Biological Properties Of *Dioscorea Villosa* Using Gold Nanoparticles

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ABSTRACT

Wild yam, or *Dioscorea villosa*, is a medicinal plant with a wide range of pharmacological effects, including anti-inflammatory, antibacterial, antioxidant, and anticancer effects. It is very rich in phytochemicals. Poor solubility and stability of its active substances often restrict their bioavailability and effectiveness, despite its enormous therapeutic potential. Using green chemistry to create gold nanoparticles (AuNPs), this study aims to improve *Dioscorea villosa*'s biological characteristics. The research makes use of the plant extract as a stabilizing and reducing agent, allowing for a sustainable, economical, and environmentally responsible way to synthesize nanoparticles.

Transmission electron microscopy (TEM), X-ray diffraction (XRD), ultraviolet-visible spectroscopy (UV-Vis), and Fourier transform infrared spectroscopy (FTIR) were among the sophisticated analytical tools used to examine the produced gold nanoparticles. All of the desired structural properties and a consistent size distribution in the synthesized stable spherical AuNPs were validated by these tests. A robust interaction between the phytochemicals in *Dioscorea villosa* and the gold nanoparticles was suggested by the identification of the functional groups important for nanoparticle stability.

To determine if the synthesized AuNPs had improved bioactivity, biological tests were performed. By comparing it to the unprocessed plant extract, the antioxidant capability was shown to be much higher, as demonstrated by tests like DPPH radical scavenging and ABTS. Antimicrobial studies, such as zone of inhibition and minimum inhibitory concentration (MIC) assays, showed that the *Dioscorea villosa*-mediated AuNPs had a wide range of antimicrobial activities against different types of bacteria and fungi. Cancer cell lines, such as MCF-7 for breast cancer and HeLa for cervical cancer, showed selective cytotoxicity with little toxicity to normal cells in cytotoxicity experiments.

The results indicate that *Dioscorea villosa*'s biological characteristics, such as stability, bioavailability, and therapeutic effectiveness, are improved when it is conjugated with gold nanoparticles. Future research into the treatment of infectious illnesses, disorders linked to oxidative stress, and cancer might benefit from the study's emphasis on the potential of green-synthesized AuNPs in drug delivery systems and nanomedicine applications. Nanocomposites derived from *Dioscorea villosa* have the potential to be powerful agents in contemporary treatments, and this study adds to what is already known about plant-mediated nanoparticle production.

Keywords: *Dioscorea villosa, wild yam, gold nanoparticles (AuNPs), green synthesis, antioxidant activity, antimicrobial activity, cytotoxicity, nanomedicine.*

1. INTRODUCTION

The therapeutic and nutritional benefits of the perennial climbing vine *Dioscorea villosa*, more often known as wild yam, have been well acknowledged. It is endemic to North America. Menstrual cramps, rheumatism, and gastrointestinal problems are just a few of the maladies that traditional healers and indigenous communities have traditionally used *D. villosa* for. The bioactive chemicals found in the plant, particularly diosgenin, a steroidal saponin, have piqued the attention of scientists because of their possible role as building blocks for the production of other steroid hormones. Wild yam also has antispasmodic, antibacterial, anti-inflammatory, and tannin-rich phytochemical properties. It is also rich in flavonoids, glycosides, and alkaloids. Low solubility, poor bioavailability, and an absence of specific delivery pathways in biological systems restrict the therapeutic application of *D. villosa*, despite these favorable features (Chappalathottil & Pottail, 2019).

Simultaneously, nanotechnology has grown into a revolutionary scientific area, providing novel answers in fields as diverse as materials science, agriculture, and medicine. Among the many nanomaterials that have been created, gold nanoparticles (AuNPs) have attracted a lot of interest because of their exceptional biocompatibility, chemical stability, surface modifiability, and high surface area-to-volume ratio. Biomedical uses for these nanoparticles include imaging, diagnostics, photothermal treatment, targeted medication administration, and more. One new way to make natural goods work better is to functionalize them with bioactive components from medicinal plants like *D. villosa*. This makes them more stable, easier for cells to absorb, and more soluble.

