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BIOLOGICAL EVALUATION OF SILVER NANOPARTICLES USING *AGLAOMORPHA QUERCIFOLIA* PLANT EXTRACT: ANTIBACTERIAL, ANTIOXIDANT, ANTICANCER, AND ANTI-INFLAMMATORY ACTIVITIES

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ABSTRACT
The present study investigates the green synthesis of silver nanoparticles (Ag-NPs) using the methanolic extract of *Aglaomorpha quercifolia* and evaluates their biological activities. The synthesized Ag-NPs exhibited significant antibacterial activity against both Gram-positive and Gram-negative bacteria, with maximum inhibition observed against Corynebacterium glutamicum at 150 µg/mL. The nanoparticles also demonstrated potent anticancer activity against HCT-116 human colorectal cancer cells, with an IC_{50} value of 57.81 ± 0.19 µg/mL and concentration-dependent cytotoxicity. Antioxidant potential was confirmed through DPPH and FRAP assays, showing enhanced free radical scavenging activity with increasing nanoparticle concentration. Furthermore, the Ag-NPs displayed remarkable anti-inflammatory activity, achieving 80% inhibition at 50 µg/mL. These findings suggest that *Aglaomorpha quercifolia*-mediated silver nanoparticles possess promising biomedical applications as antimicrobial, antioxidant, anticancer, and anti-inflammatory agents. Further in vivo studies are warranted to validate their therapeutic potential.

KEYWORDS: *Aglaomorpha quercifolia*, silver nanoparticles (Ag-NPs), Antibacterial activity, Antioxidant activity, Anticancer activity, Anti-inflammatory activity

INTRODUCTION

Nanotechnology has emerged as one of the most promising interdisciplinary fields, offering innovative solutions in medicine, pharmaceuticals, agriculture, and environmental sciences. Among various nanomaterials, silver nanoparticles (Ag-NPs) have gained considerable attention due to their unique physicochemical properties and broad-spectrum biological activities, including antimicrobial, antioxidant, anticancer, and anti-inflammatory effects [1-2]. Conventional methods for synthesizing Ag-NPs often involve hazardous chemicals, expensive equipment, and high energy consumption, which may pose environmental and health risks. Consequently, green synthesis using plant extracts has become an eco-friendly, cost-effective, and sustainable alternative for nanoparticle production. Plant-derived phytochemicals such as flavonoids, phenolics, terpenoids, alkaloids, and proteins act as natural reducing and stabilizing agents, resulting in the formation of stable and biocompatible nanoparticles with enhanced therapeutic potential [4-5].
Aglaomorpha quercifolia, a medicinal fern, is known to contain a wide range of bioactive phytochemicals with potential pharmacological properties. However, limited information is available regarding the synthesis and biological evaluation of silver nanoparticles derived from this plant. In the present study, silver nanoparticles were synthesized using the methanolic extract of *A. quercifolia* and evaluated for their antibacterial, antioxidant, anticancer, and anti-inflammatory activities. The synthesized Ag-NPs exhibited concentration-dependent antibacterial activity against Gram-positive and Gram-negative bacteria, significant cytotoxicity against HCT-116 colorectal cancer cells, effective free radical scavenging activity in DPPH and FRAP assays, and remarkable anti-inflammatory effects. These findings demonstrate the promising biomedical potential of *A. quercifolia*-mediated Ag-NPs and support their further investigation as multifunctional nanotherapeutic agents for pharmaceutical applications.

Antibacterial assay of Ag-NPs

Test Microorganisms

Overnight bacterial cultures of *Pseudomonas aeruginosa* (MTCC-2295), *Staphylococcus aureus* (MTCC-3160), *Corynebacterium glutamicum* (MTCC-2745), and *Escherichia coli* (MTCC-443), grown at 37°C, were utilized to evaluate antibacterial activity.

Agar-well diffusion method

Muller Hinton Agar Medium (1 L)

A commercially supplied medium (Hi-Media) weighing 33.9 g was dissolved in 1000 milliliters of distilled water to create Muller Hinton Agar Medium. The autoclave was used to sterilize the solution for 15 minutes at 121°C and 15 pounds of

pressure. Following sterilization, the medium was well combined and then, still molten, transferred onto 100 mm petri dishes (25–30 mL per plate).

