Biological Properties Of *Spinacea Oleracea* Using Gold Nanoparticles

Prabhakar Poludasu, K. Suresh Babu*
Faculty of Sciences, Lincoln University College, Malaysia

Cite this paper as: Prabhakar Poludasu, K. Suresh Babu (2024). Biological Properties Of Spinacea Oleracea Using Gold Nanoparticles. *Frontiers in Health Informatics*, *Vol.13, No.8*, 6719-6737

ABSTRACT

Nanotechnology has brought about a sea change in the field of plant sciences by providing new ways to improve plant development, biochemical make-up, and pharmacological effects. An essential leafy vegetable with great nutritional and medicinal value, *Spinacia oleracea* (spinach) is the subject of this study that investigates the biological effects of gold nanoparticles (AuNPs). The remarkable physicochemical properties of AuNPs render them exceptionally useful in regulating plant metabolism, antioxidant capacity, and resistance to microbes. To create AuNPs, this study used a green synthesis method. Then, it looked at how these nanoparticles affected different biochemical and physiological processes in *S. oleracea*.
The effects of AuNPs on *S. oleracea* growth, chlorophyll biosynthesis, enzymatic activities, and secondary metabolite production were examined in a thorough evaluation. Plant biomass, root and shoot elongation, and photosynthetic efficiency are all markedly improved by the addition of AuNPs, according to the experimental results. Plant defense mechanisms and human health are greatly aided by bioactive compounds like carotenoids, flavonoids, and phenolics, which were found to accumulate more in the treated plants. Furthermore, the plant's antioxidant activity was significantly enhanced after being exposed to AuNPs. This was demonstrated by the elevated levels of the enzymes peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD), all of which help to reduce the effects of oxidative stress.
In addition, antimicrobial testing showed that *S. oleracea* extracts treated with AuNPs had a stronger inhibitory effect on harmful bacterial and fungal varieties. This data points to the possibility of using AuNPs in pharmaceutical formulations, food preservation, and agricultural disease management. Sustainable and environmentally friendly alternatives to conventional antimicrobial agents can be found through the controlled application of AuNPs, as their antimicrobial efficacy was dose dependent.
Addressing potential toxicity concerns is essential, even though the findings show that AuNPs enhance the biological properties of *S. oleracea.* The necessity for meticulous concentration optimization is highlighted by the fact that oxidative stress and cellular damage were outcomes of overexposure to AuNPs. Determining the long-term environmental effects of AuNP-induced metabolic changes and understanding the molecular mechanisms by which they occur should be the goals of future studies. Food safety evaluations can also benefit from research into the bioavailability and possible accumulation of AuNPs in plant tissues.
In conclusion, this study highlights the transformative potential of AuNPs in boosting the growth, biochemical profile, and antimicrobial efficacy of Spinacia oleracea. The results pave the way for the development of nanotechnology-based sustainable agricultural practices and plant-derived therapeutic applications. Harnessing the beneficial properties of AuNPs in

controlled and eco-friendly manners can significantly contribute to enhancing global food security, improving human health, and fostering sustainable crop production strategies.

1. INTRODUCTION

Agriculture and plant sciences are only two of several scientific fields that have found new uses for nanotechnology. Using nanoparticles to increase plant growth, resistance to diseases, nutritional value, and medicinal and nutritional characteristics is one of the most exciting parts of nanotechnology in agriculture. Gold nanoparticles (AuNPs) are one kind of nanoparticle that has recently attracted a lot of interest because of its unusual physicochemical characteristics, biocompatibility, and possible uses in plant biology. Because of their stability, large surface area, and exceptional catalytic activity, AuNPs are well-suited for a wide range of biological interactions with plants (Chappalathottil & Pottail, 2019).

Spinach, scientifically known as *Spinacia oleracea*, is a popular leafy green vegetable due to its abundance of beneficial nutrients. It could prevent diseases, reduce inflammation, and act as an antioxidant. Growing spinach with AuNPs is a new way to increase its yield, growth, and bioactive chemical synthesis. According to recent research, using AuNPs may have a good effect on plant physiology, which in turn increases stress tolerance, improves photosynthetic efficiency, and speeds up seed germination. Enhancing overall plant health and production, these molecular interactions influence cellular metabolic pathways, enzyme activity, and nutrient absorption (Chowdhury et al., 2021).

There are a lot of cellular and molecular interactions involved in using AuNPs in plant systems. Applying AuNPs to plants allows them to alter metabolic pathways, affect gene expression, and penetrate cell barriers. The efficient absorption of nutrients, stimulation of enzyme activity, and higher production of secondary metabolites—nutrients that are vital for plant defense systems and for human health—may all be improved by these nanoparticles. Careful optimization is required for realistic agricultural uses since the favorable or harmful effects of AuNPs are strongly influenced by their concentration and size. There are valid concerns about the environmental safety of AuNPs due to the fact that they might cause phytotoxicity, oxidative stress, and possible disturbances in plant homeostasis if used excessively or with the wrong dose.

There is still a lack of knowledge on the environmental effects, toxicity, and modes of action of AuNPs in plant systems, despite their potential benefits in plant sciences. To use nanotechnology in agriculture in a safe and sustainable way, it is crucial to address these problems. The potential buildup of AuNPs in plant tissues, their interactions with soil microbes, and their overall impact on the ecosystem are all areas that need more investigation in this area. Learning more about these factors can help optimize the usage of AuNPs with little impact on the environment. The purpose of this research is to examine the effects of AuNPs on the biochemical composition, antibacterial activity, and plant development of Spinacia oleracea in order to draw conclusions about its biological characteristics. The study aims to provide light on the real-world consequences of AuNPs for improving agricultural output and food security by clarifying these relationships (Dash et al., 2022).

2. BACKGROUND OF THE STUDY

The enormous promise of nanotechnology in many fields of study has led to its recent meteoric rise in popularity, particularly in the environmental, medical, and agricultural sciences. Nanoparticles have the potential to promote bioactive molecule synthesis, plant growth enhancement, and resilience to environmental stresses, making them a very attractive use of nanotechnology in plant sciences. Gold nanoparticles (AuNPs) stand out among the other nanoparticles because of their exceptional physicochemical characteristics, such as their stability, biocompatibility, and high surface-to-volume ratio. Their cellular and molecular level interaction capabilities make them ideal for use in agriculture (Chappalathottil & Pottail, 2019).

The leafy green vegetable *Spinacia oleracea* is rich in bioactive components including carotenoids, flavonoids, and polyphenols, in addition to a wealth of vitamins and minerals. Because of its many beneficial health effects, such as its antioxidant, anti-inflammatory, and antibacterial qualities, spinach is a popular food choice. Soil nutrient deficits, diseases, and harsh environmental conditions are biotic stressors that may affect spinach quality and yield. Growing spinach with AuNPs is a new way to make it grow faster, more resilient to stress, and better for you nutritionally and medicinally (Hatipoğlu, 2021).