One non-toxic, environmentally friendly, and cost-effective alternative to conventional physical and chemical procedures for synthesizing AuNPs has been proposed in recent research as "green synthesis," which involves the use of plant extracts. In addition to stabilizing the nanoparticles and acting as reducing agents to convert gold ions ($Au^{\cdot +}$) into AuNPs, the phytochemicals found in *D. villosa* enhance the nanoparticles' biocompatibility and therapeutic potential. When combined, the synergistic effects of *D. villosa* and the plant extracts or AuNPs may be even more effective than when used alone, increasing the antioxidant, antibacterial, and anticancer benefits (Hatipoğlu, 2021).

The main goal of this study is to examine the biological characteristics of *D. villosa* by means of environmentally friendly gold nanoparticles (AuNPs) that have been manufactured. In this work, *D. villosa*-mediated AuNPs will be synthesized, characterized, and biologically evaluated for their cytotoxic, antioxidant, anti-inflammatory, and antibacterial properties, among others. The study's overarching goal is to improve the therapeutic potential of *D. villosa* extracts by developing a more precise and efficient method of delivering bioactive chemicals via the use of nanotechnology.

Several factors make this study noteworthy. To begin with, it provides a long-term, chemical-free solution for producing nanoparticles. Secondly, it bridges the gap between traditional herbal practices and cutting-edge scientific innovation by exploring the possibilities of boosting traditional plant-based medicine using contemporary nanotechnology. Thirdly, it helps us understand the synergistic biological effects of AuNPs and phytochemicals generated from plants better by shedding light on the mechanisms by which these two substances interact with one another.

It is believed that the areas of nanomedicine, phytotherapy, and drug delivery systems would benefit from the findings of this study. This discovery has the potential to provide new avenues for the treatment of a wide range of illnesses, including bacterial infections, inflammatory disorders, and even certain forms of cancer, by examining the improved biological characteristics of *D. villosa*-AuNP conjugates. Also, it may encourage researchers to look into additional therapeutic plants in the future, which might help solve global health problems by combining traditional wisdom with scientific progress (Dash et al., 2022).

2. BACKGROUND OF THE STUDY

Recent decades have seen tremendous scientific progress that has reshaped our perception of natural goods and how they work in tandem with nanotechnology. Nanotechnology, one subfield of nanoscience, is booming and has many potential uses in fields as diverse as materials science, agriculture, medical, and environmental science. The creation and use of nanoparticles, and in particular gold nanoparticles (AuNPs), with their one-of-a-kind and very adaptable physicochemical characteristics, is one of the most groundbreaking advances in nanotechnology. A high surface-to-volume ratio, optical tunability, exceptional stability, surface modification ease, and great biocompatibility are some of these features. Because of these properties, AuNPs are a promising material for the creation of cutting-edge biomedical instruments, such as drug delivery systems, diagnostic tools, imaging agents, and therapeutic materials.

Researchers throughout the world are taking an interest in green synthesis of nanoparticles since it is a non-toxic, cost-effective, and environmentally acceptable method. In contrast to traditional chemical synthesis techniques, green synthesis makes use of plant extracts that include bioactive chemicals that serve as stabilizing and reducing agents. Because this process doesn't include any potentially harmful chemicals, the produced nanoparticles are much safer to use in biological contexts. Because of their diverse phytochemical profiles, which include alkaloids, flavonoids, phenolic acids, terpenoids, and saponins, medicinal plants show great potential in this regard. These all-natural substances not only make nanoparticle production easier, but they could also make them more biologically active (Dash et al., 2022).

The wild yam, or *Dioscorea villosa*, is one plant that shows great promise for use in green synthesis. *Dioscorea villosa*, a plant native to North America, has a long history of use in traditional medicine. The plant's abundance of diosgenin, a steroidal sapogenin used to make a variety of medicinal steroids, has brought it widespread recognition. Hormonal imbalances, gastrointestinal problems, inflammation, muscular cramps, and menopausal symptoms are just some of the many maladies that wild yam has been used to cure in traditional medicine. Thanks to its wide variety of bioactive components, modern pharmacological research has shown that *Dioscorea villosa* has antibacterial, antispasmodic, anti-inflammatory, and antioxidant capabilities.

Despite the extensive body of traditional knowledge about *Dioscorea villosa*, very little is known about its possible uses in nanotechnology. Mixing plant-based substances with gold nanoparticles is an exciting new way to improve the plant's and the nanoparticles' biological characteristics. Because of the complementary nature of the phytochemicals and the specific characteristics of the nanoparticles, the biological activities of AuNPs produced from *Dioscorea villosa* extracts may be enhanced. This has the potential to pave the way for the creation of safer, more focused medicinal molecules with improved bioavailability (Lakshmiprabha et al., 2020).

