthesis of metal nanoparticles [10].

Although numerous plant extracts have been employed for the green synthesis of gold nanoparticles, the use of *Catunaregam spinosa* fruit extract as a renewable biomass source remains largely unexplored. Therefore, the novelty of the present study lies in the rapid and eco-friendly synthesis of stable AuNPs using *Catunaregam spinosa* fruit extract without the use of hazardous chemical reducing agents. In addition, the structural, morphological, catalytic, and antioxidant properties of the synthesized AuNPs were systematically investigated to evaluate their potential as environmentally sustainable nanomaterials for biomedical and environmental applications.

In this study, AuNPs were successfully synthesized using *Catunaregam spinosa* fruit extract through a rapid and environmentally friendly green synthesis approach. The synthesized nanoparticles were comprehensively characterized using UV–Visible spectroscopy, Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), energy-dispersive X-ray spectroscopy (EDX), and transmission electron microscopy (TEM) [11]. Furthermore, the catalytic performance of the synthesized AuNPs was evaluated through the reduction of methylene blue dye, while their antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The results demonstrate that *Catunaregam spinosa* fruit extract-mediated AuNPs are environmentally sustainable nanomaterials with promising potential for catalytic, antioxidant, and future biomedical applications.

Materials and Methods

Materials

Hydrogen tetrachloroaurate trihydrate ($HAuCl_4{\cdot}3H_2O$) was procured from Sigma-Aldrich (India). All other chemicals and reagents used in this study were of analytical grade and purchased from Merck (India). Double-distilled water was used throughout all experiments. Before use, all glassware was thoroughly cleaned with freshly prepared aqua regia, followed by repeated rinsing with double-distilled water to remove any residual contaminants.

Preparation of *Catunaregam spinosa* Fruit Extract (CSFE)

Fresh fruits of *Catunaregam spinosa* were collected from the Mannanur forest region, Mahabubnagar, Telangana, India. The fruits were thoroughly washed several times with double-distilled water to remove dust and other surface impurities. Approximately 6 g of fresh fruit material was crushed and mixed with 60 mL of double-distilled water. The mixture was heated at 64–70 °C for 50 min and then allowed to cool to room temperature. The resulting extract was filtered through Whatman No. 1 filter paper, and the clear filtrate was collected and stored for the subsequent synthesis of gold nanoparticles.

Green Synthesis of Gold Nanoparticles

Gold nanoparticles (AuNPs) were synthesized using a green, plant-mediated approach. Briefly, 5 mL of CSFE was mixed with 4 mL of 1 mM aqueous $HAuCl_4$ solution. The reaction mixture was transferred to an autoclave and maintained at 120 °C under a pressure of 15 psi for 20 min. Upon completion of the reaction, the solution developed a characteristic ruby-red color, indicating the reduction of Au^3 ions to metallic gold (Au^0) and the successful formation of gold nanoparticles [12].

Characterization of Gold Nanoparticles

The optical properties of the synthesized AuNPs were analyzed using a UV–Visible spectrophotometer (UV-3600, Shimadzu, Japan). Fourier transform infrared (FTIR) spectra were recorded using an IRAffinity-1 spectrometer (Shimadzu, Japan) to identify the functional groups responsible for the reduction, capping, and stabilization of the nanoparticles [13-14]. The crystalline structure of the synthesized AuNPs was investigated by X-ray diffraction (XRD) using a Rigaku Miniflex diffractometer equipped with Cu-Kα radiation (λ = 1.5406 Å). The morphology and particle size distribution of the nanoparticles were examined using transmission electron microscopy (TEM; JEM-1200EX, JEOL Ltd., Japan).

Antioxidant Activity

The antioxidant activity of the synthesized AuNPs was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay with slight modifications to a previously reported method. Different volumes of the AuNP suspension (6, 12, 18, 24, 30, and 36 µL) were diluted with dimethyl sulfoxide (DMSO) to a final volume of 60 µL. Subsequently, 2.56 mL of 0.17 mM DPPH solution was added to each sample. The reaction mixtures were vigorously mixed and incubated in the dark at room temperature for 40 min. The absorbance was measured at 517 nm using a UV–Visible spectrophotometer. Ascorbic acid was used as the standard reference antioxidant.

The DPPH assay was performed in triplicate (n = 3), and the results are expressed as mean ± standard deviation (SD).

