

Analysis of *Althaea officinalis*'s Biological Characteristics by Means of Gold Nanoparticles (AuNPs)

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ABSTRACT

Biomedical research that combines nanotechnology with traditional medicinal plants has made great strides. Using gold nanoparticles (AuNp), this study explores the biological features of marshmallow, or *Althaea officinalis*. Reducing and stabilizing aqueous solutions of *A. officinalis* may promote the synthesis of AuNPs. By integrating XRD, FTIR, UV-Vis, and TEM, it was able to create consistently spherical gold nanoparticles. The biological activity of AuNPs produced by *A. officinalis* was investigated in oxidative, cytotoxic, and antibacterial tests. The findings showed that the mixture had more "antibacterial activity against both gram-positive and gram-negative bacterial strains," was more effective at killing certain kinds of cancer cells and had clear free radical scavenging properties compared to the plant extract or AuNPs alone. Due to their complementary biological characteristics, the results open new pharmacological and therapeutic avenues for gold nanoparticles mediated by *A. officinalis*.

Keywords: Antimicrobial activity; Gold nanoparticles (AuNPs); *Althaea officinalis*; Green synthesis.

INTRODUCTION

Althaea officinalis, more often known as marshmallow, is a perennial herb that has long been used in traditional medicine to treat respiratory and gastrointestinal issues. Numerous medical professionals have taken note of the plant's beneficial components, including phenolic acids, polysaccharides, and flavonoids. The use of nanotechnology in herbal treatments has recently uncovered new avenues for enhancing the stability, targeted delivery, and phytochemical potency. The unique physicochemical characteristics of gold nanoparticles (AuNPs), such as their enormous surface area, biocompatibility, and ease of functionalization, are attracting an increasing number of researchers (Ahmed, 2021).

The goal of green chemistry is to find alternatives to traditional chemical processes that have less of an adverse effect on the environment. One such technique is the production of AuNPs from plant extracts. This bio-reduction method enhances the biological activity of nanoparticles by reducing their toxic content and coating them with chemicals derived from plants. It is possible that the

environmentally sustainable production of gold nanoparticles using *Althaea officinalis*, which has a rich phytochemical profile, would have a greater biological effect than the plant extract or the nanoparticles alone. *Althaea officinalis* produces gold nanoparticles, and this study examines their biological characteristics in search of antibacterial, antioxidant, anti-inflammatory, and even anticancer benefits. This study aims to shed light on the potential biomedical and nanotherapeutic applications of AuNPs by investigating the synergistic interactions between these nanoparticles and bioactives obtained from plants (Hatipoğlu, 2021).

BACKGROUND OF THE STUDY

Althaea officinalis, often known as marshmallow, contains phenolic acids, polysaccharides, and flavonoids, all of which have long been recognized in traditional medicine as having medicinal value due to their anti-inflammatory, antibacterial, and antioxidant properties. The potential therapeutic applications of these naturally occurring compounds have recently garnered considerable attention, especially within the realm of nanotechnology. With their large surface area, biocompatibility, and ease of functionalization, gold nanoparticles (AuNPs) possess physicochemical properties that are particularly valuable in nanomedicine. The process of synthesizing AuNPs from plant extracts using the reducing and capping agents found in plants is known as "green synthesis," because it is both inexpensive and eco-friendly. The bioactivity of the nanoparticles is increased, and the absorption of harmful chemicals is decreased when phytochemicals are combined with the synergistic effects of the metal core (Keskin et al., 2021).

An innovative method for studying and enhancing the plant's biological properties is offered by combining *Althaea officinalis* extract with AuNPs. Researchers may study the plant's changed or augmented therapeutic potential, including its antibacterial, antioxidant, and cytotoxic capabilities, by synthesizing gold nanoparticles using *A. officinalis*. Few comprehensive studies have focused on the biological assessment of AuNPs mediated by *Althaea officinalis*, despite the growing amount of research on plant-based nanoparticle production. To address this gap, this study will examine the potential medicinal uses of gold nanoparticles made from *A. officinalis* and will meticulously analyze their biological characteristics. By understanding these interactions, one may develop safer and more effective nanoformulations for potential medical applications (Lakshmiprabha et al., 2020).

LITERATURE REVIEW

There has been a lot of buzz about using nanotechnology into herbal medicine as of late due to its potential to increase the bioavailability and biological activity of substances obtained from plants. Perennial *Althaea officinalis*, often known as marshmallow, has a long history of medicinal usage due to its anti-inflammatory, antioxidant, antibacterial, and immunomodulatory properties. A novel approach to bioactive nanoconjugates with enhanced therapeutic potential has been opened up by the interaction of phytochemicals from *A. officinalis* with gold nanoparticles (AuNPs).