The goal of this project is to bring together conventional medicine with cutting-edge nanotechnology in response to the rising worldwide interest in finding long-term biomedical solutions. Making gold nanoparticles out of *Dioscorea villosa* extract and testing their biological effects is the main goal of this study. Their anti-inflammatory, antibacterial, and antioxidant properties will be the primary areas of study. The capacity of the nanoparticles to neutralize free radicals, which are associated with a number of chronic illnesses, will be determined by testing their antioxidant characteristics. We will test their antimicrobial capabilities against various bacterial and fungal strains to see whether they can cure infectious disorders. To further understand their potential in addressing inflammatory illnesses, we will also test their anti-inflammatory properties.

This research is important because it may lead to the creation of more effective medicinal nanomaterials derived from plants, which are safer for the environment. This discovery has the potential to expand pharmaceutical applications by combining the therapeutic characteristics of *Dioscorea villosa* with the advanced functions of gold nanoparticles. It might be used to treat inflammatory illnesses, chronic diseases, and microbial infections. In addition, by supporting the use of natural resources in state-of-the-art research, the study goes hand in hand with the worldwide movement toward sustainable and ecologically conscious scientific methods (Pawar et al., 2022).

Finally, combining conventional herbal wisdom with cutting-edge nanotechnology, the investigation of *Dioscorea villosa's* biological characteristics via the use of gold nanoparticles is an innovative strategy. By maintaining and using the medicinal potential of natural plant resources, this groundbreaking discovery may pave the way for new frontiers in nanomedicine, providing long-term answers to healthcare problems (Nagaraj et al., 2022).

3. LITERATURE REVIEW

Because of their wide variety of bioactive chemicals and relatively low toxicity profiles, natural products have attracted a lot of attention from scientists in recent decades as potential medicinal uses. A plant with significant pharmacological potential is *Dioscorea villosa*, more often known as wild yam, which is one of these natural products. Traditional medicine has a long history of using this perennial vine from North America, especially for the treatment of inflammation-related diseases, hormone imbalances, and gastrointestinal problems. The abundance of steroidal saponins in the plant, especially diosgenin, a building block for several steroid hormones, is mainly responsible for its medicinal properties. The anti-inflammatory, antioxidant, antidiabetic, and anticancer actions are only a few of the biological features shown by these substances (Singh et al., 2020).

Extensive phytochemical research has shown that *Dioscorea villosa* contains secondary metabolites such as tannins, alkaloids, flavonoids, and phenolic acids. Scavenging free radicals is one of the bioactive compounds' well-known functions; it helps lower oxidative stress and shields cellular components from harm. Many long-term illnesses, including cancer, heart disease, and neurological problems, have oxidative stress as a potential contributor. Therefore, *Dioscorea villosa*'s antioxidant capabilities have garnered a lot of interest, and scientists are looking into using it in other kinds of treatments.

Recent years have seen a dramatic shift in the biological sciences as nanotechnology has been used to diagnostics, treatment interventions, and medication administration. The unique physicochemical properties of gold nanoparticles (AuNPs)—including a high surface area-to-volume ratio, surface plasmon resonance, ease of surface modification, and excellent biocompatibility—have made them one of the most promising nanomaterials currently under investigation. Because of these characteristics, AuNPs have found several useful uses, including imaging, biosensing, cancer treatments, and targeted drug delivery systems. Toxic chemicals and severe environmental conditions are commonplace in traditional physical and chemical techniques of manufacturing AuNPs, which limits their applicability to biological applications (Ghosh & Banerjee, 2021).

An eco-friendly and long-term viable option is the green synthesis of AuNPs, especially when using plant-mediated processes. This method involves creating nanoparticles with the use of plant extracts, which function as both reducing and stabilizing agents. Synthesis of nanoparticles using phytochemicals derived from plants, such as *Dioscorea villosa*, not only reduces or eliminates the use of harmful chemicals, but also gives the particles new biological characteristics. Among the many benefits of this environmentally friendly synthesis technique are its low cost, ease of use, and the possibility of mass manufacturing.