The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = [(A_0 - A_t)/A_0] \times 100$$

where A_0 represents the absorbance of the control and A_t represents the absorbance of the test sample.

In addition to evaluating antioxidant activity, the synthesized AuNPs were further investigated for their antibacterial and anticancer activities to explore their potential biomedical applications.

Results and Discussion

Characterization of Gold Nanoparticles

The successful synthesis of gold nanoparticles (AuNPs) was initially confirmed by UV–Visible spectroscopy through the appearance of the characteristic surface plasmon resonance (SPR) absorption band. The synthesized AuNPs exhibited a distinct SPR peak in the wavelength range of 520–530 nm, which is characteristic of spherical gold nanoparticles. The influence of CSFE concentration (0.1–0.8%) on nanoparticle synthesis was investigated, and the corresponding UV–Visible spectra are presented in **Figure 1a**. As the concentration of CSFE increased, the intensity of the SPR band gradually increased, indicating enhanced reduction of Au^{3-} ions and increased formation of AuNPs. This observation suggests that the phytochemical constituents present in the fruit extract play a vital

ISSN: 2380-5021

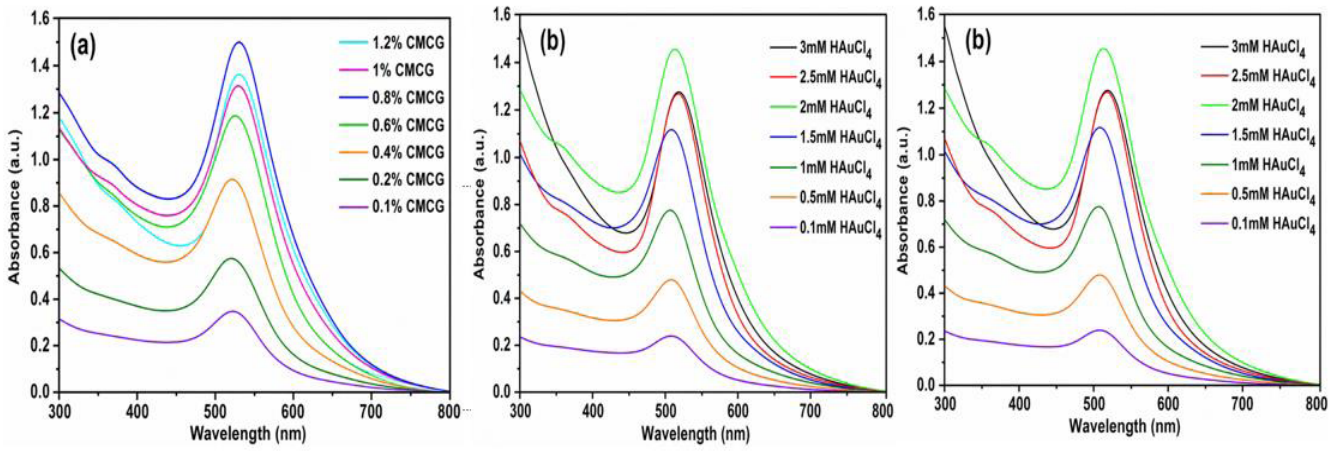

Figure 1: UV–Visible absorption spectra of AuNPs synthesized using (a) different concentrations of CSFE, (b) different concentrations of $HAuCl_4$, and (c) different autoclave reaction times.

role in the reduction and stabilization of gold nanoparticles [15].

The effect of the gold precursor concentration on nanoparticle synthesis was also investigated using different concentrations of $HAuCl_4$, as shown in Figure 1b. An increase in $HAuCl_4$ concentration resulted in a corresponding increase in the intensity of the SPR band, indicating a higher yield of AuNPs under increased precursor concentrations [16]. The influence of autoclave reaction time on nanoparticle formation was evaluated and is presented in Figure 1c. As the reaction time increased from 0.5 to 20 min, the SPR peak intensity progressively increased, reflecting the formation of a greater number of AuNPs. However, extending the reaction time beyond 20 min did not produce any significant changes in either the intensity or the shape of the SPR band, indicating that the reduction reaction had reached completion. These results demonstrate that the concentrations of both the plant extract and the gold precursor, together with the reaction time, play crucial roles in determining the efficiency of AuNP synthesis [17-18].