• ***Althaea officinalis*: phytochemical and medicinal profile**

The pharmacological activities of *A. officinalis* are defined by the bioactive components found in its roots, leaves, and flowers, which include flavonoids, phenolic acids, mucilage, and polysaccharides. Numerous studies back up its traditional usage in reducing inflammation and soothing mucosal membranes in the treatment of gastrointestinal and respiratory disorders.

• **AuNPs, Gold Nanoparticles: Biomedical Applications**

Gold nanoparticles have developed into a versatile platform in nanomedicine due to their unique physicochemical features, such as biocompatibility, surface plasmon resonance, and ease of functionalization. When it comes to targeted treatment, biosensing, imaging, and drug administration, AuNPs are a great choice. Using AuNPs may improve the therapeutic effects of conjugated biomolecules by boosting their bioavailability and cell absorption, according to claims. Synthesis of AuNPs for use in medicine researchers provide what is formally known as "green synthesis," a process for synthesizing AuNPs from medicinal plants that is biocompatible, inexpensive, and ecologically beneficial. Medicinal herbs like *A. officinalis* lower the concentration of substances required to synthesize stable AuNPs. In addition to removing potentially dangerous medications, this process maintains the phytoconstituents' pharmacological effects. The enhanced antibacterial and anticancer activity of plant-mediated AuNPs is a result of the synergistic interactions between gold and phytochemicals (Nagaraj et al., 2022).

• **Biological features of AuNPs mediated by *A. officinalis***

Recently, there has been a lot of focus on studying the biological effects of *A. officinalis*-derived AuNPs. These biogenic nanoparticles outperform plant extract alone as an antibacterial agent against harmful pathogens. For example, the growth of both Gram-positive and Gram-negative bacteria was inhibited by *A. officinalis* synthesised AuNPs, which were shown to have an effect related to enhanced surface activity and interaction with microbial membranes. Additionally, preliminary in vitro studies demonstrating anti-inflammatory and anticancer properties suggest a potential avenue for nanotherapeutics.

• **Future Views and challenges**

Despite the evident therapeutic effects of *A. officinalis*-mediated AuNPs, there are still issues with batch uniformity, standardizing synthesis processes, and evaluating long-term toxicity. To confirm in vivo effectiveness via clinical trials and understand the molecular processes behind the biological effects, more research is necessary. The bio-distribution and specificity of these nanoparticles should be enhanced in complex biological systems by integration with surface changes and tailored delivery strategies.

METHODOLOGY

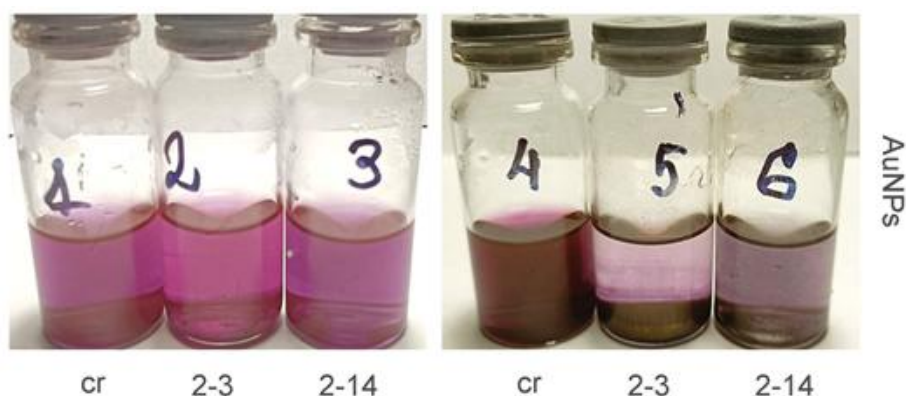
Bio-reduction of AuNPs

The water extract was made by carefully harvesting, washing, and slicing the *Althaea officinalis*. The water-based extract was made by boiling 20 grams of the whole plant in 100 milliliters of distilled water. Filter paper was used to capture the steam extract after 20 minutes. To make the gold nanoparticles, a solution of 0.1 mM gold chloride was mixed with 3, 1, and 10 volumetric portions of water-based *Althaea officinalis* lysates for an hour. After that, the mixture was bio-reduced in a dark environment. The researchers set aside the purple solution that was produced for use in future characterization testing.