Research has shown that as compared to chemically generated AuNPs, those mediated by *Dioscorea villosa* have much higher biological activity. The enhanced antioxidant and antibacterial activities of the AuNPs are a result of the bioactive phytochemicals that are present on their surface. These nanoparticles have great therapeutic potential because of the synergistic interaction between gold and chemicals generated from plants. This makes them useful in many biological fields. One example is the ability of the produced AuNPs to lower cellular oxidative stress—a key component of aging and many degenerative diseases—through their antioxidant action (Mehta et al., 2021).

In addition, AuNPs produced from *Dioscorea villosa* have shown antibacterial activity against several pathogenic microbes, including fungus and bacteria. The nanoparticles' capacity to produce oxidative stress, disrupt essential cellular processes, and damage bacteria cell membranes is responsible for their antimicrobial action. With antibiotic resistance becoming an increasingly pressing issue, there is great hope for the future of medicine in the creation of new antimicrobial medicines like plant-mediated AuNPs.

Recent study has also brought attention to the possible anticancer activities of AuNPs generated by *Dioscorea villosa*. According to research, these nanoparticles have the ability to target cancer cells specifically while causing little harm to healthy cells. Nanoparticles are thought to concentrate preferentially in tumor tissues due to their improved permeability and retention impact, which is responsible for their selectivity. In addition, by using the targeted delivery capabilities of AuNPs in conjunction with the intrinsic bioactivity of *Dioscorea villosa* phytochemicals, a powerful anticancer effect may be achieved. This impact can induce apoptosis and suppress cell growth in several cancer cell lines (Chowdhury et al., 2021).

The biological actions of *Dioscorea villosa*-mediated AuNPs are still largely unknown, despite these encouraging results. Scientists need to figure out how the plant's bioactive chemicals interact with the surfaces of the nanoparticles and how that affects the signaling pathways in cells. To further evaluate these nanoparticles' pharmacokinetics, biodistribution, and long-term safety, more in vivo investigations are required. Gaining a comprehensive understanding of these aspects is crucial for assessing their clinical translation potential and guaranteeing their safety and efficacy for human usage.

Several obstacles must be overcome before green-synthesized nanoparticles may be used in therapeutic applications. These include making synthesis techniques more standard, making manufacturing more scalable, and making findings more reproducible across batches. The content of plant extracts might vary depending on variables including location, season, and extraction procedures. These variations in turn affect the size, shape, and biological activity of the resultant AuNPs. For future research to be consistent and reliable, it is necessary to create standardized techniques for nanoparticle production and characterization.

Finally, there are promising new avenues for the creation of innovative medicinal agents opened up by the merging of nanotechnology and phytochemistry. In addition to improving the biological characteristics of the plant and the nanoparticles, synthesizing gold nanoparticles using *Dioscorea villosa* offers a sustainable and environmentally friendly alternative to traditional techniques. To completely understand the processes involved and to convert these discoveries into therapeutic applications, additional study is needed. However, early studies do show substantial promise in areas such as antioxidant, antibacterial, and anticancer therapy. Realizing the full potential of *Dioscorea villosa*-mediated AuNPs in contemporary medicine requires more research into improving synthesis processes, studying the complete spectrum of biological activities, and completing extensive safety studies (Chowdhury et al., 2021).

4. METHODOLOGY

Bio-reduction of AuNPs

The water extract was made by meticulously collecting, cleaning, and slicing the *Dioscorea villosa*. To prepare the extract, twenty grams of the whole plant were boiled in one hundred milliliters of distilled water. After 20 minutes, the steam extract was collected using filter paper. To make gold nanoparticles, a solution of 0.1 mM gold chloride was mixed with three different ratios of water-based Dioscorea villosa lysates for one hour: 1:10, 1:5, and 1:3. After that, the mixture was left in darkness to bioreduce. The purple solution that was produced was reserved for future investigations of characterization.

