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(Research Article)

Plant assisted synthesis of gold nanoparticles from leaf aqueous source of *Glinus oppositifolius*; characterization, and evaluation of antibacterial and DPPH activities efficacy

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Abstract

Synthesizing metal nanoparticles through Green methods has become an essential alternative to conventional methods. In the present study, gold nanoparticles (AuNPs) were synthesized using the aqueous leaf extract of *Glinus oppositifolius* as a reducing and stabilizing agent. The formation of AuNPs was confirmed by a characteristic surface plasmon resonance peak at 533 nm using UV–Visible spectroscopy. Particle size analysis by dynamic light scattering (DLS) revealed an average size of 99.4 nm, while the zeta potential value of −31.2 mV indicated good stability of the synthesized nanoparticles. The biological activities of the synthesized AuNPs were evaluated for antibacterial and antioxidant potential. Antibacterial activity was assessed against *Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis,* and *Enterococcus faecalis* using the agar well diffusion method. Among the tested strains, the highest zone of inhibition was observed against Bacillus subtilis (20.5 mm), indicating strong antibacterial efficacy. Additionally, the antioxidant activity was determined using the DPPH radical scavenging assay, where the AuNPs exhibited significant activity with 83.44% inhibition at 100 µL concentration. By the results, this study demonstrates that Glinus oppositifolius-mediated synthesis of gold nanoparticles is a simple and sustainable approach, yielding stable nanoparticles with notable antibacterial and antioxidant properties, suggesting their potential applications in biomedical and pharmaceutical fields.

Keywords: Gold nanoparticles; *Glinus oppositifolius*; Antibacterial; Antioxidant activity

1. Introduction

The nanotechnology sector has proven to be one of the most active research fields (Sakhare et al., 2021). Nanotechnology has recently come to light as a promising tactic that can address issues with the current types of therapies (Haleem et al., 2023 and Kush et al., 2021). Various types of metal nanoparticles have already been synthesized using physical, chemical, electrochemical reduction, photochemical reduction, heat evaporation, and biological approaches. Traditionally, the nanoparticles have been developed by physical and chemical approaches (Shah et al., 2015); however, these routes are laborious and potentially dangerous to the environment and human health (Kumar et al., 2008). Physical method is hazardous, capital intensive and is inefficient in materials and energy use (Granma Yeh Rad et al., 2011) Chemical method involves use of toxic and harmful chemicals that get adsorbed on the surface and are accountable for various biological hazards (Kundu et al., 2020).

Amongst all these methods plant mediated synthesis of GNPs as gaining more importance owing to (a) easy synthesis (b) rapid rate of synthesis (c) biocompatibility (d) non-hazardous (e) higher potential for reduction (f) water is used as

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solvent (g) zero contamination (h) does not need special conditions like temperature, pressure, hazardous chemicals and (i) functions as a reducing and capping agent at the same time (Bordoloi et al., 2020). As a result, the study of gold nanoparticles synthesis utilizing plant extract has generated a lot of interest. GNPs derived from plant extract are employed in biomedicine for a variety of applications, including leukemia therapy, biomolecular immobilization and biosensor creation. GNPs have also been used as anti-angiogenesis, anti-malaria and anti-arthritic medicines. In contrast, the biological approach for synthesizing various nanoparticles is safer, eco-friendly, and cost-effective (Ramanathan et al., 2018; Sumitha et al., 2019; Wang et al., 2018). Many biological sources including plants, bacteria, fungi, and algae were used to synthesize of nanoparticles. Among the biological approaches, plant-mediated synthesis of nanoparticles, including gold nanoparticles (AuNPs), has greater advantages than microbial approaches because of its high reaction rate, reduced cost, and large-scale production (Park et al., 2011).

The most commonly used metals to synthesize NPs are platinum, palladium, silver, and gold (Asiya et al., 2020). Among these common metals, gold is possessing a high ionic conductivity, and synthesized gold nanoparticles (AuNPs) can be adjusted based on their surface state, shape, and size (Raji et al., 2020; AL Jabali et al., 2021; Ahmed et al., 2020; Asiya et al., 2020; Bawazeer AL Jabali et al., 2022; Ahmad et al., 2017; Huang et al., 2007; Kasthuri et al., 2007). Gold is a noble metal that has long been associated with power and wealth. Egyptians employed gold metal solubilized water for mental and spiritual purification in the 18th century.