The Fourier transform infrared (FTIR) spectra of the CSFE and the biosynthesized AuNPs were compared to identify the functional groups involved in the reduction, capping, and stabilization of the nanoparticles (Figure 2a). The observed shifts in peak positions and changes in peak intensities after nanoparticle synthesis confirm the interaction of phytochemical constituents with the surface of the AuNPs.

A broad absorption band in the region of 3400–3300 cm^{-1} was assigned to the stretching vibration of hydroxyl (–OH) groups present in phenolic compounds, flavonoids, tannins, carbohydrates, and alcohols. Following nanoparticle synthesis, this band became broader and shifted slightly toward a lower wavenumber, indicating the involvement of hydroxyl groups in coordinating with the gold nanoparticle surface. These hydroxyl-containing phytochemicals likely donated electrons to reduce Au^{3+} ions while simultaneously stabilizing the synthesized nanoparticles.

The absorption bands observed in the 2920–2850 cm^{-1} region correspond to the asymmetric and symmetric stretching vibrations of aliphatic $–CH_2$ and $–CH_3$ groups present in proteins and other organic constituents. Minor variations in these bands after nanoparticle synthesis suggest that aliphatic functional groups are associated with the nanoparticle surface but play only a limited role in the reduction process.

The absorption band located around **1650–1600 cm^{-1}** is attributed to the stretching vibration of carbonyl (C=O) groups of proteins, polyphenols, and conjugated ketones and may also include amide I vibrations. The shift of this band toward a lower wavenumber following nanoparticle formation indicates the coordination of carbonyl oxygen atoms with the AuNP surface, thereby contributing to nanoparticle stabilization.

A band observed near 1540–1510 cm^{-1} corresponds to amide II vibrations and N–H bending modes. The slight shift and reduction in intensity of this band suggest the participation of amino groups from proteins or amino acids in binding to the nanoparticle surface through nitrogen-containing functional groups.

The absorption bands in the 1450–1380 cm^{-1} region arise from the bending vibrations of methyl groups and the symmetric stretching of carboxylate (COO^-) ions. The observed shifts in these bands indicate the interaction of carboxyl groups from organic acids and proteins with the AuNP surface, thereby enhancing the colloidal stability of the nanoparticles.

The absorption bands observed between 1250 and 1000 cm^{-1} are characteristic of C–O–C and C–O stretching vibrations associated with alcohols, polysaccharides, glycosides, and flavonoids. A reduction in band intensity accompanied by slight peak shifts after nanoparticle synthesis indicates that these oxygen-containing functional groups were adsorbed onto the nanoparticle surface and acted as natural capping agents.

Overall, the FTIR analysis demonstrates that hydroxyl, carbonyl, carboxyl, amide, and C–O functional groups present in the phytochemicals of CSFE actively participated in the reduction of Au^{3-} ions and remained adsorbed on the surface of the synthesized AuNPs as natural reducing, capping, and stabilizing agents.

The crystalline nature of the synthesized AuNPs was investigated by X-ray diffraction (XRD), and the corresponding diffraction pattern is presented in Figure 2b. Distinct diffraction peaks were observed at