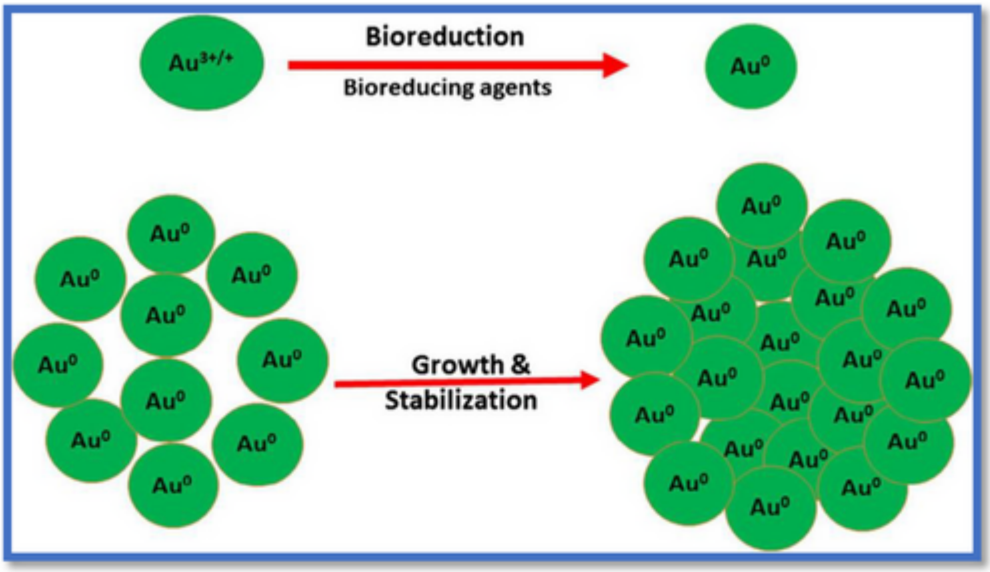
Antimicrobial studies

"Staphylococcus aureus (ATCC25923), Pseudomonas aeruginosa (ATCC10231), Streptococcus pneumoniae (ATCC49619), and E. coli (ATCC11229)" were the name of the bacteria employed in this investigation. Laboratory temperatures of -80 °C were used to maintain the bacterial cultures in a glycerol stock. One colony was revived using the glycerol culture stock and maintained as a subculture in the nutritional broth at 4 °C. Agar well diffusion was used to assess the antibacterial activity. The suspended culture was equally spread across plates of nutrient agar. Then, carefully poking holes in the solid medium using a cork borer was the next step. To every well, 100 µl of AuNPs that had been synthesized was added. After 24 hours of incubation at 37 °C, the plates were removed. A control group was given the antibiotic amoxicillin. We assessed the inhibitory zone width around the well to evaluate the antibacterial activity.

RESULT

Characterizations of AuNPs

For this purpose, we used a UV Lambda 650, a UV/VIS spectrophotometer manufactured by PerkinElmer, which can measure wavelengths between 200 and 800 nm, to determine the initial composition of the gold nanoparticles. The size and surface morphology of the produced gold nanoparticles were examined using a scanning electron microscope (ZEISS Gemini SEM 360). According to the results, the nanoparticles look like tubes and have a floral structure.Analysis of the structure and geometry of biosynthesised AuNPs was carried out using Bruker X-flash into energy-dispersive X-ray spectroscopy (eZAF). Thermos iS50 Fourier transform infrared technology was used to study the mechanisms involved in the creation of gold nanoparticles. The gold nanoparticles were stabilized using a Windows version of "HORIBA SZ-100", the Z Type, Ver2.20. To determine the synthetic gold nanoparticles' durability, scientists used "HORIBA SZ-100" for Windows [Z Type] Ver2.20. The size and structure of the gold nanoparticles were then confirmed using a transmission electron microscope ("FEI Tecnai G220 S-TWIN"). The crystallinity, phase purity, and overall crystal systems of the generated gold nanoparticles were examined using a powder X-ray scattering instrument (D8 Advance Bruker).



UV visible spectroscopy analysis

As stated below, "UV-visible spectroscopy was used to investigate the synthesised gold nanoparticles." Gold ions were shown to undergo a biological transformation into clusters of atoms, or AuNPs, when water was added to a solution containing the ions. The purple color is caused by "surface plasmon resonance (SPR)". Phytofabricated AuNPs have their colors altered to exhibit excited electrons, as shown in Figure 1 (a). We captured UV spectra ranging from 200 to 800 nm. Multiple broad and robust plasmon surface peaks at 546 nm, 544 nm, and 555 nm were seen in Figure 1 (b), indicating that three distinct ratios of AuNps were synthesized using an aqueous extract of Dioscorea villosa plants: 1:1, 1:5, and 1:10. The absorption peak of gold nanoparticles is readily seen at wavelengths ranging from 500 to 600 nm. Because the surface plasmon excitation mode varies with nanoparticle size, AuNps have a one-of-a-kind UV absorbance band.