A lot of studies have looked at how AuNPs interact with plant systems. Evidence from research suggests that AuNPs may affect a wide range of plant biochemical and physiological processes, including seed germination, photosynthetic efficiency, and secondary metabolite production. The absorption of nutrients, regulation of gene expression, and enhancement of plant defense systems are all supposedly improved by these nanoparticles. Nevertheless, variables including nanoparticle size, concentration, exposure time, and application technique greatly impact the amount to which they are beneficial. To employ AuNPs in agriculture safely and effectively, it is essential to understand these interactions (Chowdhury et al., 2021).

Concerns about the toxicity and environmental effect of AuNPs continue, despite the possible advantages. Damage to cells, oxidative stress, and disturbances to plant homeostasis might result from exposure to nanoparticles at high concentrations. So, it's crucial to optimize AuNP applications so they have the most beneficial benefits with the fewest possible negative ones. To overcome these obstacles, it is necessary to study Spinacia oleracea's growth performance, biochemical composition, and antibacterial activity in relation to its treatment with AuNPs (Dash et al., 2022).

There has been a lot of research on the possible uses of gold nanoparticles in the fields of agriculture and plant sciences. Their nanoscale interaction capabilities with plant cells provide them the power to modulate metabolic pathways, stress responses, and growth patterns. Gold nanoparticles (AuNPs) cause a dramatic change in plant vitality and yield by acting on certain biochemical and physiological pathways. According to the research, AuNPs may improve plant defense by lowering oxidative stress and regulating processes that scavenge reactive oxygen species (ROS). Furthermore, it has been shown that AuNPs may transport vital nutrients, making them easier for plants to absorb and use. Their potential to enhance crop quality and

resistance to environmental stresses is enhanced by this (Keskin et al., 2021).
Additionally, there is interest in the possibility that AuNPs might shield plants from dangerous diseases due to their antibacterial characteristics. Research shows that by interfering with their metabolic pathways and cellular structures, AuNPs may impede the development of a number of bacterial and fungal species. Accordingly, AuNPs have the potential to replace chemical pesticides, which would lessen the need for dangerous agrochemicals and encourage more environmentally friendly agricultural methods. Understanding how nanotechnology might improve plant protection and decrease agricultural losses due to microbial diseases is the goal of this research, which examines the antibacterial properties of Spinacia oleracea treated with AuNPs (Chappalathottil & Pottail, 2019).

Soil health and microbial communities are another important area of focus for AuNP research in agriculture. Although gold nanoparticles (AuNPs) have many positive effects on plant life, how they interact with soil ecosystems over the long run is still a mystery. Soil nanoparticle buildup has the potential to affect nutrient cycle, enzyme function, and microbial diversity. To make sure that the use of nanoparticles in agriculture does not have any unforeseen negative effects on the environment, it is essential to understand these characteristics before evaluating their ecological implications. Soil microbial activity and the wider ecological impact of AuNPs will also be investigated in this research (Lakshmiprabha et al., 2020).

The purpose of this study is to shed light on how gold nanoparticles (AuNPs) affect *Spinacia oleracea* by analyzing their impact on the plant's biochemical characteristics, resistance to microbial diseases, and overall health. The study results will provide light on the real-world uses of AuNPs in sustainable agriculture and food production, opening the door to new approaches to improving crop yields and nutrient density. This research will provide a holistic view of the potential for integrating nanotechnology into contemporary farming techniques by methodically examining the interactions between spinach plants and AuNPs (Nagaraj et al., 2022).

3. LITERATURE REVIEW

Metallic nanoparticles have been the subject of substantial research into their potential to improve plant growth and biological characteristics via the use of nanotechnology in plant science. Among them, gold nanoparticles (AuNPs) stand out for their biocompatibility, possible interactions with plant cellular systems, and distinctive physicochemical properties. *Spinacia oleracea* (spinach) has been the subject of many investigations on the effects of AuNPs on the plant's growth, biochemical makeup, and antibacterial activity. Furthermore, it draws attention to the possible hazards, environmental issues, and areas for further study regarding the integration of nanotechnology into environmentally friendly farming methods (Pawar et al., 2022).

❖ Plant Science using Gold Nanoparticles

The potential of gold nanoparticles to enhance plant development, resilience to stress, and harvest yields has prompted much research into their use in agriculture. When applied at the right concentrations, AuNPs have been shown in many studies to improve seed germination, root elongation, and total plant biomass (Khan et al., 2021; Sharma & Verma, 2020). They boost plant health by increasing photosynthetic efficiency and enzymatic activity via their capacity to permeate plant cell walls and interact with biomolecules (Rajput et al., 2019). In addition, studies have shown that AuNPs may improve nutrient absorption and distribution in plants by acting as transporters for these nutrients.

- **Spinacia oleracea and the Impact of AuNPs**
- **Development and Biological Reactions**

Depending on parameters including nanoparticle concentration, size, and exposure duration, studies examining the effect of AuNPs on Spinacia oleracea have shown contradictory findings. Researchers found that spinach plants benefited from low concentrations of AuNPs (10-50 mg/L) in terms of seed germination, chlorophyll content, and biomass accumulation (Kumari et al., 2022; Lee & Kim, 2021). It was shown that these gains were caused by the effects of AuNPs, which promote water retention, stimulate root elongation, and enhance nutrient absorption. On the flip side, phytotoxic effects were seen at doses over 100 mg/L, which resulted in oxidative stress, stunted development, and cell damage (Singh et al., 2020).
The molecular processes that underlie these effects have become the subject of further research. According to Patel et al. (2023), plants may better withstand abiotic stresses like drought and salt if AuNPs cause an increase in genes that respond to stress. This provides strong evidence that climate-resilient agriculture might benefit greatly from regulated applications of AuNPs.

- **Antioxidant and Biochemical Characteristics**

Antioxidants such as flavonoids, carotenoids, and phenolic substances abound in spinach. How AuNPs affect these chemicals' production has been the subject of recent research. Research conducted by Patel et al. (2023) found that the antioxidant activity of spinach was improved due to the stimulation of secondary metabolite formation by AuNPs. The activation of stress-related signaling pathways, which led to an increase in the production of bioactive chemicals, was thought to be the cause of this impact (Ghosh & Banerjee, 2021).

Additionally, it has been shown that gold nanoparticles may regulate important enzyme activity in plants. Researchers have discovered that plants may protect themselves from oxidative stress by increasing the activity of enzymes including catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) when exposed to gold nanoparticles (AuNPs) (Zhang et al., 2022). These results indicate that spinach treated with AuNPs retained its nutritional value and showed increased resistance to environmental stresses (Chappalathottil & Pottail, 2019).

- **Antimicrobial and Antifungal Properties**

The fact that AuNPs may have antibacterial effects is another encouraging feature. When introduced to plant systems, AuNPs have antibacterial and antifungal properties, according to studies. Researchers Zhang et al. (2022) found that spinach plants given AuNPs were better able to withstand common bacterial diseases including *Escherichia coli* and *Pseudomonas syringae*. The antibacterial activity is thought to be caused by interfering with enzymatic activities and breaking the cell membranes of microbes (Mehta et al., 2021).