Nutrient broth (1L)

A nutrient broth was made by heating 13 g of commercially available nutrient medium (Hi-Media) until it was completely dissolved in 1000 mL of distilled water. After that, the medium was dispensed as needed and autoclaved for 15 minutes at 121°C and 15 pounds of pressure to sanitize it.

1. Chloramphenicol (standard antibacterial agent)

Muller Hinton agar media (Hi-Media) was made by dissolving it in water and then autoclaving the mixture in a 100 mL conical flask for 15 minutes at 121°C and 15 lbs of pressure. The medium was poured into sterile petri plates after it had been sterilized. The antibacterial activity was assessed using DMSO as a negative control and chloramphenicol as a positive control. The agar well diffusion method was used to evaluate the silver nanoparticles antibacterial activity. The inocula was uniformly distributed over the agar surface using sterile glass spreaders. Five wells were made on the agar plates at equal intervals using a sterile cork borer. A final concentration of 100 mg/mL was made in order to test the purified compound's antibacterial activity. The wells were filled with varying amounts of the compound: 80 µg/mL, 100 µg/mL, and 150 µg/mL. After that, the plates were incubated for 24 hours at 37°C. To assess the compound's antibacterial efficacy, the diameter of the transparent inhibitory zones that developed around each well after incubation was determined in millimeters. This technique gave information on the compound's potential as an antibacterial agent by comparing its antimicrobial activity to that of the bacterial strains utilized in the investigation.

Anticancer activity of nanoparticles

Preparation of HCT 116 cell lines suspension

The HCT 116 cell line was trypsinized following subculturing in Dulbecco's Modified Eagle's Medium (DMEM). After the cells were disintegrated, 25 mL of DMEM supplemented with 10% Fetal Calf Serum (FCS) was added to the flask holding the cells. To ensure equal dispersion, the mixture was homogenized after the cells were properly suspended in the medium using a pipette. This process allowed for proper cell suspension for further cultivation and study.

Seeding of cells

Each well of a 24-well growth plate received one milliliter of the homogenized cell suspension with varying concentrations of sample (10–200 g/mL). The plates were then incubated at 37°C in a humidified CO2 incubator with 5% CO2. Following a 48-hour incubation period, the cells were examined at x40 magnification using an Olympus inverted tissue culture microscope.

Cytotoxicity assay

The chemical 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), which is broken down by mitochondrial succinate dehydrogenase and reductase in living cells, was used in the assay. The purple formazan product that is produced as a result of this enzyme activity is inversely correlated with cytotoxicity and directly correlated with the number of viable cells. MTT was added to each well following a 48-hour incubation period, and the plates were then allowed to sit at room temperature for three hours in order to allow the formazan crystals to form. Following the incubation period, 100 µL of DMSO was used to dissolve the formazan crystals after the contents of each well were extracted using a pipette. A Robonik microplate reader was then used to measure the absorbance at 540 nm. Cell viability and cytotoxicity were quantified by calculating the percentage of growth inhibition, which was based on the absorbance difference between the treated and control groups. With lower absorbance signifying increased cytotoxicity and greater inhibition of cell development, this assay made it possible to evaluate the compound's impact on cell proliferation.

$$\text{Percentage of growth inhibition} = \frac{\text{OD of Test}}{\text{OD of Control}} \times 100$$

Usually, inhibition concentration 50% (IC50) is chosen as the best biological marker of cytotoxicity. The IC50 value represents the concentration of the tested extract that causes 50% of cell inhibition.

Anti-oxidant activity of Ag-NPs

Diphenyl picryl hydrazyl radical scavenging Assay (DPPH)

The 1,1, dipheny 1-2 picrylhydrazyl (DPPH) scavenging potential of the silver nanoparticles from *Aglaomorpha quercifolia* rhizome extract was determined using the method of Mensor et al., [5]. Different concentrations (50, 75, 100, 125 and 150 µg/mL) of the silver nanoparticles and the standard (Ascorbic acid) were taken in different test tubes. Thereafter, 1 ml of freshly prepared DPPH (0.3 mM) was added to each test tube and vortexed thoroughly. Finally, the solution was incubated in dark place for 30 min. For control, 2 ml of methanol was added instead of silver nanoparticles and run simultaneously with the test. After 30 min incubation at 37°C absorbance was measured at 517 nm against control using a spectrophotometer. Ascorbic acid was used as the reference materials. The percentage of inhibition was calculated by comparing the absorbance values of the test samples with those of the controls. The inhibition percentage (I) was calculated as radical scavenging activity as follows