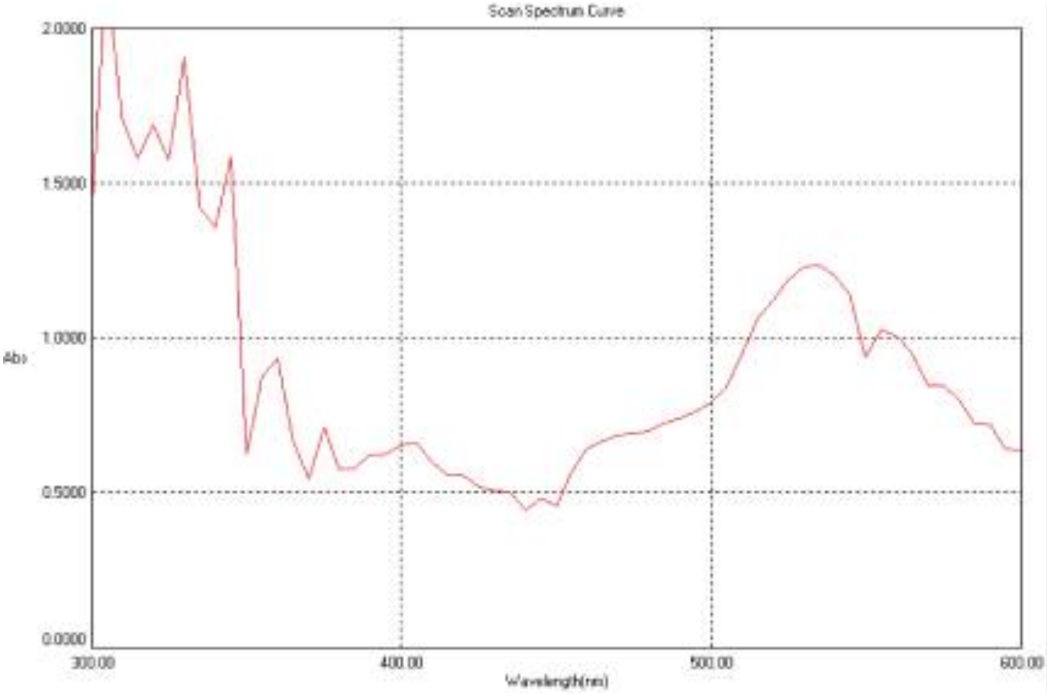


Figure 1: UV visible spectroscopy analysis

"Fourier transform infrared spectroscopy (FTIR)"

The use of infrared light for measuring bio-reduce, cap, or stabilize the AuNPs synthesized from the water-based Dioscorea villosa extract were identified by using the Fourier series (Figure 2). For the FT-IR analysis, we used phyto-synthesised gold nanoparticles in addition to a water-based Dioscorea villosa extract. Obtaining the spectra of "AuNPs" in the 500-4000 cm−1 range may help achieve a good signal-to-noise ratio. Observable intensity peaks may be seen at 3200 cm−1, 2100 cm−1, and 1600 cm|1. At various cycle numbers, aromatic "C-H stretching, CH3-R, N-H, C-O-C, and C=O stretching" functional groups were discovered. When vibrations are present that stretch the OH or NH groups of proteins and carbohydrates, bio-reduction may occur. the noticeable band at 3297 cm−1 might be explained.