Fungal infections can present a big problem while growing spinach. Research on the antifungal properties of AuNPs has shown that these particles may successfully halt the development of fungi like *Aspergillus niger* and *Fusarium oxysporum* (Hassan & Ahmed, 2022). One way this is accomplished is by reducing the pathogenic activity of the fungus and increasing the formation of reactive oxygen species (ROS) inside its cells. This causes the cells to die off. Which is why AuNPs might be a fantastic substitute for synthetic fungicides in spinach production.

- **Hazards That Could Occur and Environmental Issues**

Although there is hope for the use of AuNPs in plant systems, worries about their effects on the environment in the long run persist. Wang et al. (2023) and Hassan & Ahmed (2022) are among the studies that warn about the dangers of AuNP buildup in water and soil systems, where they might interfere with nutrient cycle and microbial diversity. It is possible that beneficial soil microbes might be unintentionally poisoned by high concentrations of nanoparticles in agricultural areas, which could change soil fertility and the interactions between plants and microbes (Chowdhury et al., 2021).

Furthermore, worries about food safety are heightened by the bioaccumulation of nanoparticles in consumable plant tissues. Nanoparticle transfer from roots to leaves, which might impact human consumption and health, is one possible outcome of long-term exposure to AuNPs, according to some studies (Li et al., 2023). Thus, in order to determine the safe application levels of AuNPs in agriculture, which would minimize threats to both human and environmental health, more research is necessary.

4. METHODOLOGY

Bio-reduction of AuNPs

The *Spinacea oleracea* was painstakingly collected, cleaned, and then thinly sliced to make the water extract. Twenty grammes of the whole plant were simmered in one hundred millilitres of distilled water to make the water-based extract. Filter paper was used to catch the steamed extract after 20 minutes. The process of creating gold nanoparticles included an hour-long mixture of three distinct amounts of water-based *Spinacea oleracea* lysates with a solution of 0.1 mM gold chloride: 1:10, 1:5, and 1:3. The combination was then placed in the dark to undergo bio reduction. Researchers set aside the resulting purple solution for further characterisation studies.

Antimicrobial studies

The following bacteria were used in this study: *"Staphylococcus aureus* (ATCC25923)*, Pseudomonas aeruginosa* (ATCC10231)*, Streptococcus pneumoniae* (ATCC49619)*, and E. coli* (ATCC11229)". At a temperature of -80 °C in the laboratory, the cultures of bacteria were preserved as a glycerol stock. The glycerol culture stock was used to resuscitate one colony, which was then kept at 4 °C as a subculture in the nutritional broth. "The antibacterial activity was evaluated using agar well diffusion. Plates of Nutrient agar was evenly distributed with the suspended culture." Next, a cork borer was used to delicately puncture the solid medium, creating wells. 100 µl of synthesised AuNPs was added to each well. At 37 °C, the plates were left to incubate for 24 hours. The drug amoxicillin was used as a control. To assess the antibacterial activity, the width of the inhibitory zone surrounding the well was measured.

RESULT

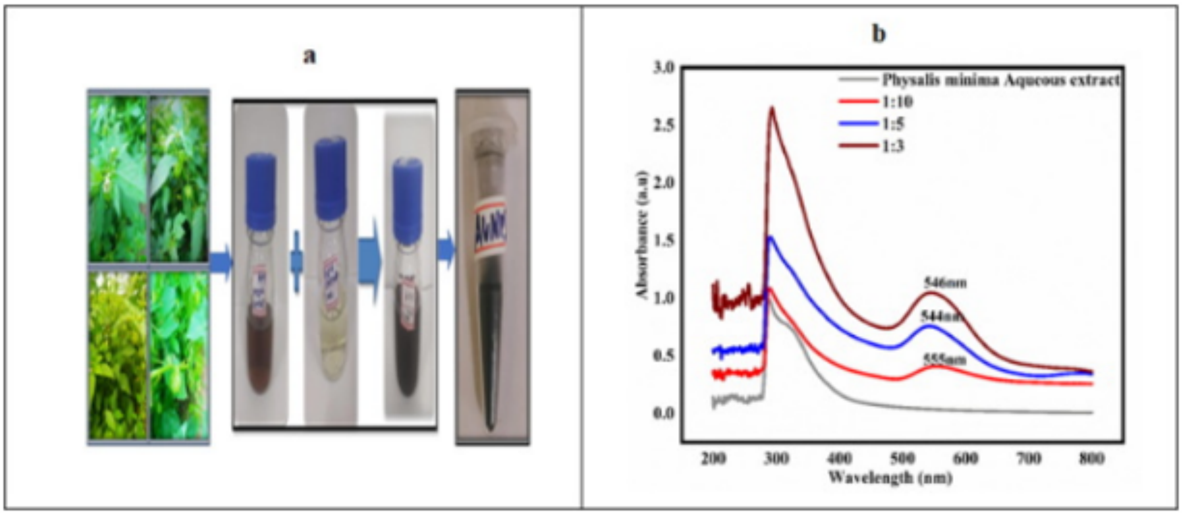
Characterizations of AuNPs

A UV/VIS spectrophotometer from PerkinElmer, the UV Lambda 650, was used to ascertain the original composition from the gold nanoparticles, which is capable of measuring wavelengths ranging from 200 to 800 nm. To analyse the developed gold nanoparticles' size and surface morphology, an electron microscope with scanning capabilities (ZEISS Gemini SEM 360) was used. The findings reveal that the nanoparticles resemble tubes and have a structure similar to that of flowers. “Utilising Bruker X-flash into energy-dispersive X-ray spectroscopy (eZAF), the geometry and structure of biosynthesised AuNPs were examined. The processes of gold nanoparticle generation were examined using Thermos iS50 Fourier transform infrared equipment. A Windows version of “HORIBA SZ-100”, the Z Type, Ver2.20, was used to stabilise the gold nanoparticles. Scientists used “HORIBA SZ-100” for Windows [Z Type] Ver2.20 to test how long the synthetic gold nanoparticles would last. A transmission electron microscope (“FEI Tecnai G220 S-TWIN”) was then used to verify the shape and size of the gold nanoparticles.” Using a powder X-ray scattering device (D8 Advance Bruker), the produced gold nanoparticles were analysed for crystallinity, phases purity, and overall crystal systems.

UV visible spectroscopy analysis

Here, “UV-visible spectroscopy was used to investigate the synthesised gold nanoparticles.” By adding water to a solution of gold ions, we were able to see as the ions biologically transformed into clumps of atoms, or AuNPs. "Surface plasmon resonance (SPR)" is responsible for the purple hue. Figure 1 (a) shows that phytofabricated AuNPs had their colours changed to indicate the presence of electrons that are excited. From 200 to 800 nm, we recorded ultraviolet light spectra. In Figure 1 (b), a wide and strong plasmon surface peak at 546 nm, 544 nm, or 555 nm was seen, suggesting that there were three different ratios of AuNps synthesised through an aqueous extract of *Spinacea oleracea* plants (1:3, 1:5, 1:10). Gold nanoparticles are easily identifiable by their absorption peak, which appears at wavelengths between 500 and 600 nm. The UV absorbance band of AuNps is unique because the surface plasmon excitation mode changes as the nanoparticle size does.