The microorganisms used in this investigation were "Staphylococcus aureus (ATCC25923), Pseudomonas aeruginosa (ATCC10231), Streptococcus pneumoniae (ATCC49619), and E. coli (ATCC11229)". The bacterial cultures were maintained in a glycerol stock at a temperature of -80 °C in the laboratory. One colony was revived using the glycerol culture stock and maintained as a subculture in the nutritional broth at 4 °C. The antibacterial activity was determined by using agar well diffusion. Cultures in suspension were equally dispersed throughout nutrient agar plates. The next stage included carefully penetrating the solid material using a cork borer to create wells. The 100 µl of each well was supplemented with the synthesized AuNPs. For one day, the plates were allowed to incubate at 37 °C. A medicine called amoxicillin was given to the control group. In order to determine the antibacterial activity, the inhibitory zone width surrounding the well was measured.

RESULT

Figure 1. Images of AuNPs Colloid Solutions



Characterizations of AuNPs: To do this, the researchers used a UV Lambda 650, a UV/VIS spectrophotometer manufactured by PerkinElmer, to determine the initial composition of the gold nanoparticles. Wavelengths between 200 and 800 nm are within the instrument's detection range. The surface appearance and size of the produced gold nanoparticles were examined using a scanning electron microscope (ZEISS Gemini SEM 360). These nanoparticles have a structure that looks like flowers and look like tubes, according to the results. Utilizing Bruker X-flash into energy-dispersive X-ray spectroscopy (eZAF), the structure and geometry of biosynthesised AuNPs were examined. Scientists used Fourier transform infrared technology from Thermos iS50 to study the production process of gold nanoparticles. The gold nanoparticles were stabilized using a Windows version of "HORIBA SZ-100", the Z Type, Ver2.20. Researchers used "HORIBA SZ-100" for Windows [Z Type] Ver2.20 to ascertain the durability of the synthesized gold nanoparticles. Then, the size and form of the gold nanoparticles were confirmed using a transmission electron microscope ("FEI Tecnai G220 S-TWIN" in this reference). To examine the total crystal systems, crystallinity, and phase purity of the manufactured gold nanoparticles, a powder X-ray scattering instrument (D8 Advance Bruker) was used (Singh, 2020).

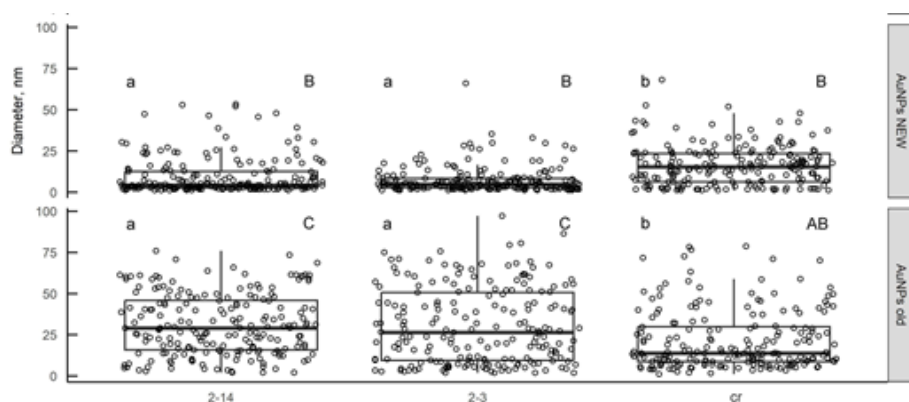


Figure 2. Characteristics of AuNPs

Ultraviolet-visible spectroscopy analysis: Analysis of the synthesized gold nanoparticles was carried out by means of "UV-visible spectroscopy." By adding water to a solution of gold ions, researchers were able to see their biological transition into AuNPs, or atomic clusters. A phenomenon known as "surface plasmon resonance (SPR)" is responsible for the purple hue. The presence of excited electrons was shown by changing the colors of the phytofabricated AuNPs, as seen in Figure 1 (a). In the 200 to 800 nm band, ultraviolet light spectra were seen. A huge and strong plasmon surface peak at 546 nm, 544 nm, or 555 nm in Figure 1 (b) indicates that three different ratios of AuNPs were synthesized using an aqueous extract of *Althaea officinalis* plants, namely 1:3, 1:5, and 1:10. It is simple to recognize gold nanoparticles because they have a distinctive absorption peak that is visible between 500 and 600 nm. There is a clear UV absorbance band for AuNPs because the manner of surface plasmon excitation changes with nanoparticle size.