Percentage of inhibition (I) = (Absorbance of Control- Absorbance of Test)/ Absorbance of control ×100

Ferric reducing or antioxidant power assay (FRAP)

Using the Pulido et al., 2000 [6] technique, ferric reducing antioxidant power (FRAP) tests were carried out. Different concentrations (50, 75, 100, 125 and 150 µg/mL) of the silver nanoparticles and the standard (Ascorbic acid) were taken in different test tubes. After adding 100µl of test extract to 3.0 ml of FRAP working reagent in a test tube and vortex mixing the combination, which was carefully vortexed before being tentatively incubated for 30 minutes at 37°C and then the absorbance was measured at 593 nm.

Percentage of inhibition (I) = (Absorbance of Control- Absorbance of Test)/ Absorbance of control ×100

***In vitro* Anti-inflammatory activity of nanoparticles**

The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (pH 6.4) and 2 mL of varying concentrations of Ag-NPs (50, 25, 12.5, 6.25 and 3.125 µg/mL). Similar volume of distilled water served as control. The mixture was incubated at 37°C in a biochemical oxygen demand incubator for 15 min then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm using distilled water as blank. Aspirin was used as reference drug and treated similarly for determination of absorbance.

The absorbance of control - Absorbance of sample × 100/ Absorbance of control

RESULTS AND DISCUSSION

Antibacterial activity of Ag-NPs

The agar well diffusion method was employed for screening the antibacterial activity of Ag-NPs against Gram-positive and Gram- negative bacteria. The green route synthesized Ag-NPs showed good antibacterial activity in all concentrations (80, 100, and 150µg/mL) against all microorganisms and also it indicated the dose depended on mechanisms. The antibacterial activity of Ag-NPs was increasing with the increasing the concentration of Ag-NPs against test pathogens. The maximum antibacterial activity of Ag-NPs (19.86±0.41) against *Corynebacterium glutamicum* at the concentration of 150µg/mL (Fig. 1B). Well-diffusion method was opted to evaluate the antibacterial activity. The inhibitory action of silver compounds may be attributed to the strong interaction of silver with thiol groups present in key respiratory enzymes in bacteria [6].