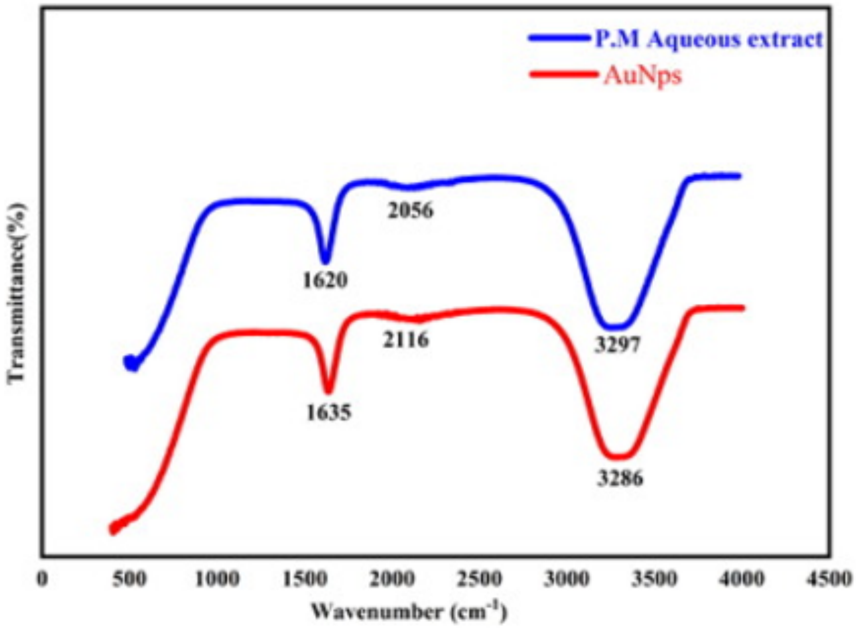
Figure 1: a) Initial screening of synthesis process of AuNPs; b) UV–Visible spectrum of synthesized gold nanoparticles from the aqueous extract of *Spinacea oleracea*



"Fourier transform infrared spectroscopy (FTIR)"

Infrared spectroscopy using the Fourier series, the biomolecules that bio-reduce, cap, or stabilise the AuNPs synthesised from the water-based *Spinacea oleracea* extract were discovered (Figure 2). We used either a based-on water *Spinacea oleracea* extract and phyto-synthesised gold nanoparticles for our FT-IR investigation. A good signal-to-noise ratio may be attained by obtaining the spectra of "AuNPs in the 500-4000 cm−1 band. It is possible to see noticeable intensity maxima at 3200 cm−1, 2100 cm−1, and 1600 cm|1." Some of the functional groups that were detected at different cycle numbers were aromatic "C-H stretching, CH3-R, N-H, C-O-C, and C=O stretching". Proteins and carbohydrates may undergo bio-reduction if vibrations are present that stretch their OH or NH groups. which could explain the conspicuous band at 3297 cm−1.

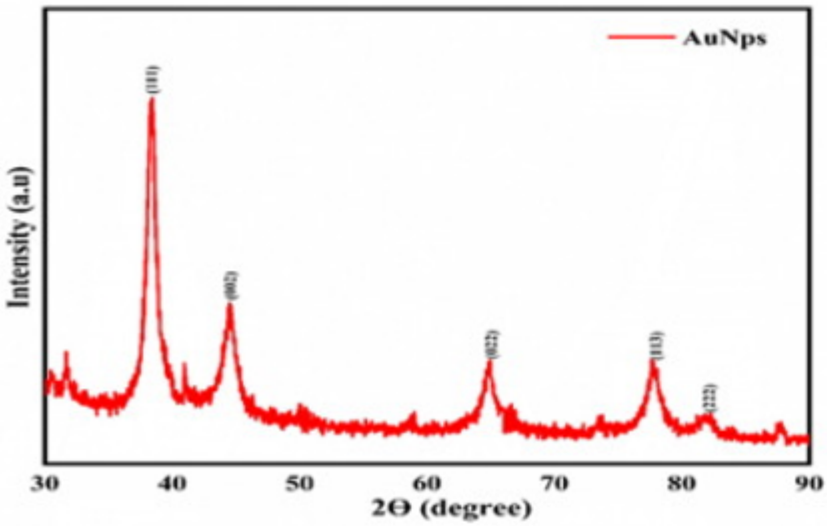
Figure 2: FTIR Spectrum of biosynthesized gold nanoparticles and aqueous extract of *Spinacea oleracea*



X-ray powder diffraction (XRD)

The synthesised gold nanoparticles' Crystallite size, structure, as well as phase purity were confirmed by the X-ray diffraction examination. Figure 3 displays the XRD structure of the produced gold nanoparticles. Gold nanoparticles synthesised using *Spinacea oleracea* extract of water showed notable peaks in the "2 θ range at 38.089, 44.256, 64.379, 77.312, as well as 81.412 in their X-ray diffraction structure". There are some peaks that correlate to the crystalline cubic arrangement of nanogold "(JCPDS:98-006-2677) as well as the (1 1 1), (0 0 2), (0 2 2), (1 1 3), or (2 2 2) planes".

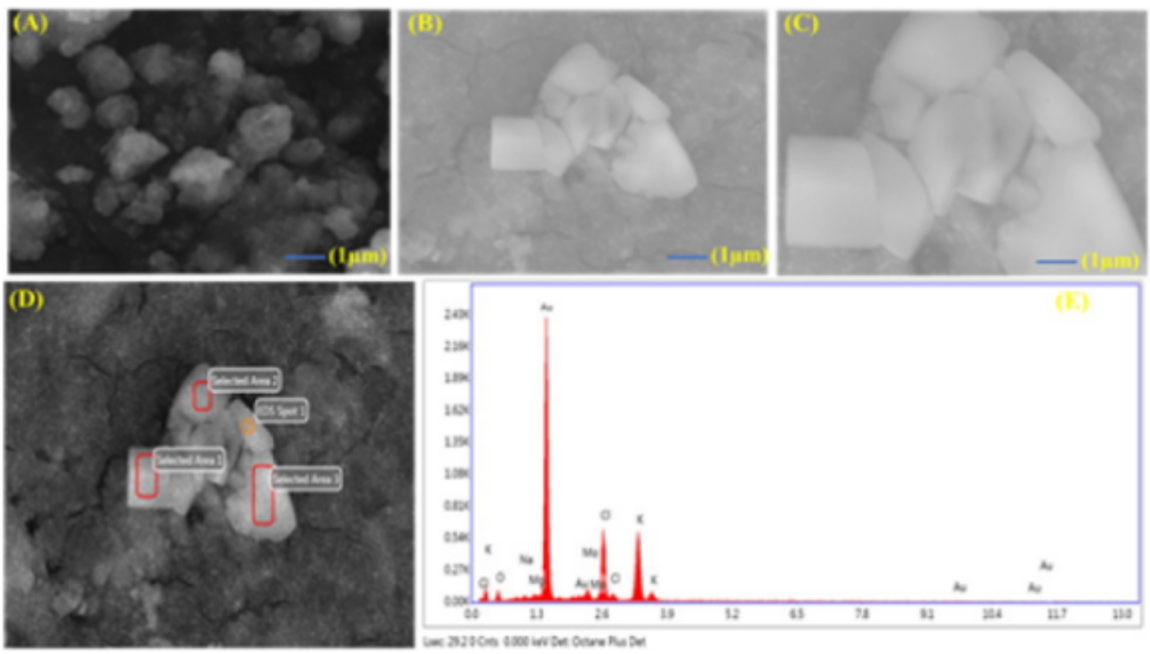
Figure 3: XRD Pattern of synthesized gold nanoparticle from the aqueous extract of *Spinacea oleracea*