Gold was used to treat ailments like smallpox, skin ulcers, syphilis, and measles in many ancient societies (Egypt, India, and China). Gold Nanoparticles (GNPs) are one of the prominent metal nanoparticles with a wide variety of applications in various fields of science and technology. In recent past years different types of nanoparticles are synthesized successfully by the greener approach using plant materials like Indium oxide nanoparticles from *Aloe vera* (Maen Siria et al., 2008), Iron oxide nanoparticles from *Medicago sativa* (Herrera-Becerra et al., 2008], Palladium nanoparticles from *Cinnamomum camphor* (Yang et al., 2009), Copper nanoparticles from *Magnolia kobus* (Lee et al., 2013), Cadmium Oxide Nanoparticles from *Achillea Wilhelmi* (Karimi Andeani and Mohsen Zadeh, 2013). Calcium nanoparticles from *Boswellia ovalifoliolata* (Yugandhar and Savithramma 2013), and gold nanoparticles from Stem Bark of *Walsura trifoliata* (A. Juss) Harms Characterization and Validation (Venkata Subbaiah Kedam and Savithramma Nataru, 2022).

The *Glinus oppositifoliu*s, the local name is Sadaodin, is included in the family of Solanaceae. This plant is widely spread throughout all regions of Thailand. The shoot or whole plant of *Glinus oppositifolius* is eaten as a vegetable. It is a small herb, widely used in the traditional medicine of Bangladesh in treatment of a variety of diseases and disorders such as insomnia, pain, inflammation, jaundice, and fever locally called as "Gima Shak." This plant is extensively used by the local people as a vegetable and the traditional healers as an essential ingredient. In treatment of a wide range of diseases and disorders including pain, inflammation, jaundice, diarrhea, boils, and malaria [Hoque et al., 2011]. This herb is also useful for its diuretic, CNS depressant, anthelmintic, and antiviral properties in the folk medicine of this country [Pattana yak et al., 2012]. In recent years, several studies have been conducted to identify the phytochemical constituents of this plant and a number of phytochemicals including L-(−) -(N-trans-cinnamoyl)-arginine, kaempferol 3-O-galactopyranoside, isorhamnetin 3-O-β-D-Lopiano sly-(1→2)-β-D-glucopyranoside, and-phenylalanyl (Sahakitpichan et al., 2010). In this study *Glinus oppositifolius* aqueous extract from leaves was used as stabilize material in biological way for synthesis of GNPs.

2. Material and Methods

2.1. Chemicals

Gold salt ($HAuCl4 .3H2 O$, 99%), 1,1-diphenyl-2- picrylhydrazyl (DPPH), ascorbic acid (Vitamin C), and DMSO (molecular biology grade were obtained from Sigma Aldrich, India. Sodium hydroxide and hydrochloric acid were prepared from Merck, Germany. All chemicals used in this study were prepared with the highest purity.

2.2. Collection of plant material and extraction

The matured eaves of *Glinus oppositifolius* collected at Japalitheertham, Tirumala; Andhra Pradesh. The leaves transferred in to sterile polythene cover, then brought in to the laboratory and washed with tap water for three times followed by sterile distilled water. The material was cut into pieces with the help of sterile scissor and dry up its moisture content with papers and shade dried for 14 days at sterile conditions. Then this ground fine powder, afterwards 5grams of powder was taken into the 250ml of sterile conical flask; added 100mL of sterile distilled water, and kept on water both for 20min. at 1000C, then this filtered through Whatman No. 1 sterile filter paper. The extraction was kept at 50C until the synthesis.

2.3. Preparation of $HAuCl_4$ solution

Gold salt ($HAuCl_4 .3H_2O$ 99%), acquired from Sigma Aldrich, India. 1mM of $HAucl_4.3H_2O$ solution was prepared by sterile double distilled water.

2.4. Biosynthesis of Gold nanoparticles (AuNPs)

The procedure established by Banerjee et al. (2014) successfully outlines the synthesis of gold nanoparticles (AuNPs). In this process, 10 ml of leaf extract is incorporated into a 250 ml sterile conical flask. Following this, 100 ml of a 1mM tetra chloroauric acid ($HAuCl_4·3H_2O$) solution is added. The mixture is stirred at a controlled temperature ranging from 60 to 80°C. The transformation of the solution's color from brown to purple is a clear and reliable indication of the successful synthesis of gold nanoparticles.