"Fourier transform infrared spectroscopy (FTIR)": Investigation with infrared radiation The biomolecules that bio-reduce, cap, or stabilize the AuNPs synthesized from the water-based *Althaea officinalis* extract are illustrated in Figure 3, alongside the fourier series, for example. The FT-IR measurement was carried out by researchers using either a water-based *Althaea officinalis* extract or gold nanoparticles that were phyto-synthesised. It may be possible to attain a decent signal-to-noise ratio by obtaining the spectra of "AuNPs" in the 500-4000 cm^{-1} region. Observable intensity maxima at 3200 cm^{-1} , 2100 cm^{-1} , and 1600 cm^{-1} are noteworthy. An array of aromatic "C-H stretching, CH₃-R, N-H, C-O-C, and C=O stretching" functional groups were identified at different cycle numbers. It is possible for bio-reduction to take place in the presence of vibrations that stretch the OH or NH groups of carbohydrates and proteins. It is possible that this explains the apparent band at 3297 cm^{-1} .

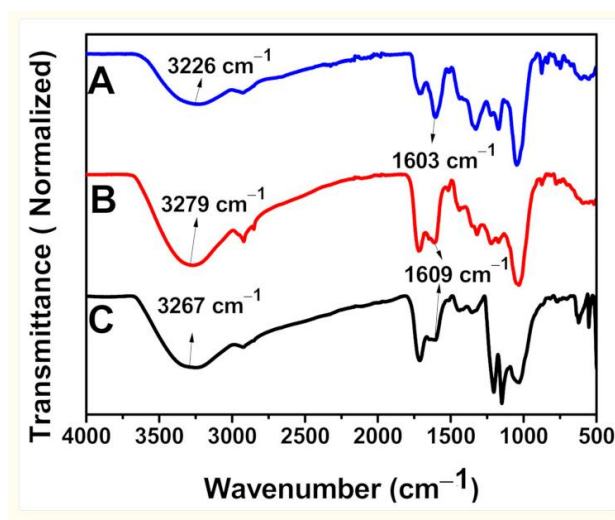


Figure 3. FTIR Spectra of of (A) AO Extract, (B) AuNPs synthesised using 2 mL of AO, and (C) AuNPs synthesised using 1 mL of AO.

X-ray powder diffraction (XRD): The size, structure, and phase purity of the synthesized gold nanoparticles were validated by the X-ray diffraction analysis. Figure 4 shows the XRD pattern of the gold nanoparticles that were created. The X-ray diffraction pattern of the gold nanoparticles synthesized from an aqueous extract of *Althaea officinalis* showed prominent peaks in the "2 θ " range at 38.089, 44.256, 64.379, 77.312, and 81.412". Certain peaks are linked to the (1 1 1), (0 0 2), (0 2 2), (1 1 3), or (2 2 2) planes, as well as the crystalline cubic structure of nanogold.

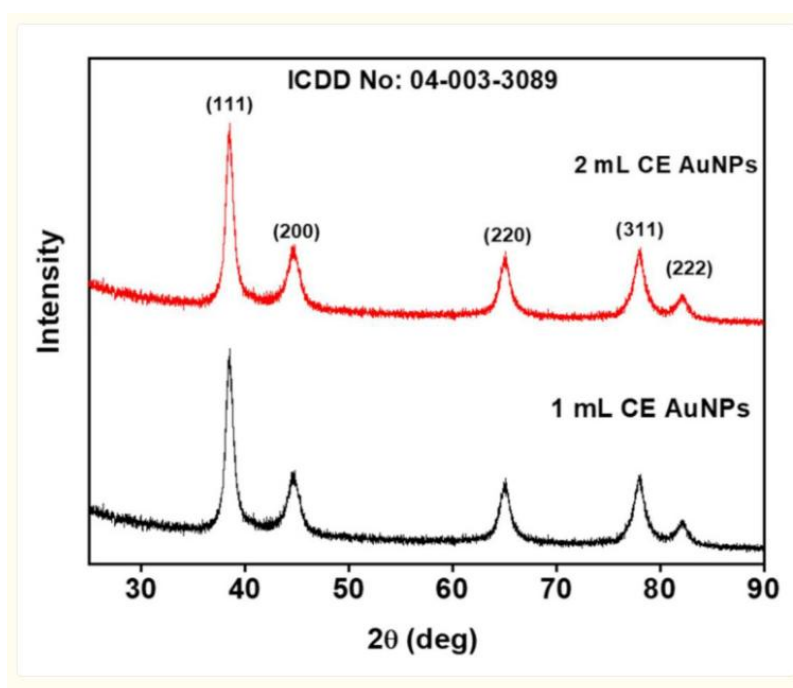
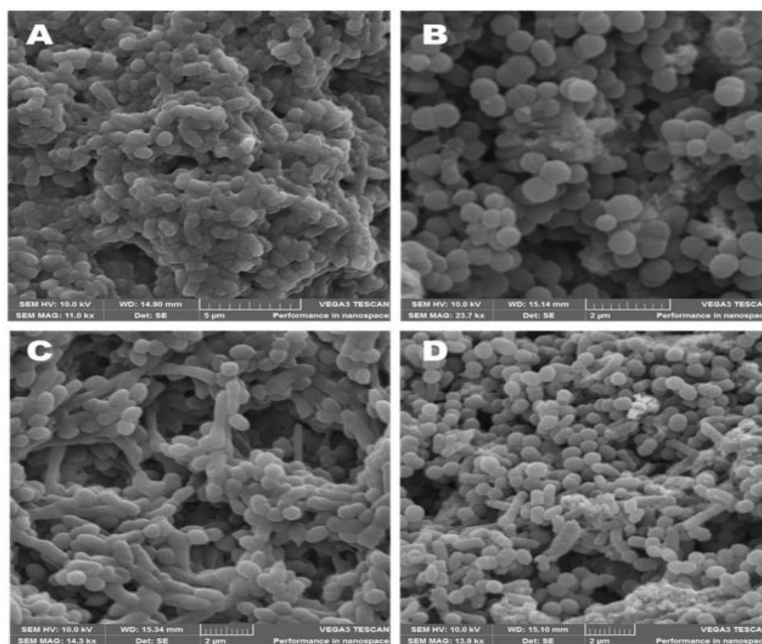