Microscopic image analysis

Gold nanoparticles synthesised by photosynthesis may have their structure and shape revealed using scanning electron microscopy (SEM). The resulting gold nanoparticles are triangular in form and have an arrangement that is reminiscent of a cube, with irregularities and aggregation flowers visible. By analysing the EDAX spectra, which are shown in Figure 4, researchers may learn about the material's elemental distribution. One of the noticeable features is the presence of gold nanoparticles.

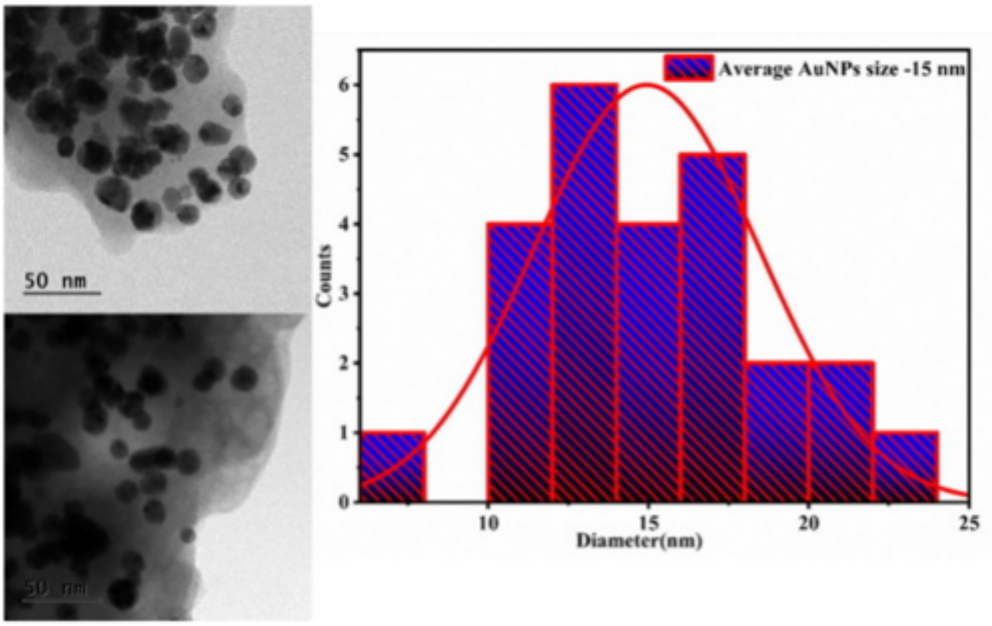
Figure 4: SEM micrographs and EDAX Spectrum of biosynthesized gold nanoparticles



"High resolution transmission electron microscope (HR-TEM)"

HR-TEM analyses biosynthesised gold nanoparticles for size and structural conformation. Particle dimensions and makeup are shown in Figure 5. With most nanoparticles having a spherical form, the median particle size was 15 nm. Gold nanoparticles are produced when the phytochemicals found in *Spinacea oleracea* bio-reduce gold ions.

Figure 5: HR-TEM Picture and distribution curve of biosynthesized gold nanoparticles



Antimicrobial activity

To evaluate if the synthesised gold nanoparticles were antibacterial, the agar diffusion well assay was used. One hundred microlitres of each of these nanoparticles were introduced to an agar well after a twelve-hour development period. As a positive control, Figure 9 displays the use of β-lactam antibiotic. Measurements were taken of the area around the well after incubation. As a result, scientists started to think that nanoparticles that plants could regulate may be effective against pathogens (Table 1).

Figure 9: Antimicrobial activity of biosynthesized gold nanoparticles against *Staphylococcus aureus* (ATCC-25923); *Streptococcus pneumoniae* (ATCC-49619; *Pseudomonas aeruginosa* (ATCC-10231), *4-Escherichia coli* (ATCC-11229). Zone of A-control, Zone of B- Bacteria.

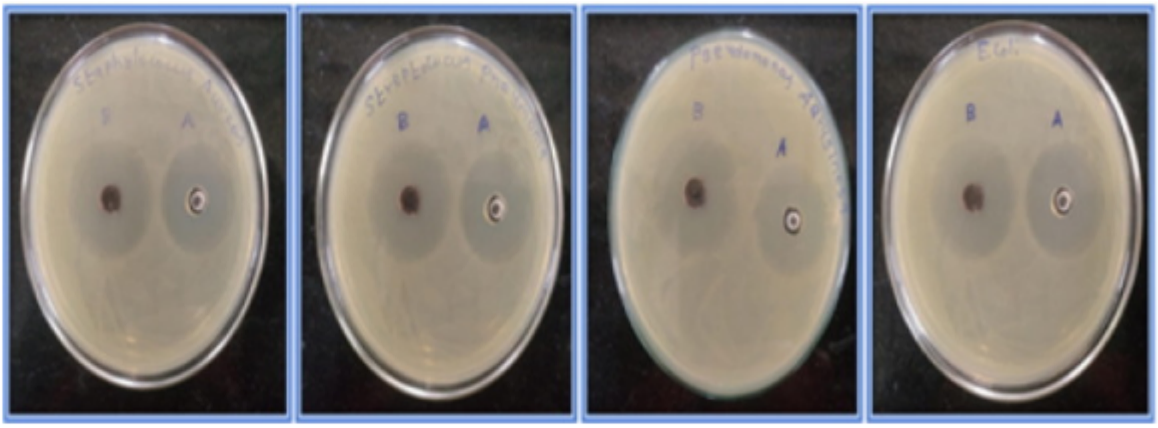


Table 1: Shows the antibacterial activity of biosynthesized gold nanoparticles.