After the initial incubation period of 24 hours, which allowed for the bio-reduction of gold chloride ($HAuCl_4$) to gold nanoparticles (AuNPs) using the aqueous leaf extract, the mixture was then subjected to centrifugation at 15,000 rpm for 20 minutes. This step was crucial for the removal of any biological admixtures present in the reaction mixture. Following centrifugation, the resulting supernatant was utilized for characterization and validation of the synthesized AuNPs. This was incubated for 24 hours for bio- reduction of gold chloride ($HAuCl_4$) to gold nanoparticles (AuNPs) from leaf extract, then it was centrifuged at 15000 rpm for 20 min. to removal of presence biological admixture in the reaction mixture, and then it was used characterization and validation.

2.5. Characterization of AuNPs

The UV-visible absorption spectrum of the synthesized gold nanoparticles (AuNPs) was confidently measured using a Nano Drop 800 nm Spectrophotometer. To accurately assess the size and size distribution of the particles in the aqueous AuNPs solution, we conducted a thorough Dynamic Light Scattering (DLS) analysis with a Malvern Zeta analyzer, ensuring precise characterization of our samples.

2.6. Anti-bacterial studies of AuNPs

The biosynthesized nanoparticles were thoroughly investigated for their antimicrobial prowess against a range of pathogens, specifically two Gram-negative bacteria, *Escherichia coli* and *Klebsiella pneumoniae*, alongside two Gram-positive bacteria, *Bacillus subtilis* and *Enterococcus faecalis*Employing the disc diffusion assay method, as meticulously described by Cruickshank (1986), we assessed the antimicrobial capabilities of the gold nanoparticles (AuNPs) with precision and rigor.

For the experiment, we crafted 7 mm sterile discs from high-quality Whatman no. 1 filter paper. Each disc was judiciously loaded with 20 µl of the plant extract, 1 mM tetra chloroauric acid ($HAuCl_4$) solution, and 10 µg/disc of streptomycin, ensuring that each one was evenly saturated. After allowing them to dry for a full 60 minutes in a sterile environment to maintain purity, we introduced freshly prepared nutrient agar media into sterile Petri plates, letting it solidify for 30 minutes into a firm, even consistency. Next, we carefully swabbed 60 µl of distinct bacterial cultures onto the surface of the plates, ensuring even distribution, before placing the meticulously prepared discs atop the agar.

These plates were then incubated at a precise temperature of 37°C for a 24-hour period, creating ideal conditions for bacterial growth. After incubation, we carefully measured the zones of inhibition using a precise scale, meticulously recording each result for thorough analysis. Significantly, our findings indicated that the nanoparticles not only exhibited compelling antimicrobial activity but also enhanced the expression of genes associated with redox processes, effectively leading to the eradication of both fungal and bacterial cells (Nagy et al., 2011). This comprehensive study underscores the potential of biosynthesized gold nanoparticles as formidable agents in combating microbial pathogens.

2.7. Anti-oxidant activity (DPPH)

DPPH stock solution was prepared by dissolving 10mg in 100mL of Methanol, which yielded a solution mixture with an absorbance of around 1.305 at 517nm in the test tubes. 3mL DPPH workable solutions (1 mL of DPPH stock solution+2mL of Methanol) were combined with 100µL of leaf extract. As a standard, 3mL of DPPH workable solution often mixed with 100 µL of Methanol. After 30 min incubation in complete darkness, the absorbance was therefore determined at 517nm. The following formula was used to compute the percentage of antioxidants (Baliyan et al., 2022).

Percentage of antioxidants =[(AC-AS+AC]X100where Ac- Controle reaction absorbance; As- Testing specimen absorbance

3. Results and Discussion

3.1. UV-Visible Spectroscopy studies

The UV-visible absorption spectrum of the synthesized gold nanoparticles (AuNPs) was effectively analyzed using a Nano Drop 800 nm Spectrophotometer. The transition from brown to purple color unmistakably confirms the successful synthesis of AuNPs. This study serves as a robust confirmatory tool for detecting the surface Plasmon resonance (SPR) property and indicating of AuNPs. Remarkably, an absorbance peak was observed at 533 nm when analyzing the Glines oppositionist leaf extract, further validating the efficacy of this method. Similar types of results were observed in gold nanoparticles via tangerine peel extract (Seyedeh Masoumeh Ghoreishi and Sobhan Mortazavi-Dera kola, 2025).