ISSN: 2380-5021

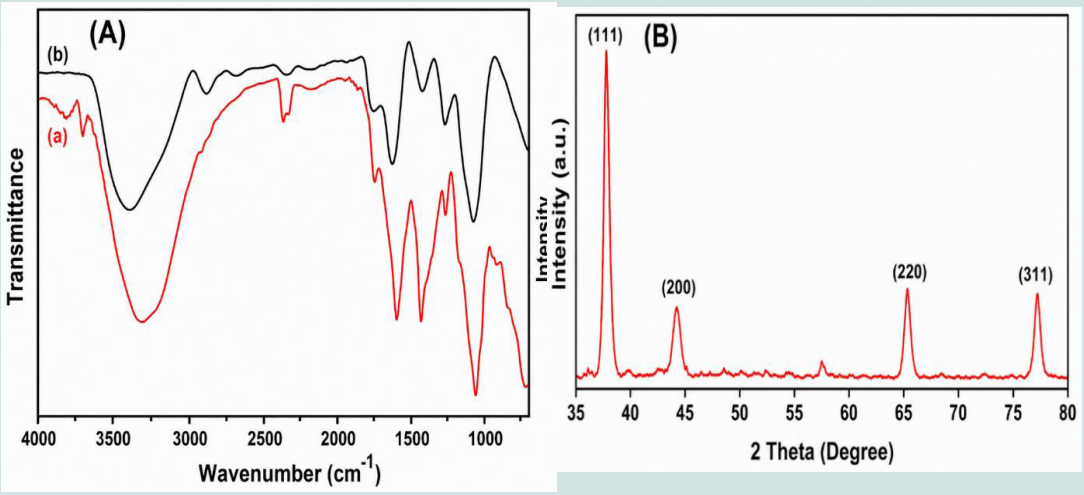

Figure 2: (A) FTIR spectra of (a) CSFE and (b) CSFE-capped gold nanoparticles (AuNPs). (B) X-ray diffraction (XRD) pattern of the synthesized AuNPs.

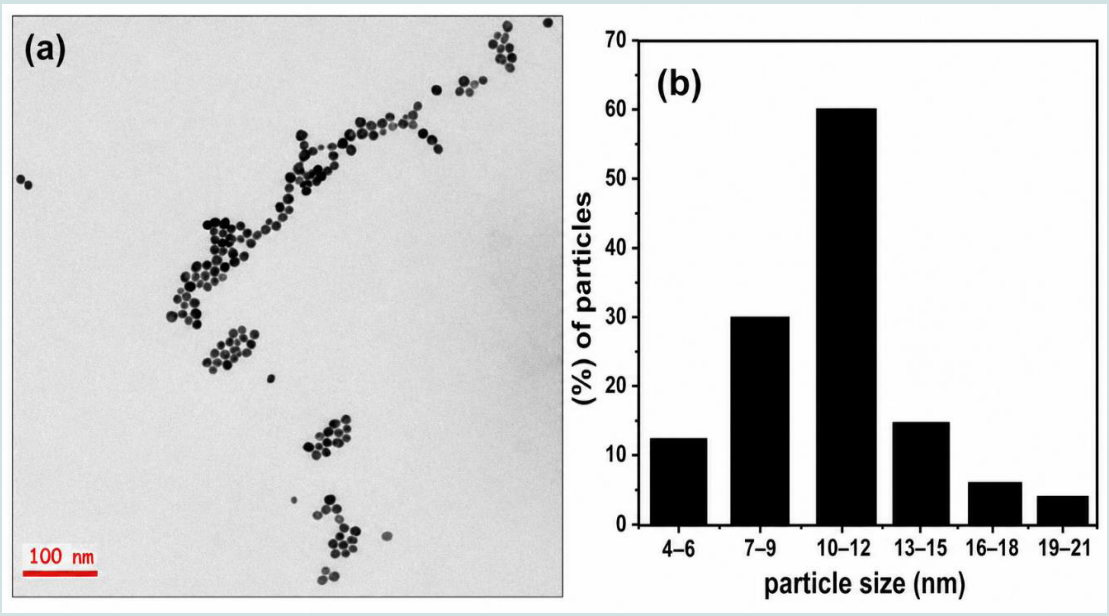

Figure 3: (a) TEM micrograph of the synthesized CSFE AuNPs and (b) particle size distribution histogram of the synthesized AuNPs.

2θ values of 38.15°, 44.50°, 64.51°, and 77.42°, corresponding to the (111), (200), (220), and (311) crystallographic planes, respectively. These diffraction peaks are characteristic of the face-centered cubic (FCC) crystal structure of metallic gold, confirming the successful formation of crystalline AuNPs [19-21].

The average crystallite size of the synthesized AuNPs was estimated using the Debye–Scherrer equation and was found to be approximately 8.5 nm. This value is in good agreement with the particle size obtained from transmission electron microscopy (TEM), indicating the successful synthesis of nanoscale crystalline gold nanoparticles.

The morphology and particle size distribution of the synthesized AuNPs were further investigated using TEM. Representative TEM micrographs (Figure 3a) revealed that the nanoparticles were predominantly spherical, well dispersed, and exhibited minimal aggregation [22–23]. The average particle size was determined to be 10 ± 2 nm.

The particle size distribution histogram obtained from the TEM measurements (Figure 3b) showed a relatively narrow size distribution, indicating the uniform formation of AuNPs. Furthermore, the particle size determined from TEM analysis closely matched the crystallite size estimated from XRD, confirming the successful synthesis of uniformly distributed nanoscale gold nanoparticles through the green synthesis route using CSFE.