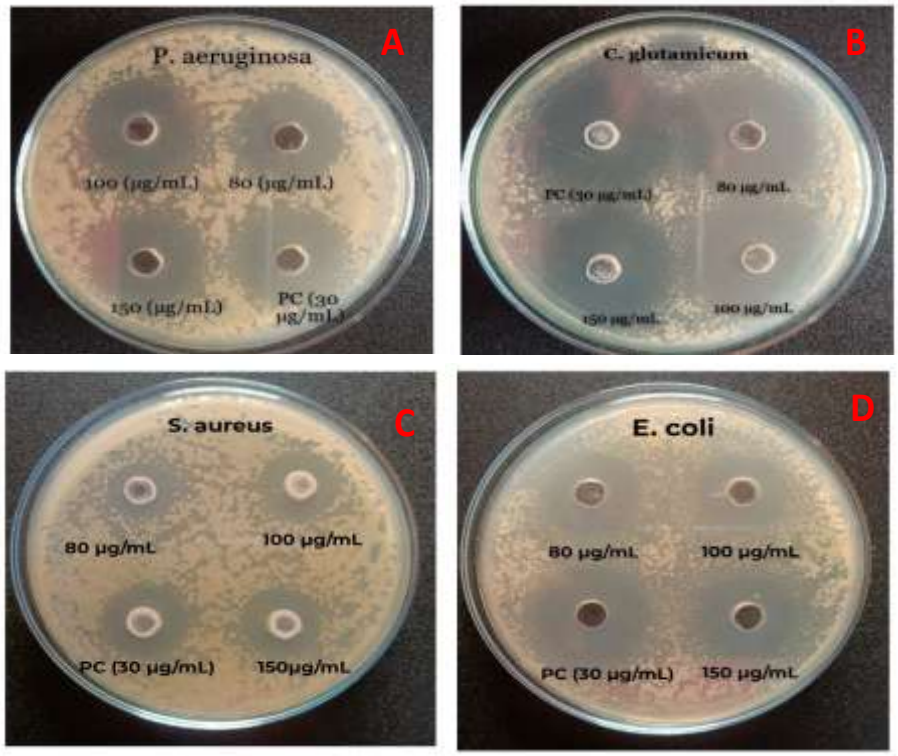

Fig. 1 Antibacterial activity of Ag-NPs against bacteria (A) *Pseudomonas aeruginosa*, B) *Corynebacterium glutamicum*, C) *Staphylococcus aureus*, D) *Escherichia coli*

Table. 1 Antibacterial activity of Ag-NPs against bacteria

S.No	Test organism	Zone of inhibition (mm)			
		Ag-NPs (µg/mL)			
		80	100	150	Positive control (Chloramphenicol) 30µg/mL

1	*Pseudomonas aeruginosa* (MTCC-2295)	12.16±0.76	14.76±1.25	16.23±0.83	18.83±2.02
2	*Corynibacterium glutamicum* (MTCC-2745)	14.43±1.42	17.23±0.83	19.86±0.41	22.86±1.89
3	*Staphylococcus aureus* (MTCC-3160)	9.43±0.72	11.33±0.67	13.46±0.22	14.66±1.68
4	*Escherichia coli* (MTCC-443)	13.14±0.56	15.37±1.23	17.23±0.87	19.83±2.05

Bacterial strains *B.subtilis*, *S.typhi* and *S.aureus* and fungal strains *C.lunata*, *C.albicans* and *A.niger* were used to study the antimicrobial activity from the synthesized *D. quercifolia* methanol extract. The *in vitro* antimicrobial activity showed that the methanol extract had substantial activity against all the microorganisms studied, especially on high-concentration of *S.typhi*, *S.aureus* and *C.lunata* (60 µl), but there was activity in the lower concentrations also. The results of antimicrobial activity from the synthesized *D. quercifolia* methanol extract showed excellent effect on all organisms. The maximum inhibition zone on *C.lunata* (20 mm) was observed in the rhizome extract in methanol synthesized with a concentration of 60 µl and a minimum inhibition zone observed on *C.albicans* (14 mm) and *A.niger* (14 mm). The antimicrobial activity of nanoparticles also depends on the nanoparticle shapes. This attribute can be further verified by using various formed nanoparticles to study the inhibition of bacterial growth [7].

Anticancer activity

Ag-NPs' effectiveness against specific cancer cells, such as HCT 116 cell lines. Ag-NPs have a notable impact on cell wall rupture and harm the polymer components of the cell membrane and concentration of Ag-NPs. The anticancer activity of the nanoparticles against HCT 116 cell lines was assessed, and the results showed that the chemical was effective against the cell lines. Additionally, the cytotoxicity was a phenomenon that was depending on concentration. The MTT experiment revealed an IC50 value of 57.81±0.19µg/mL (Table.2) for the cytotoxicity of the synthesized silver nanoparticles on HCT 116 cell lines. An increase in the concentration of the purified compound led to a noticeable rise in the number of dead cells. Microscopic examination of the treated cell lines revealed distinct morphological alterations. When observed under an inverted tissue culture microscope at 40x magnification, the cells exhibited a shrunken appearance and were detached from the substrate, indicating a significant disruption of cellular integrity. These morphological changes are indicative of cytotoxic effects, suggesting that the compound has a detrimental impact on the viability of the cells (Fig. 2A-D).