3.2. Dynamic Light Scattering (DLS) Zeta potential studies

The size and distribution of gold nanoparticles (AuNPs) in aqueous solution were analyzed using Dynamic Light Scattering (DLS) with a Malvern Zeta-analyzer. This technique measures the scattering of light due to the Brownian movement of nanoparticles, producing intensity fluctuations that allow for particle size estimation. In the present study, the synthesized AuNPs exhibited an average size of 99.4 nm (Fig. 1b) and a zeta potential value of -31.2 mV (Fig. 1c). These results indicate that the nanoparticles were well-dispersed and stable, demonstrating a polydispersity distribution in the colloidal solution. These findings indicate that the selected medicinal plant and the employed protocol are highly effective for the eco-friendly synthesis of AuNPs. The observed negative zeta potential value may be attributed to the capping effect of biomolecules present in the leaf extract of Glines oppositionist, which enhances the stability and dispersion of the nanoparticles.

4. Applications of Bio-synthesized AUNPS

4.1. Antibacterial studies of AuNPs

The antibacterial activity of green-synthesized gold nanoparticles (AuNPs) was evaluated using two Gram-negative and two Gram-positive bacterial strains cultured on nutrient agar medium. The test organisms included Escherichia coli and Klebsiella pneumoniae (Gram-negative bacteria), as well as Bacillus subtilis and Enterococcus (Gram-positive bacteria). Zones of inhibition produced by each extract were compared to those produced by the standard antibiotic streptomycin, while the HAuCl4 solution served as a negative control. The AuNPs synthesized using Glines oppositionist exhibited significant inhibition zones against all tested bacterial strains, indicating broad-spectrum antibacterial efficacy (Figure 2 and Table 1). These kinds of results were observed in the earlier study of AuNPs synthesized from bark aqueous extract of Walsura trifoliata (Venkata Subbaiah Kedam and Savithramma Natarus, 2022).

4.2. Anti-oxidant activity (DPPH)

The antioxidant potential of the synthesized AuNPs was assessed using the DPPH assay. DPPH is a stable free radical commonly used to evaluate antioxidant activity, as it can accept hydrogen atoms or electrons from antioxidant substances (Kumar R and Shikha D, 2025). Upon interaction with an antioxidant, DPPH undergoes a color change from violet to yellow, indicating its reduction to a stable diamagnetic molecule (Kumar, R. et al., 2025). In this study, the radical-scavenging activity of AuNPs against DPPH increased in a concentration-dependent manner, reaching its maximum at higher concentrations (Figure 3 and Table 2). Similar type of results was observed in leaf sourced AuNPs prepared by Eruca sativa (Hazar, A.M et al., 2025).