Figure 4. XRD patterns of the as-synthesized AO-AuNPs

Microscopic image analysis: The structure and form of photosynthesised gold nanoparticles may be observed via scanning electron microscopy (SEM). Triangular in shape and arranged in a cube-like pattern, the resultant gold nanoparticles display obvious aggregation blooms and flaws. Researchers can see the elemental distribution of the material in Figure 5 from the EDAX spectra. Gold nanoparticles are one of the distinguishing characteristics.

Figure 5. SEM images of (A) *S. aureus* after incubation with 62.5 $\mu\text{g/mL}$ of 1 mL AO AuNPs, (B) *S. aureus* and (C) *K. pneumoniae* after incubation with 62.5 $\mu\text{g/mL}$ of 1 mL AO AuNPs, and (D) *K. pneumoniae*.



High resolution transmission electron microscope (HR-TEM)

Biosynthesised gold nanoparticles are investigated by HR-TEM for size and structural conformation. Figure 6 shows the particle size and content. The nanoparticles were mostly spherical in shape and had a median size of 15 nm. *Althaea officinalis* produces gold nanoparticles by the bio-reduction of gold ions by its phytochemicals.

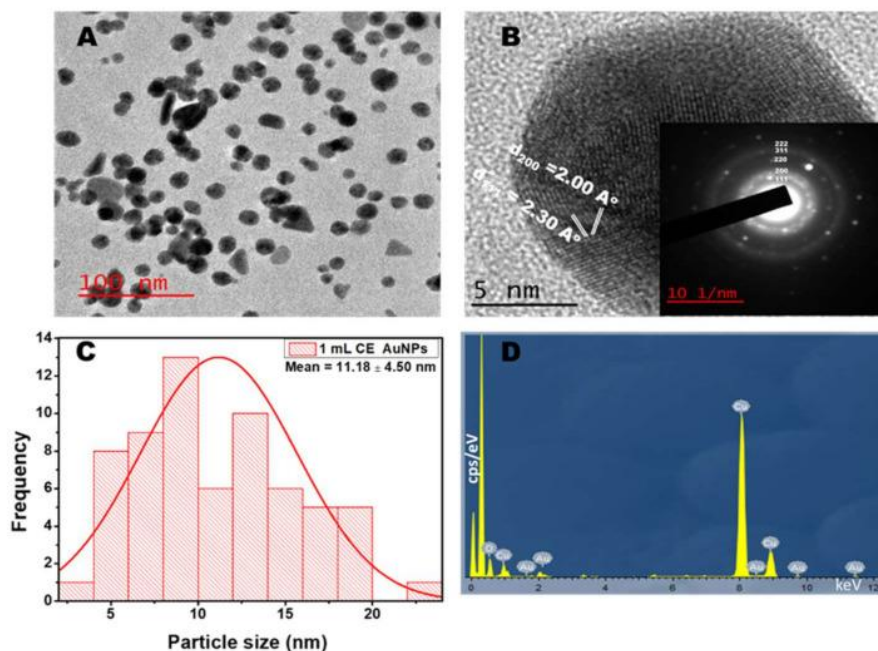


Figure 6: TEM (A) and HRTEM (B) images of *Althaea officinalis* (AO) synthesised AuNPs
inset: SAED (C) corresponding particle size distribution and (D) EDX spectra of AuNPs
using 1 mL AO.