The observed shrinkage of the cells, accompanied by detachment from the surface, points to the compound's ability to induce cell stress and apoptosis, ultimately leading to cell death. Such alterations are characteristic of cellular responses to toxic agents, where structural components of the cell undergo significant changes, resulting in loss of normal function. The detachment from the substrate further implies that the compound may interfere with the cell's ability to maintain adhesion, a crucial factor in sustaining cell structure and function. According to earlier research, biosynthesized Ag-NPs have a great deal of potential to combat cancer cell lines [9-10]. Ag-NPs made using green synthesis techniques can stop the growth of MCF-7 breast cancer cells, A549 lung cancer cells, and human glioblastoma cells. Ag-NPs' precise mode of action against cancer cells is not entirely understood. However, Xu *et al.,* 2012 discovered that a silver nanoparticle hydrogel caused DNA damage and reactive oxygen species (ROS) production in cancer cells, which ultimately resulted in cell death [11-12. Silver nanoparticles made from Andrographis echioides leaf extract also exhibit potential anticancer activity by inducing necrosis and apoptosis, two processes that cause cell death. Its bio-cytotoxicity is demonstrated by the transfer of electrons from molecular oxygen [13].

Table 2 *In vitro* cytotoxicity of nanoparticles on HeLa cell lines

S.No	Nanoparticles Concentration (µg/mL)	% Cell viability of HCT 116 cell lines
1	0	100.00
2	12.5	95.67
3	25	84.70
4	50	73.48
5	75	56.81
6	100	47.20
7	150	32.70
8	200	26.40
9	IC50	57.81

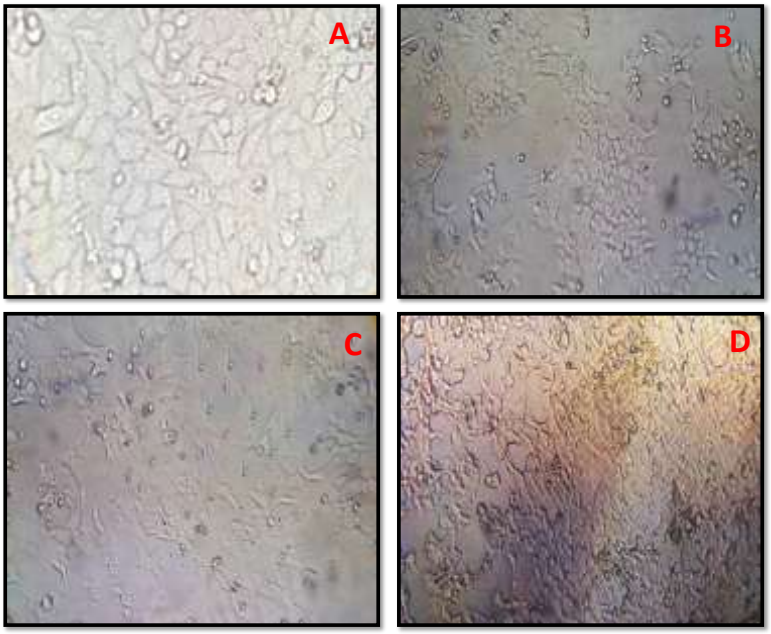

Fig. 2 Anticancer activity of Ag-NPs against HeLa cell lines (A) Control (Without treatment, B) Treatment with Ag-NPs (100µg/mL), C) Treatment with Ag-NPs (150µg/mL), D) Treatment with Ag-NPs (200µg/mL)