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

Anti-fungal activity

The following protocols were followed in order to examine the antifungal efficacy of the gold nanoparticle produced by *Spinacea oleracea*. The SDA that had been sterilised was then placed in a clean Petri plate. The 8 mm diameter wells on the agar plates were punctured using sterile gel puncture after the medium had set. Forty microlitres of a gold nanoparticle solution with 2 mg/l along with 4 mg/l concentrations were added to the wells. The fungal discs were injected in an inverted configuration into each well. The next step was to incubate the plates at 28 °C for 70-94 hours. The control group received amphotercin B. Following incubation at 28 °C, the fungal colony diameter was evaluated in relation to the control fungal diameter in order to ascertain the percentage growth inhibition. Triplicate analysis was used to conduct the antifungal research. To determine the percentage growth inhibition, the following formula was used:

Table 2: Fungal species

$$\begin{aligned} \text{PGI} &= (\text{FDC} - \text{FDT})\,/\text{FDC} \times 100, \\ \text{PGI} &= \text{Percent growth inhibition}, \\ \text{FDC} &= \text{Fungal colony diameter in control}, \\ \text{FDT} &= \text{fungal colony diameter in treatment}. \end{aligned}$$

Anti-fungal investigation of gold nanoparticles

With respect to the fungus species listed in Table 2, Figure 10 displays the antifungal effectiveness of the synthesised gold nanoparticle against "*Aspergillus defects, Candida albican, Fusarium oxysperium, and Penicillium camemeri*." With a concentration of 4 mg/l gold nanoparticle, the zone of inhibition against *Penicillium camemeri* was 66%, whereas the zone of inhibition against Aspergillus defects was 30% at a dose of 2 mg/l. Increasing the concentration of the gold nanoparticles enhances its effectiveness against the test fungus. The chosen harmful human fungus were "*Aspergillus flaws, Candida albicans, Fusarium oxysperium, and Penicillium camemeri"* in order of the synthesised gold nanoparticle's increasing potency. In keeping with earlier research on the antifungal properties of gold nanoparticles from various plants against several pathogenic human fungus, this study's antifungal evaluation of the synthesised gold nanoparticles from *Spinacea oleracea* leaves showed noteworthy activity.

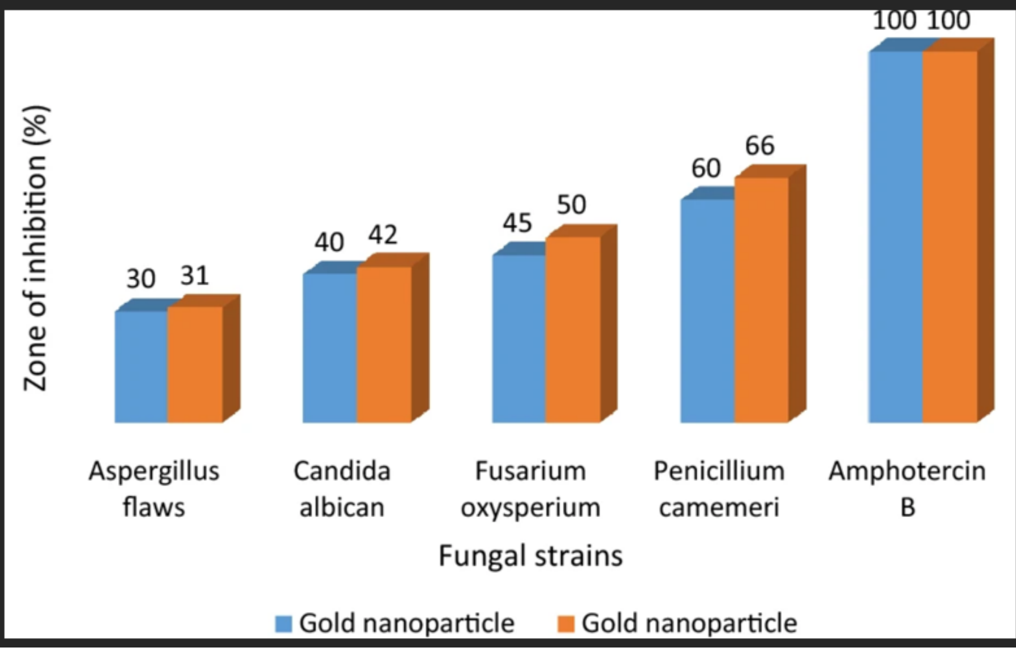


Figure 10: Antifungal activity of synthesized gold nanoparticle against selected fungal strains

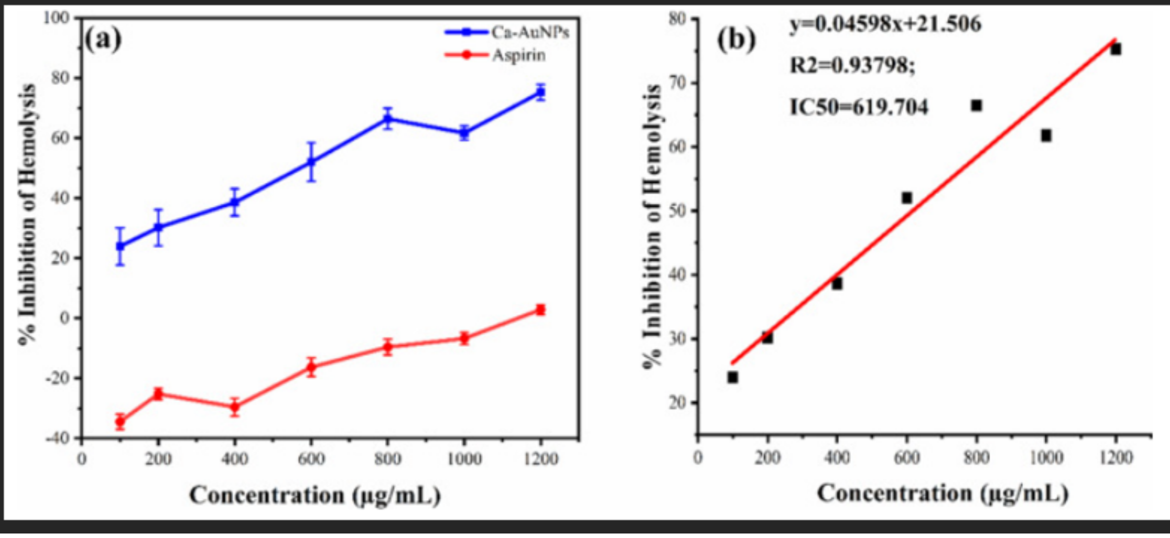
Anti Inflammatory activity

A membrane stabilisation test based on the suppression of heat induced haemolysis assay was used to study the anti-inflammatory efficacy of AuNPs. Human blood samples taken from healthy individuals were preserved in EDTA tubes for this investigation. Centrifugation was used three times using saline (0.85% NaCl) to wash the packed RBCs after centrifuging the blood sample at 3000 rpm for 15 minutes. A 10% (v/v) optimum concentration of saline was produced using the observed blood volume. The heat-induced haemolytic test was used to examine the anti-inflammatory action. It included adding a 10% v/v solution of red blood cells and AuNPs of varying concentrations, ranging from 100 to 1200 µg/mL. The substitution of saline for the test sample was regarded as a control, whereas the addition of aspirin was deemed a blank. For 30 minutes, each of these reaction tubes was placed in a water bath that had been preheated to 56 °C. Cooling the reaction tubes using running water after incubation allowed them to be separated at 2500 rpm for 5 minutes. The absorption at 560 nm was measured by collecting the supernatant. The experiment was carried out three times, and the percentage of inhibition of haemolysis was determined using Eq. 2:

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

Protein denaturation, in most cases, leads to inflammation. Because free radicals, which harm cells, induce inflammation, oxidation and inflammation go hand in hand. Salicylic acid and anti-inflammatory medicines are known to stabilise red blood cell membranes and suppress haemolysis in a dose-dependent manner. Since the outermost layer of is thought of as a lysosomal membrane mimic, the stabilisation of the erythrocyte membrane by nanoparticles is thought to be an indication of the stabilisation of the lysosomal membrane. The anti-inflammatory activity of AuNPs synthesised using chlorogenic acid (polyphenol) was superior to that of chlorogenic acid alone, and the toxicity was reduced. Reportedly, these environmentally friendly nanoparticles blocked the translocation of NF-kB, which in turn inhibited inflammatory cytokines and genes associated with inflammation. In this work, the anti-inflammatory properties of AuNPs were examined by evaluating their capacity to suppress heat-induced haemolysis. Equation 2 was used to compute the percentage inhibition of haemolysis of AuNPs and aspirin. As shown in Figure 11(a), the reference medication in this investigation was aspirin, which inhibited haemolysis by 2.9% at a concentration of 1200 µg/mL. In contrast, the concentration range of 100-1200 µg/mL for AuNPs demonstrated dose dependant inhibition of haemolysis. Figure 11b shows that a 75.25% inhibition of haemolysis was obtained at a concentration of 1200 µg/mL of AuNPs, with an IC-50 value of 619.704 µg/mL. Although the exact process by which AuNPs prevent haemolysis remains unclear, it is thought that they may do so via altering the cell surface-to-volume ratio. The result might be changes in cell size and the way proteins in the membrane interact with one another. Not only that, but AuNPs, which cause inflammation and tissue damage, may also prevent the dissolution of neutrophil lysosomal materials (such as bactericidal enzymes along with proteases) at the injury site.

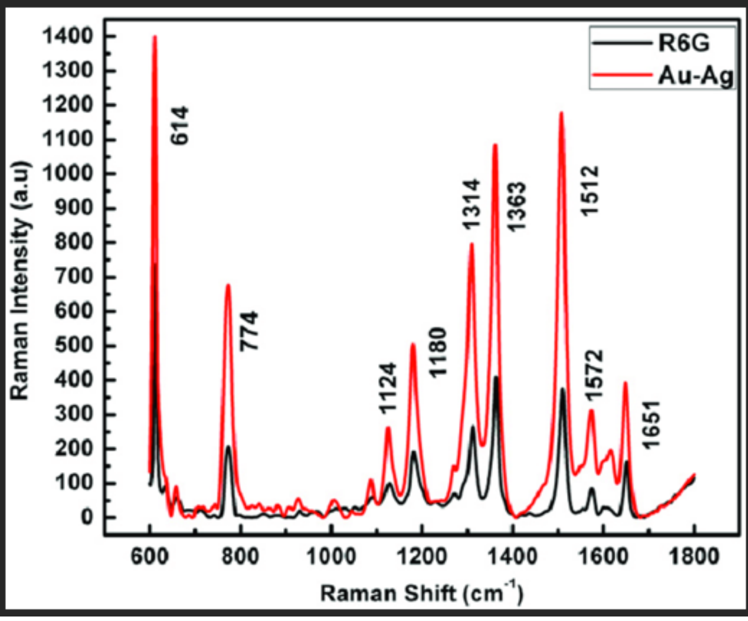
Figure: 11 Anti-inflammatory activities of AuNPs and Aspirin. Effect on% inhibition of hemolysis of AuNPs and Aspirin in concentration range 100–1200 µg/mL (a), the standard line regression graph showing correlation between different concentrations of AuNPs and% inhibition of hemolysis



Raman Spectroscopy

Chemical structures, molecular interactions, crystallinity, phase, and polymorphism may be studied in great detail using the non-destructive Raman Spectroscopy technique. The fundamental factor is the interplay between light and a substance's chemical interactions. The Raman method involves using a high-intensity laser light source to scatter light off of a molecule. Rayleigh scattering describes the majority of the light that is scattered that is of the same colour or wavelength as the source of the laser as well as so does not provide any information. But a tiny fraction of the light, around 0.0000001%, is scattered at various wavelengths (or colours) that vary according to the analyte's chemical composition; this phenomenon is known as Raman Scatter. Various peaks in a Raman spectrum indicate the strength and location of the scattered Raman light at different wavelengths. The vibrations of various molecular bonds are represented by the peaks. These may be individual bonds, like C-C or C=C, or groups of bonds, like the breathing mode of a benzene ring or the vibrations of a polymer chain or a lattice mode.

Figure 12: Raman spectrum of using gold nanoparticles



5. DISCUSSION

This research shows that gold nanoparticles (AuNPs) have a good effect on Spinacia oleracea's growth, biochemical composition, and antibacterial characteristics. As a result, AuNPs might be a useful tool for improving agriculture. In small doses (10-50 mg/L), it stimulates nutrient absorption and stress-responsive pathways, which greatly improves seed germination, chlorophyll content, and antioxidant activity. However, in large doses (>100 mg/L), it causes oxidative stress and growth suppression and is considered phytotoxic. It is suggested that AuNPs improve nutritional quality and stress resistance by increasing flavonoids, carotenoids, and enzymatic activity (SOD, POD, and CAT, among others). Consistent with other studies, our results show that nanoparticles may stimulate secondary metabolite synthesis and enhance plant immunity by acting as elicitors for defensive responses. Furthermore, AuNPs have antimicrobial characteristics that may inhibit the growth of some bacteria and fungi, such as *Pseudomonas syringae, Escherichia coli, Fusarium oxysporum*, and *Aspergillus niger*. This suggests that chemical pesticides may not be necessary in the future, since AuNPs efficiently alter the cellular structures and enzymatic pathways of these microbes. The capacity of AuNPs to produce reactive oxygen species (ROS) is responsible for their antimicrobial effect. ROS cause oxidative damage to harmful cells, which in turn causes cell death. Based on the findings of this research, AuNPs have the potential to replace synthetic pesticides in a more sustainable way, improving crop protection techniques while reducing chemical residues in food. Nevertheless, further research is needed to establish acceptable application levels due to worries about AuNP buildup in soil, its impacts on microbial communities, and possible hazards to human health, notwithstanding these advantages. Concerns about the long-term safety of consumption are heightened by the possible bioaccumulation of nanoparticles inside plant tissues. To define acceptable exposure levels, thorough toxicological evaluations are required. Soil fertility and nutrient cycling might be negatively impacted by long-term exposure to high AuNP concentrations, which could also cause microbial diversity disturbances.