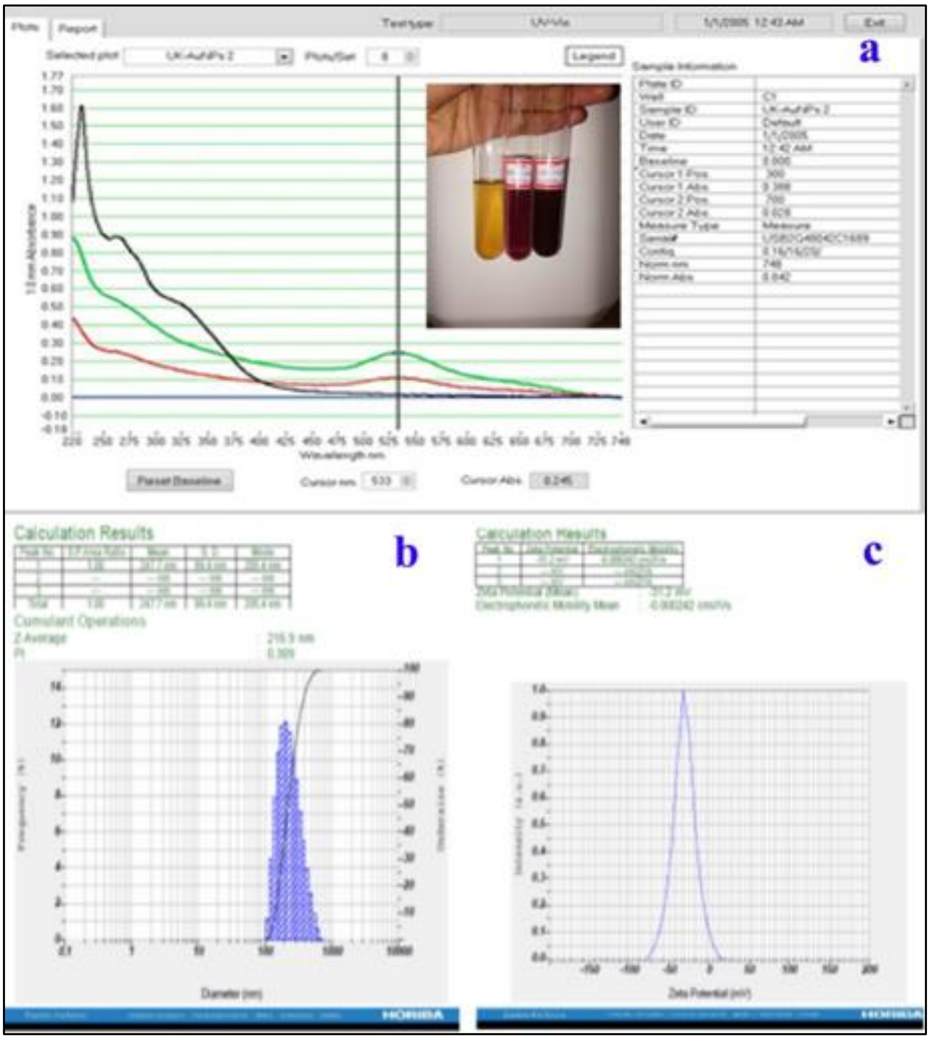

Figure 1 a) UV-graph, b), and c). DLS particle size of synthesis of AuNPs from leaves

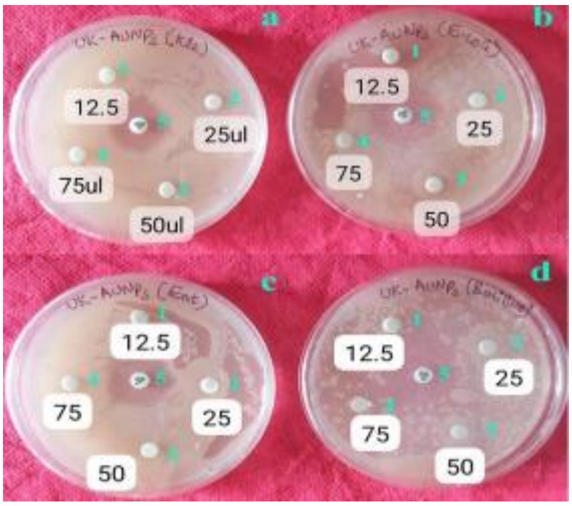

Figure 2 Antibacterial activity of AuNPs from leaf of Glines oppositionist on selected four bacteria against standard drug streptomycin

a). Klebsiella pneumoniae b). Escherichia coli c). Enterococcus d). Bacillus subtilis; 1). Au solution 2). Plant extract 25 µL 3). AuNPs 50 µL 4). AuNPs 75 µL 5). Streptomycin

Table 1 Effect of Synthesized Glines oppositionist leaf sourced AuNPs on selected Bacterial Species. ± SE of Three samples

Name of the Organism	**Zone of Inhibition**				
	Plant extract	$HAuCl_4$ solution	AuNPS (25µl)	AuNPS (75µl)	Streptomycin
B. subtilis	10.9±0.44	8.1±0.094	9.8±0.094	20.5±0.169	21.4±0.216
Enterococcus	8.1±0.094	10.1±0.165	8.9 ±0.103	16.4±0.216	22.3±0.225
K. pneumonia	11.9±0.047	9.2±0.124	9.3±0.141	17.1±0.047	28.9±0.291
E. coli	10.2±0.169	11.3±0.141	9.5±0.144	14.3±0.163	28.5±0.287