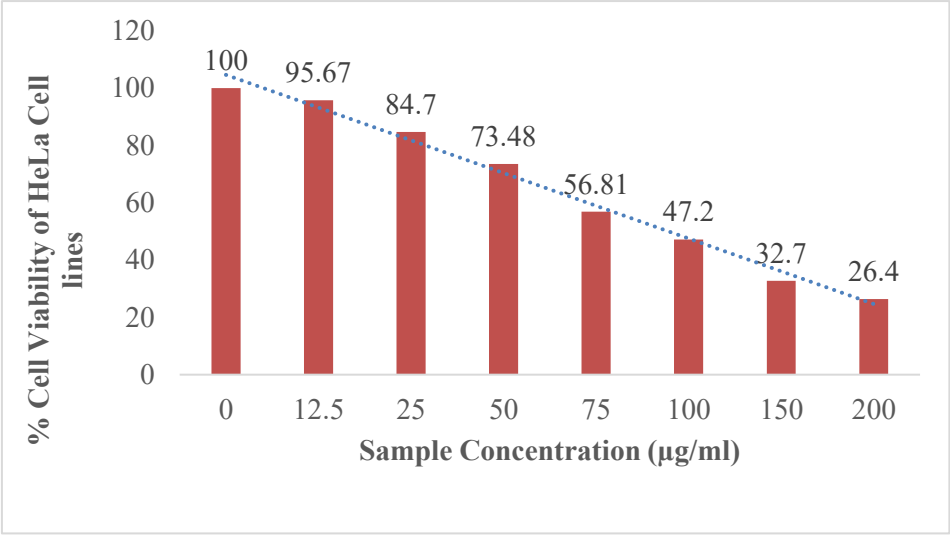

Fig. 3 Anticancer activity of nanoparticles against HCT 116 cell lines

Antioxidant activity of silver nanoparticles

DPPH radical scavenging activity of nanoparticles

The DPPH radial scavenging activity, reductive potential, and total flavonoid and phenolic content of the produced Ag-NPs at varying doses ranging from 50 to 150 µg/ml were the characteristics that were examined. A persistent organic free radical, DPPH has been used to study the antioxidant and free radical properties of a variety of natural items. The dose response for the synthesized Ag-NPs' DPPH scavenging activity is displayed in Figure 4. The Ag-NPs produced from the methanolic extract of *Aglaomorpha quercifolia* have effective inhibitory action in a dose-dependent manner, making them suitable scavengers of free radicals. Significant DPPH scavenging occurred at different concentrations of Ag-NPs (50, 75, 100, 125, and 150 µg/ml) by 45, 55, 68, 79, and 85%, respectively. These activities, however, fall short of those of ascorbic acid, the commonly used reference.

The antioxidant activity of substances can be evaluated using a variety of techniques. An easy, quick, and sensitive way to screen plant extracts for antioxidants is to use the DPPH free radical scavenging test. The absorbance drops when an antioxidant is present because the DPPH radical gains one extra electron [14]. The FRAP assay and the DPPH free radical were used to measure the antioxidant activity of the produced Ag-NPs. A common method for assessing antioxidant activity is to utilize DPPH, a stable molecule that can be lowered by taking hydrogen or electrons [15]. Using the DPPH radical scavenging assay, which has an IC50 of 30.04µg/mL, Ag-NPs' noteworthy antioxidant capability was assessed. At different extract concentrations, the *D. quercifolia* methanol extract's overall antioxidant activity was assessed. The total antioxidant activity rose with higher extract concentrations. The increased antioxidative activity of Ag-NPs may be attributed to the presence of different functional groups on their surface. Antioxidants are substances that scavenge free radicals and protect against diseases that cause degeneration, such as cancer, Parkinson's disease, Alzheimer's disease, and atherosclerosis, which are due to oxidative stress (excess production of free radicals) [16].

Table. 3 DPPH radical scavenging activity of nanoparticles

S. No	Nanoparticles concentration in µg/ml	% of radical scavenging activity	Ascorbic acid
1	50	45	57
2	75	55	65
3	100	68	76
4	125	79	84
5	150	85	91