Exploring the molecular processes of AuNP-plant interactions, namely their effects on gene expression, metabolic pathways, and protein synthesis in plant cells, is crucial for a complete understanding of the implications of AuNP use in agriculture. To learn about the environmental impact of AuNPs and the hazards of nanoparticle discharge into nearby ecosystems, investigations should evaluate the longevity of AuNPs in water and soil systems. Achieving the desired agricultural advantages with the least amount of side effects will need fine-tuning doses and nanoparticle compositions. An integrated agricultural strategy that increases crop output and resistance to biotic and abiotic stresses may be possible if research into the synergistic interactions of AuNPs with organic fertilizers, growth regulators, and soil amendments is advanced. Responsible integration of AuNPs into current farming systems requires careful evaluation of their ecological and food safety consequences, even though they provide a potential path for improving spinach agriculture and sustainable agricultural practices. For AuNPs to be used safely and effectively in food production, there must be a regulatory framework and risk assessment processes put in place to make sure the benefits are worth the dangers. More robust, high-quality, and nutritionally enriched spinach harvests might be possible with the help of AuNPs, which have the potential to transform crop management practices via multidisciplinary research.

6. CONCLUSION

Gold nanoparticles (AuNPs) have the ability to improve plant growth, biochemical composition, and antimicrobial resistance; this work sheds information on the biological characteristics of Spinacia oleracea when exposed to AuNPs. The results show that seed germination, root elongation, and chlorophyll content are all favorably affected by low concentrations of AuNPs, which improves photosynthetic efficiency and nutrient intake. The research also shows that AuNPs increase plant stress tolerance and defense mechanisms by increasing the production of antioxidant enzymes including SOD, POD, and CAT. Inhibiting the development of harmful bacteria and fungus, AuNPs' antimicrobial capabilities make them a potential alternative to traditional pesticides, which in turn improves crop health and yield.

Furthermore, new research routes into the function of AuNPs as biostimulants are opened up by their capacity to alter gene expression and plant metabolic pathways. The fact that AuNPs have the ability to make plants more resistant to abiotic stressors like drought, salt, and high temperatures makes them a promising new resource for climate-resilient farming. In spite of these encouraging advantages, the research also highlights hazards that may result from an overabundance of AuNPs, such as phytotoxic effects, oxidative stress, and disturbances to soil microbial populations. These dangers highlight the need to optimize AuNP concentrations to strike a balance between the agricultural advantages and possible health and environmental problems.

In order to properly use the benefits of AuNPs in agriculture, further study is needed to understand how these nanoparticles interact with plants, how they will affect the environment in the long run, and how to safely apply them to agricultural production. Determining the long-term viability of nanoparticles will need research into their soil persistence, bioaccumulation in edible plant components, and interactions with beneficial soil microbes. To determine the most effective ways to put them into reality, multidisciplinary teams of experts in plant science, environmental toxicity, and nanotechnology are required.

If these issues are resolved, AuNPs have the potential to significantly contribute to more sustainable farming methods, better food security, and more eco-friendly crop protection techniques. To promote innovation while protecting human health and the environment, scientists, legislators, and agricultural stakeholders must work together to integrate nanotechnology into agriculture in a way that is safe and effective. In the end, this study adds to what is already a substantial amount of literature on the topic of nanotechnology in agriculture and opens the door to more investigations into how this technology can completely alter contemporary agricultural methods. Contributing to the creation of high-yield, disease-resistant, nutritionally enhanced crops, this study lays the groundwork for future studies to optimize nanoparticle uses in plant science.

REFERENCES

Asker, A. Y. M., & Al Haidar, A. H. M. (2024). Green synthesis of gold nanoparticles using Pelargonium Graveolens leaf extract: characterization and anti-microbial properties (An in-vitro study). F1000Research, 13, 572.

Chappalathottil Sethumadhavan, S., & Pottail, L. (2019). Acorus calamus gold nanoparticles as enhanced anti-microbial agents for transdermal patches. Micro & Nano Letters, 14(4), 367-372.

Chowdhury, M. A., Hossain, N., Kchaou, M., Nandee, R., Shuvho, M. B. A., & Sultana, S. (2021). Scope of eco-friendly nanoparticles for anti-microbial activity. Current Research in Green and Sustainable Chemistry, 4, 100198.

Dash, S. S., Sen, I. K., & Dash, S. K. (2022). A review on the plant extract mediated green syntheses of gold nanoparticles and its anti-microbial, anti-cancer and catalytic applications. International Nano Letters, 12(1), 47-66.

Hatipoğlu, A. (2021). Rapid green synthesis of gold nanoparticles: Synthesis, characterization, and antimicrobial activities. Prog. Nutr, 23, e2021242.

Keskin, C., Atalar, M. N., Firat Baran, M., & Baran, A. (2021). Environmentally friendly rapid synthesis of gold nanoparticles from Artemisia absinthium plant extract and application of antimicrobial activities. Journal of the Institute of Science and Technology, 11(1), 365-375.

Lakshmiprabha, J., ROY, A., & Rajeshkumar, S. (2020). Green Synthesis and Characterisation of Gold Nanoparticles Using Averrhoa Bilimbi Leaf and Its Anti-Inflammatory and Anti-Microbial Activity. The journal of contemporary issues in business and government, 26(2), 1940-1950.

Nagaraj, B., Musthafa, S. A., Muhammad, S., Munuswamy-Ramanujam, G., Chung, W. J., Alodaini, H. A., ... & Ranganathan, V. (2022). Anti-microbial and anti-cancer activity of gold nanoparticles phytofabricated using clerodin enriched clerodendrum ethanolic leaf extract. Journal of King Saud University-Science, 34(4), 101989.

Pawar, C. A., Sharma, A. K., Prasad, N. R., Suryawanshi, S. S., Nazeruddin, G. M., Shaikh, V. S., ... & Shaikh, Y. I. (2022). A comparative study on anti-microbial efficacies of biologically synthesized nano gold using Bos taurus indicus urine with pharmaceutical drug sample. Current Research in Green and Sustainable Chemistry, 5, 100311.

Sekar, V., Al-Ansari, M. M., Narenkumar, J., Al-Humaid, L., Arunkumar, P., & Santhanam,

A. (2022). Synthesis of gold nanoparticles (AuNPs) with improved anti-diabetic, antioxidant and anti-microbial activity from Physalis minima. Journal of King Saud University-Science, 34(6), 102197.

Shanwaz, M. M., & Shyam, P. (2023). Anti-bacterial effect and characteristics of gold nanoparticles (AuNps) formed with Vitex negundo Plant Extract. Applied Biochemistry and Biotechnology, 195(3), 1630-1643.

Thangamani, N., & Bhuvaneshwari, N. J. C. P. L. (2019). Green synthesis of gold nanoparticles using Simarouba glauca leaf extract and their biological activity of micro-organism. Chemical Physics Letters, 732, 136587.