Overall, the spectroscopic and microscopic characterization results demonstrate that CSFE serves as an efficient natural reducing, capping, and stabilizing agent for the rapid green synthesis of stable, crystalline, and predominantly spherical gold nanoparticles.

ISSN: 2380-5021

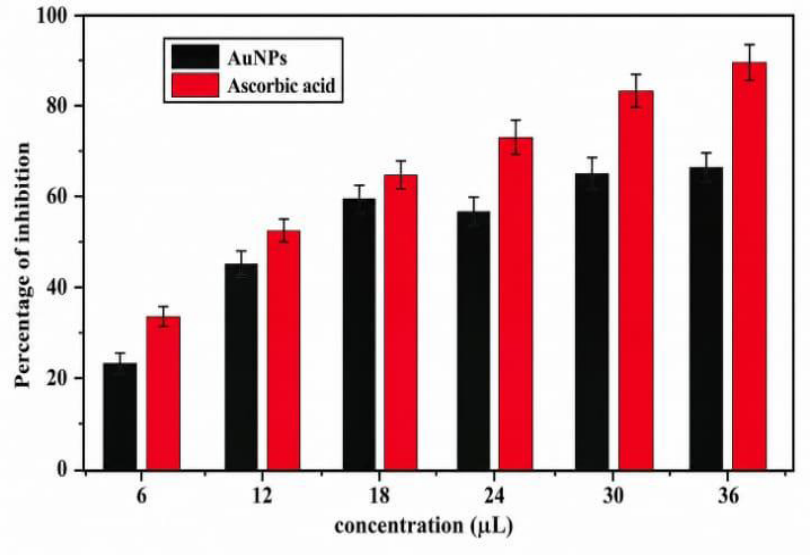

Figure 4: DPPH free radical scavenging activity of biosynthesized CSFE AuNPs at different concentrations compared with ascorbic acid. Data are presented as mean ± SD (n = 3). Error bars represent the standard deviation.

Antioxidant Activity

The antioxidant activity of the biosynthesized AuNPs was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, with ascorbic acid serving as the standard reference antioxidant. DPPH is a stable nitrogen-centered free radical that accepts electrons or hydrogen atoms from antioxidant molecules, resulting in its reduction and a characteristic color change from deep purple to yellow [24-25].

The DPPH assay was performed in triplicate (n = 3), and the results are presented as mean ± standard deviation (SD). Error bars representing the standard deviation are included in Figure 4 to demonstrate the reproducibility and reliability of the experimental results.

As shown in Figure 4, the free radical scavenging activity of the synthesized AuNPs increased with increasing nanoparticle concentration, indicating concentration-dependent antioxidant activity. The percentage inhibition values observed for the AuNPs were 23.2%, 45.6%, 59.1%, 56.3%, 63.1%, and 65.25% at concentrations of 6, 12, 18, 24, 30, and 36 µL, respectively. These results demonstrate that the biosynthesized AuNPs possess significant antioxidant activity, which can be attributed to the phytochemical constituents adsorbed on the nanoparticle surface. The antioxidant performance of the synthesized AuNPs was comparable to that of the standard antioxidant, ascorbic acid, particularly at higher concentrations.

Conclusion

In the present study, gold nanoparticles (AuNPs) were successfully synthesized through a simple, rapid, and environmentally friendly green synthesis approach using *Catunaregam spinosa* fruit extract. The phytochemicals present in the extract acted as natural reducing, capping, and stabilizing agents, enabling the formation of stable AuNPs without the use of hazardous chemicals. Comprehensive characterization by UV–Visible spectroscopy, FTIR, XRD, and TEM confirmed the successful synthesis of crystalline, predominantly spherical AuNPs with an average particle size of **10 ± 2 nm**. The synthesized nanoparticles exhibited significant concentration-dependent antioxidant activity, demonstrating their potential as effective free radical scavengers. Overall, the findings of this study highlight the potential of *Catunaregam spinosa*-mediated AuNPs as environmentally sustainable nanomaterials with promising applications in catalysis, antioxidant therapy, and other biomedical fields. Further investigations on their antibacterial, anticancer, and in vivo biological activities are warranted to fully explore their therapeutic potential.

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