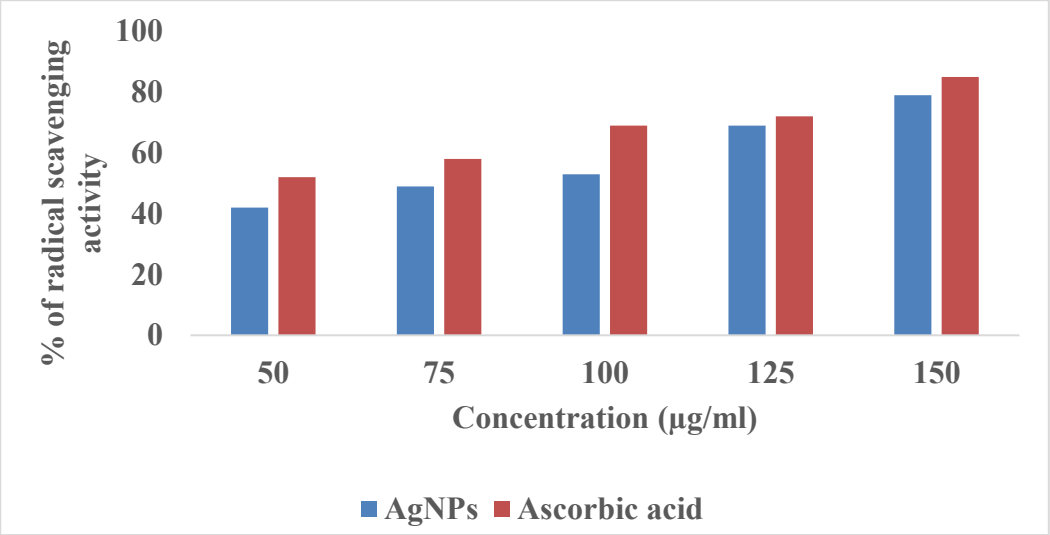

Fig. 4 DPPH radical scavenging activity of nanoparticles

FRAP activity of silver nanoparticles

The properties that were investigated included the total flavonoid and phenolic content, FRAP radial scavenging activity, and reductive potential of the generated Ag-NPs at different concentrations between 50 and 150 µg/ml. The results of the experiment are summarized in Table 4. The activity increased in tandem with the concentration of silver nanoparticles and the standard. The results showed a concentration-dependent radical scavenging action, with the maximum activity occurring at 150 µg/ml (Fig. 5). This assay is based on the ability of antioxidants to facilitate the reduction of Fe^{3+} to Fe^{2+} ions in the presence of TPTZ. The methanolic extract of Aglaomorpha quercifolia yields Ag-NPs, which are suitable scavengers of free radicals due to their efficient inhibitory action in a dose-dependent manner. At varying doses of Ag-NPs (50, 75, 100, 125, and 150 µg/ml), 42, 49, 53, 69, and 79%, respectively, demonstrated significant FRAP scavenging. However, these activities are not as high as those of the widely used reference, ascorbic acid. According to reports, R. japonica contains phenolic chemicals, which are used extensively in traditional remedies to treat a variety of illnesses, such as inflammation, cancer, and several pathological disorders. The study's findings indicate that Ag-NPs high antioxidant activity is caused by the presence of bioactive substances, most likely phenolics, on their surface. Thus, we propose that the therapy of the aforementioned human diseases may involve the use of an appropriate formulation of Ag-NPs [17].

Table. 4 FRAP activity of silver nanoparticles

S. No	Nanoparticles concentration in µg/ml	% of radical scavenging activity	Ascorbic acid
1	50	42	52
2	75	49	58
3	100	53	69
4	125	69	72
5	150	79	85