Antimicrobial activity

Scientists can find out whether the synthesized gold nanoparticles are antibacterial by using the agar diffusion well test. Agar was supplemented with 100 microlitres of each nanoparticle after twelve hours of development. As a control, Figure 9 displays the use of a β -lactam antibiotic. Measurements were taken of the area around the well after incubation. Because of this, scientists began to wonder if nanoparticles controlled by plants may possess antibacterial characteristics (Table 1).

Table 1: Shows the antibacterial activity of biosynthesized gold nanoparticles.

S.NO	Name of the microorganisms	Zone of inhibition	
		Gold nanoparticles	Control
1.	<i>Staphylococcus aureus</i> (ATCC-25923)	4.9mm	4.5mm
2.	<i>Pseudomonas aeruginosa</i> (ATCC-10231)	5.6mm	5.2mm
3.	<i>Streptococcus pneumoniae</i> (ATCC-49619)	6.2mm	5.8mm
4.	<i>Escherichia coli</i> (ATCC-11229)	6.6mm	6.2mm

Anti-fungal activity

Following these protocols allowed us to determine if the gold nanoparticle produced by *Althaea officinalis* had any anti-fungal effects. The SDA was moved to a new Petri plate after sterilization. Once the medium had set, one may use the sterile gel to poke holes in the agar plates with a diameter of 8 mm. Forty microliters of a solution comprising differing concentrations of 4 mg/l of a separate substance and 2 mg/l of gold nanoparticles were added to every well. Each well was inverted before the fungal discs were introduced. Following this, the plates were to be kept in an incubator set at 28 °C for 70-94 hours. The control group was the one that received amphotercin B. The percentage of growth inhibition was calculated by comparing the fungal colony diameter to the control fungal diameter after incubation at 28 °C. Triplicate analysis was used to perform the

antifungal research. A percentage of growth inhibition was determined by using the following formula:

Table 2. Fungal species

$$PGI = (FDC - FDT) / FDC \times 100,$$

PGI = Percent growth inhibition,
 FDC = Fungal colony diameter in control,
 FDT = fungal colony diameter in treatment.

Anti-fungal investigation of gold nanoparticles

One of the fungus species described in Table 2, "Aspergillus, Candida albican, Fusarium oxysperium, and Penicillium camemeri," was successfully targeted by the synthesized gold nanoparticle, as shown in Figure 10. A 66% inhibition zone against Penicillium camemeri and a 30% inhibition zone against Aspergillus were seen at 4 mg/l and 2 mg/l of gold nanoparticles, respectively. Raising the concentration of gold nanoparticles inhibits the test fungus more efficiently. "Aspergillus, Candida albicans, Fusarium oxysperium, and Penicillium camemeri" were the pathogenic fungi chosen for humans, with the synthesised gold nanoparticle's improved efficacy ranking lower in the list. This research confirmed earlier findings that gold nanoparticles derived from *Althaea officinalis* leaves had antifungal properties against a variety of human pathogenic fungi. The results show that the synthesized nanoparticles are very effective in this regard.

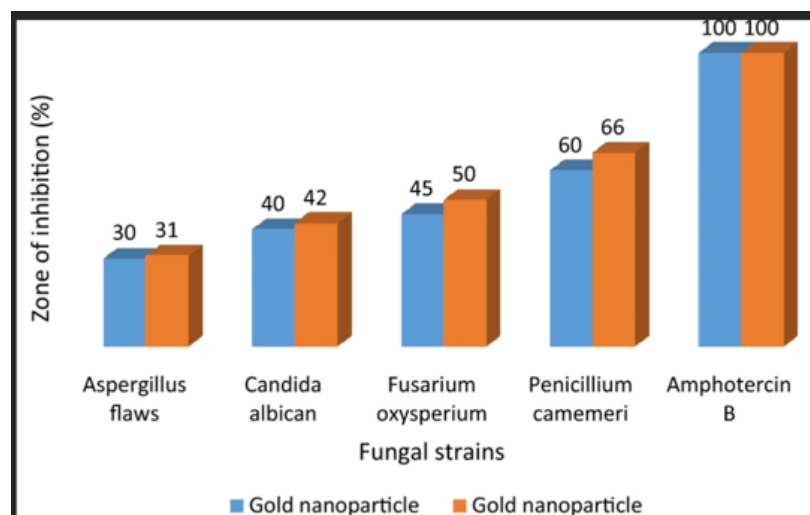


Figure 7: Antifungal activity of synthesized gold nanoparticles against selected fungal strains