Antibacterial activity of AuNPs from leaf material of Glines oppositionist

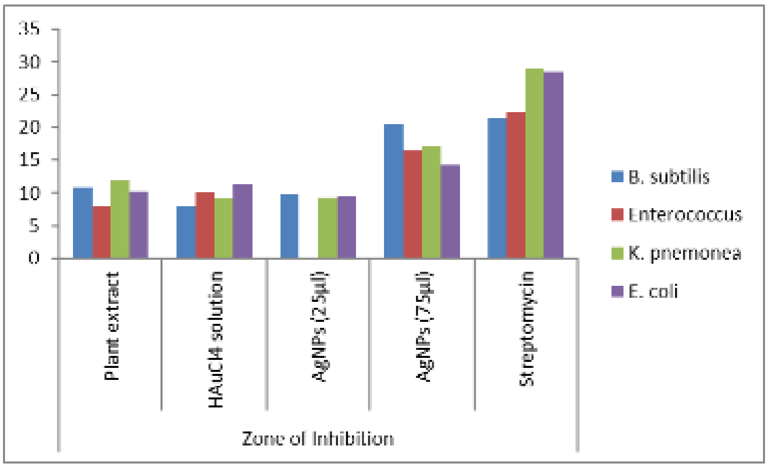

Figure 3 Antimicrobial activity of AuNPs from Glines oppositionist

Table 2 DPPH anti-oxidants activity of AuNPs from Glines oppositionist

Samples	**25µl**	**% activity**	**50 µl**	**% activity**	**100 µl**	**% activity**
Extract	1.157±0.002	11.34±0.008	0.756±0.003	42.07±0.019	0.378±0.003	71.03±0.021
AuNPs	1.083±0.012	17.01±0.031	0.866±0.017	33.63±0.006	0.216±0.023	83.44±0.035

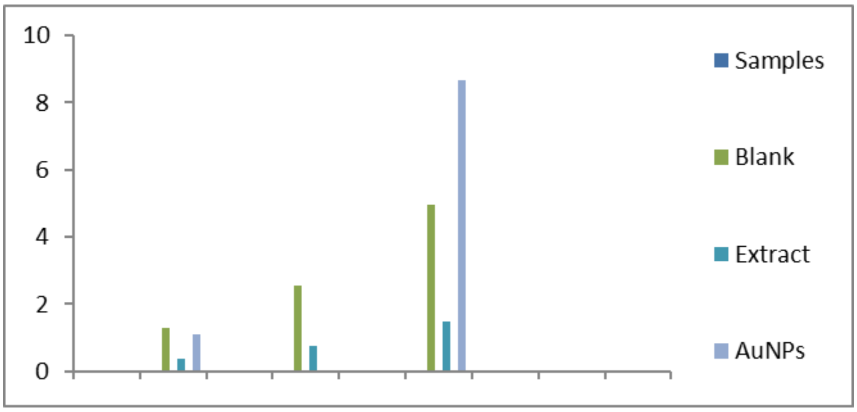

Figure 4 DPPH anti-oxidants activity of AuNPs from Glines oppositionist

5. Conclusion

The present study demonstrated an eco-friendly, inexpensive, facile, and rapid synthesis of gold nanoparticles (AuNPs) using the aqueous leaf extract of *Glinus oppositifolius,* a plant species widely recognized in traditional medicine. Characterization techniques such as UV-visible absorption spectroscopy and Dynamic Light Scattering (DLS) confirmed the successful formation of nanoparticles in the reaction mixture, highlighting the role of phytochemicals from the extract as both reducing and capping agents in this green synthesis method. Biological evaluation of the AuNPs revealed strong bactericidal properties against two Gram-negative bacteria (Escherichia coli and Klebsiella pneumoniae) and two Gram-positive bacteria (Bacillus subtilis and Enterococcus), all of which are common pathogens implicated in infectious skin diseases. The synthesized AuNPs also exhibited significant antioxidant activity. Additionally, the study assessed the potential of these nanoparticles as highly effective antibacterial agents (Ajaykumar et al., 2023), with the results indicating stronger action against Gram-negative bacteria compared to Gram-positive bacteria.The demonstrated effectiveness of this green synthesis approach underscores its significance in promoting environmentally conscious practices. The nanoparticles exhibited antibacterial activity by damaging bacterial cell walls, oxidizing cell membrane fatty acids, and interacting with vital cellular macromolecules (such as DNA and proteins), ultimately leading to bacterial cell death. Therefore, our as-synthesized AuNPs hold great potential for use as antibacterial agents against a broad spectrum of bacteria responsible for infectious diseases. The efficient production of high-quality nanoparticles using small quantities of plant extract further supports the value of Glinus oppositifolius, a plant already esteemed in traditional medicine systems. The present study can help to formulate new drugs in the large-scale production with a little amount of plant material.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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