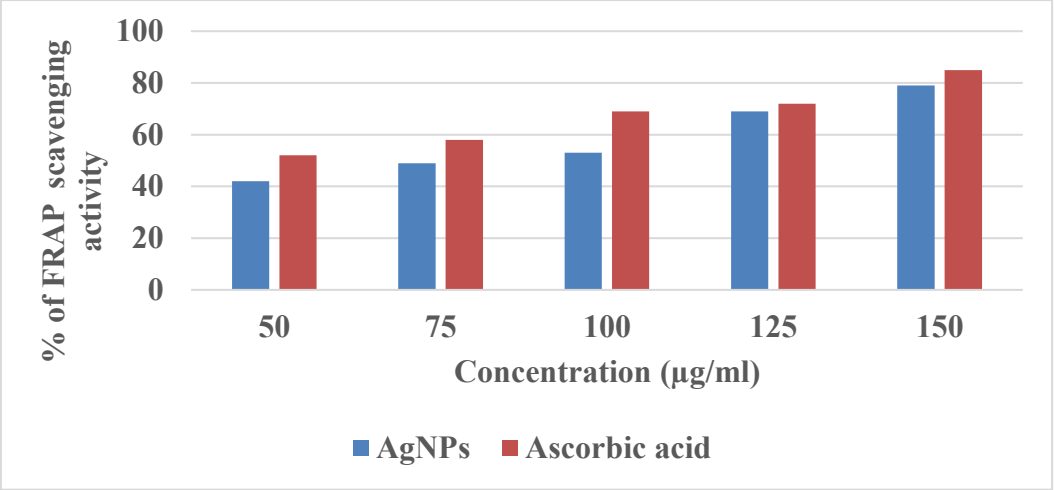

Fig. 5 FRAP activity of silver nanoparticles

Anti-inflammatory activity

The anti-inflammatory potential of the synthesized silver nanoparticles (Ag-NPs) was evaluated to assess their effectiveness in reducing inflammation. In vitro studies were conducted to examine the anti-inflammatory activity of the Ag-NPs, with a particular focus on their antioxidant properties, which are often closely related to anti-inflammatory effects. The Ag-NPs were tested at varying concentrations ranging from 10 to 50 µg/mL to determine their ability to modulate inflammatory responses. Figure 6 illustrates the dose-response relationship for the anti-inflammatory activity of the synthesized Ag-NPs. As expected, the Ag-NPs demonstrated a dose-dependent anti-inflammatory effect, with increased inhibition observed as the concentration of Ag-NPs was raised. Specifically, the synthesized Ag-NPs, derived from the methanolic extract of Aglaomorpha quercifolia, exhibited significant anti-inflammatory activity across all tested concentrations. The anti-inflammatory activity was measured at concentrations of 10, 20, 30, 40, and 50 µg/mL, and the results showed the following levels of inhibition: 58%, 61%, 72%, 79%, and 80%, respectively. These findings indicate that the Ag-NPs are effective in reducing inflammation, and their activity increases in a concentration-dependent manner. At the highest tested concentration of 50 µg/mL, the Ag-NPs exhibited the most substantial anti-inflammatory effect, with an 80% inhibition rate. This suggests that the Ag-NPs synthesized from Aglaomorpha quercifolia have considerable potential as an anti-inflammatory agent, with efficacy that grows stronger as the concentration increases.

According to the study, silver nanoparticles made from plant extract have anti-inflammatory properties. The spectra showed that the extract at 660 nm had the maximum absorbance at a concentration of 20 µL, which was 81%. This indicated that the albumin denaturation was significant. Zinc oxide nanoparticles made with grape seed extract demonstrated the maximum absorbance value at 50µL concentration in a related study [18-19]. Therefore, anti-inflammatory medicines that can stop protein denaturation can be utilized. The study found that plant extract-derived silver nanoparticles have anti-inflammatory qualities.

Table .5 Anti-inflammatory activity of Ag-NPs

S. No	Nanoparticles concentration in µg/ml	Percentage of Inhibition
1	10	58
2	20	61
3	30	72
4	40	79
5	50	80

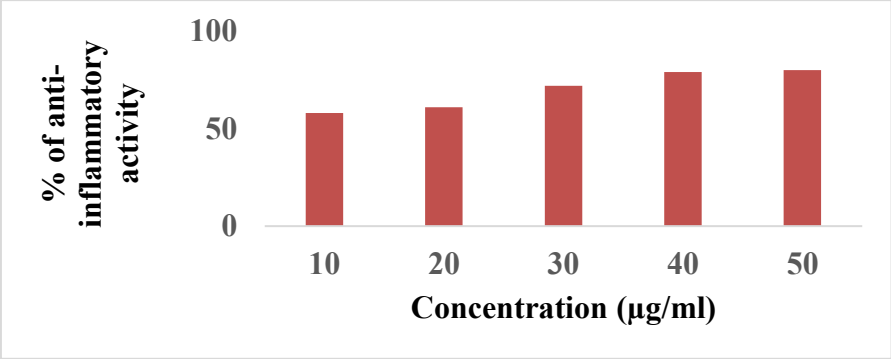

Fig. 6 Anti-inflammatory activity of silver nanoparticles

CONCLUSION

The present study demonstrates that silver nanoparticles synthesized using the methanolic extract of *Aglaomorpha quercifolia* possess significant biological activities. The synthesized Ag-NPs exhibited strong antibacterial activity against both Gram-positive and Gram-negative bacteria, effective antioxidant capacity through DPPH and FRAP assays, promising anticancer activity against HCT-116 cells, and considerable anti-inflammatory potential. All activities showed a concentration-dependent response, highlighting the therapeutic potential of these green-synthesized nanoparticles. The findings support the use of *Aglaomorpha quercifolia* as a valuable natural source for nanoparticle synthesis and suggest that these Ag-NPs may serve as promising candidates for future pharmaceutical and biomedical applications. Additional in vivo studies and toxicity assessments are recommended before clinical applications.

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