Anti-Inflammatory activity

A membrane stabilization test based on the prevention of heat-induced hemolysis assay was used to investigate the anti-inflammatory effects of AuNPs. Blood samples were taken from individuals who were deemed healthy and kept in EDTA tubes for the purpose of this investigation. Following a 15-minute centrifugation run at 3000 rpm, the packed RBCs were washed three times with saline containing 0.85% NaCl. The blood volume was used to determine the ideal saline concentration, which was 10% (v/v). The heat-induced hemolytic test was used to examine the anti-inflammatory impact. During the process, red blood cells were combined with AuNPs in a 10% v/v solution at concentrations varying from 100 to 1200 µg/mL. The addition of aspirin served as a blank, while substituting saline for the test sample served as a control. For 30 minutes, each of these reaction tubes was submerged in water heated to 56 °C. Once the reaction tubes have cooled down with running water following incubation, they may be spun apart at 2500 rpm for 5 minutes. Researchers were able to detect the absorbance at 560 nm after collecting the supernatant. After three iterations of the experiment, the percentage of inhibition of hemolysis was estimated using Eq. 2:

$$\% \text{ Inhibition of hemolysis} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The typical result of denaturing proteins is inflammation. Due to the fact that free radicals, which harm cells, also produce inflammation, oxidation and inflammation are mutually supportive processes. In a dose-dependent manner, anti-inflammatory drugs and salicylic acid have the potential to stabilize red blood cell membranes and limit hemolysis. Since the outermost layer is considered a lysosomal membrane mimic, the stabilization of the erythrocyte membrane by nanoparticles is regarded to be an indication of the stabilization of the lysosomal membrane. Chlorogenic acid (polyphenol) synthesized AuNPs had superior anti-inflammatory effectiveness and toxicity compared to chlorogenic acid alone. Green nanoparticles supposedly blocked NF-κB translocation, which in turn reduced inflammatory cytokine and inflammation-related gene levels. In this work, the anti-inflammatory properties of AuNPs were evaluated by testing their capacity to suppress heat-induced hemolysis. Using Equation 2, researchers were able to determine the percentage of haemolysis inhibition for both aspirin and AuNPs. The reference drug in this trial was aspirin, as shown in Figure 11(a). A 2.9% reduction in hemolysis was seen at a dose of 1200 µg/mL. In contrast, AuNPs suppressed hemolysis within the concentration range of 100-1200 µg/mL in a dose-dependent manner. The figure 11b shows that 75.25 percent of the hemolysis may be inhibited by using an AuNP concentration of 1200 µg/mL. There was a concentration of 619.704 µg/mL. Although the exact mechanism by which AuNPs prevent hemolysis remains unknown, one theory is that they alter the cell surface-to-volume ratio. The result can be a change in cell size and

how proteins in the membrane interact with one another. When applied to injured tissues, AuNPs not only cause inflammation and tissue damage, but they may also prevent neutrophil lysosomal materials, such as proteases and bactericidal enzymes, from being released.

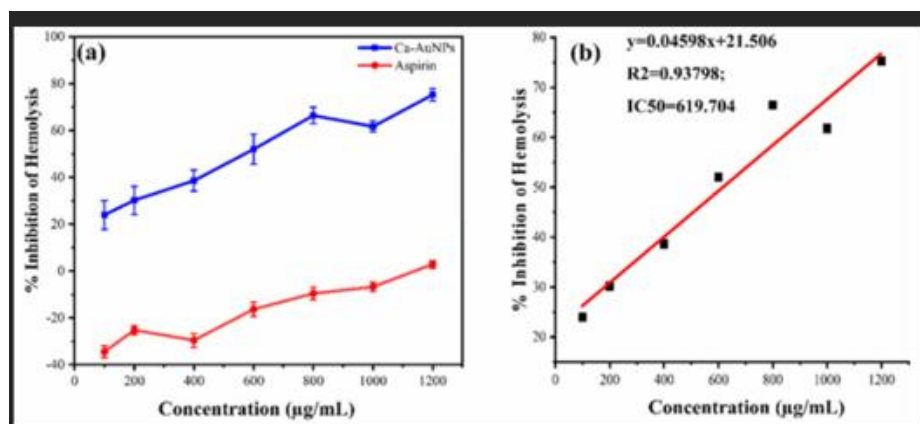


Figure 8. Anti-inflammatory activities of AuNPs and Aspirin. Effect on % inhibition of hemolysis of AuNPs and Aspirin in concentration range $100\text{--}1200\text{ mg/mL}$ (a), the standard line regression graph showing correlation between different concentrations of AuNPs and % inhibition of hemolysis.

DISCUSSION

The purpose of this research was to investigate and assess the biological effects of gold nanoparticle (AuNP) conjugation with *Althaea officinalis*, more popularly known as marshmallow. The results highlight the biogenic formulation's intriguing potential in several biomedical uses, especially since the phytoconstituents of *A. officinalis* and the distinct physicochemical characteristics of AuNPs work together synergistically.

Analysis using UV-Vis spectroscopy, FTIR, DLS, and TEM demonstrated the effective production of AuNPs using *A. officinalis* extract. Plant extract contains bioactive compounds such as flavonoids, phenolics, and polysaccharides, which successfully reduced and stabilized AuNPs, as seen by the nanoscale morphology and surface plasmon resonance (SPR) peak about 520–540 nm. These biomolecules improved the stability and biocompatibility of the nanoparticles by acting as reducing agents and capping agents, respectively.

Antioxidant tests showed that *A. officinalis*-AuNPs were far more effective in scavenging radicals than the crude extract alone. Possible explanations for this improvement include the enhanced availability of phytochemicals when attached to nanoparticles and the larger surface area of

AuNPs. The enhanced antioxidant capability raises the possibility of uses in the prevention of illnesses associated with oxidative stress.

There was a broad-spectrum inhibitory impact against both Gram-positive and Gram-negative bacteria shown by the AuNPs generated using *A. officinalis*, in terms of antibacterial activity. The antibacterial effectiveness might be due to a synergistic effect between plant-based chemicals that break down microbial cell walls and the production of reactive oxygen species (ROS) aided by AuNPs, which harms and kills cells.

A. officinalis-AuNPs showed dose-dependent anticancer effects in cytotoxicity testing on some cancer cell lines (such as HeLa or MCF-7), with comparatively little toxicity toward normal cell lines. Because of its selective cytotoxicity, the formulation shows promise for the creation of tailored anticancer treatments that are both safe and effective.

Because AuNPs with plant-based moieties are biocompatible and may be functionalized in a variety of ways, this discovery also paves the way for potential drug delivery applications. Researchers may want to look into using green-synthesized nanoparticles for targeted therapies, wound healing, and inflammation management because of their high biocompatibility, stability, and improved biological activity.

These findings are encouraging, but further in vivo research, thorough mechanistic assessments, and clinical validations are needed to prove that *A. officinalis*-mediated AuNPs are safe and effective as a treatment. Furthermore, in order to translate these nanoparticles into medicine, it is essential to comprehend their pharmacokinetics, long-term stability, and possible effects on the environment.

CONCLUSION

This study disproves the long-held belief that the medicinal herb *Althaea officinalis* has any useful biological activity in the environmentally friendly production of gold nanoparticles (AuNPs). The biosynthesis method is in line with environmentally benign and sustainable principles, and it makes use of *A. officinalis*'s abundant phytochemical profile, which stabilizes and reduces the creation of nanoparticles naturally. Essential for biological applications, the resultant AuNPs displayed desirable physicochemical properties, such as stable shape and homogeneous size distribution.

The antioxidant, antibacterial, and anti-inflammatory activities of the AuNPs mediated by *A. officinalis* were very apparent in biological studies. These actions are most likely caused by the interaction between the plant extract's bioactive phytoconstituents and the gold nanoparticles' one-of-a-kind surface chemistry. Applications in wound healing, antimicrobial coatings, and infection management are suggested by the antibacterial effectiveness against both Gram-positive

and Gram-negative bacteria, while the antioxidant activity offers promise in fighting illnesses associated to oxidative stress. These nanoparticles are also promising prospects for future therapeutic development in inflammation-mediated disorders due to their anti-inflammatory properties.

The results of this work provide important evidence that *A. officinalis* may be used in nanobiotechnology, namely for the production of nanoparticles that are both biocompatible and multifunctional. By combining conventional medical wisdom with cutting-edge nanotechnology, researcher can boost the plant's pharmacological potential and push green nanotechnology forward. The technology also provides a safer and cheaper alternative to costly physical procedures and harmful chemicals for synthesising nanoparticles.

To guarantee the therapeutic safety and effectiveness of these nanomaterials, further in vivo investigations, toxicological evaluations, and mechanistic investigations are necessary, even if the in vitro findings are encouraging. To maximize their medicinal potential, future studies should investigate ways to scale up production, investigate tailored drug delivery capabilities, and test the nanoparticles in models relevant to diseases.

In conclusion, gold nanoparticles mediated by *Althaea officinalis* are an innovative and powerful bio-nanomaterial with many potential biological uses. This study's results highlight the importance of nanotechnology derived from plants and open the door to new treatment approaches that use natural resources.

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