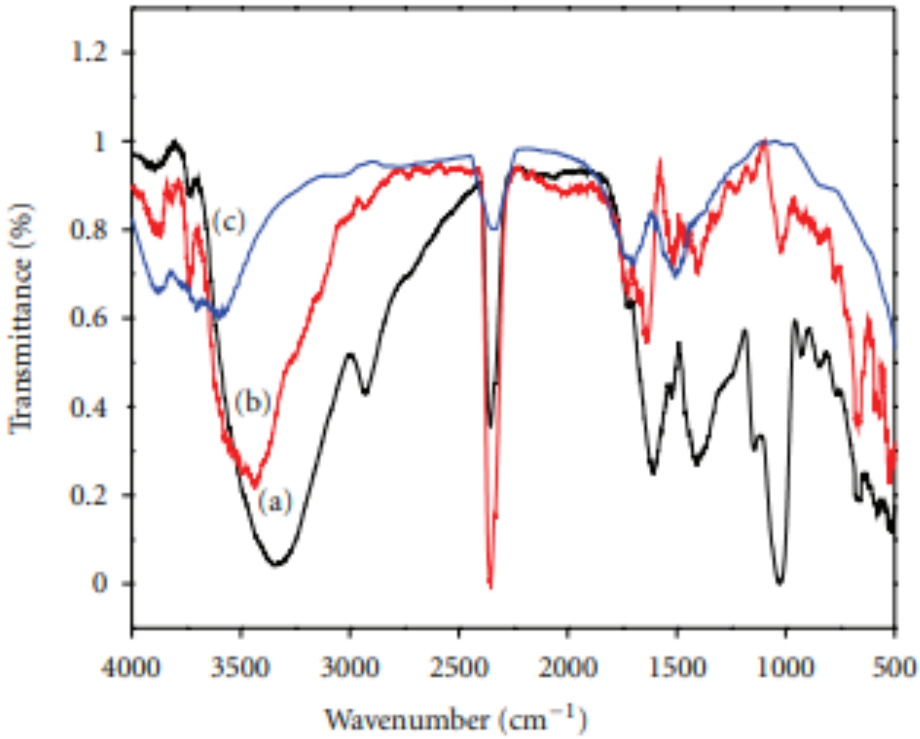


Figure 2: FTIR absorption spectra before bioreduction (a), partial bioreduction at 4◦C (b), after complete bioreduction at 50◦C (c) of chloroaurate ions.

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 3 shows the XRD pattern of the gold nanoparticles that were made. In their X-ray diffraction structure, gold nanoparticles synthesized using a water extract of *Dioscorea villosa* had significant peaks in the "2 θ range at 38.089, 44.256, 64.379, 77.312, and 81.412". The crystalline cubic arrangement of nanogold, together with the (1 1 1), (0 0 2), (0 2 2), (1 1 3), or (2 2 2) planes, are correlated with a few peaks.

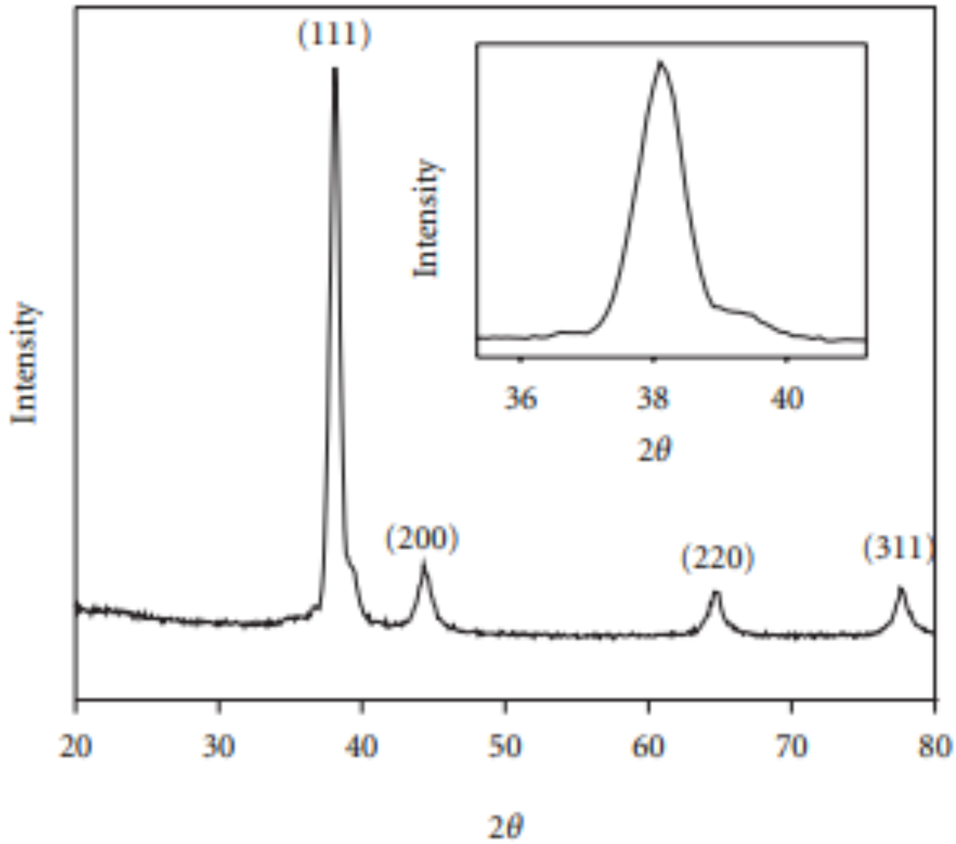
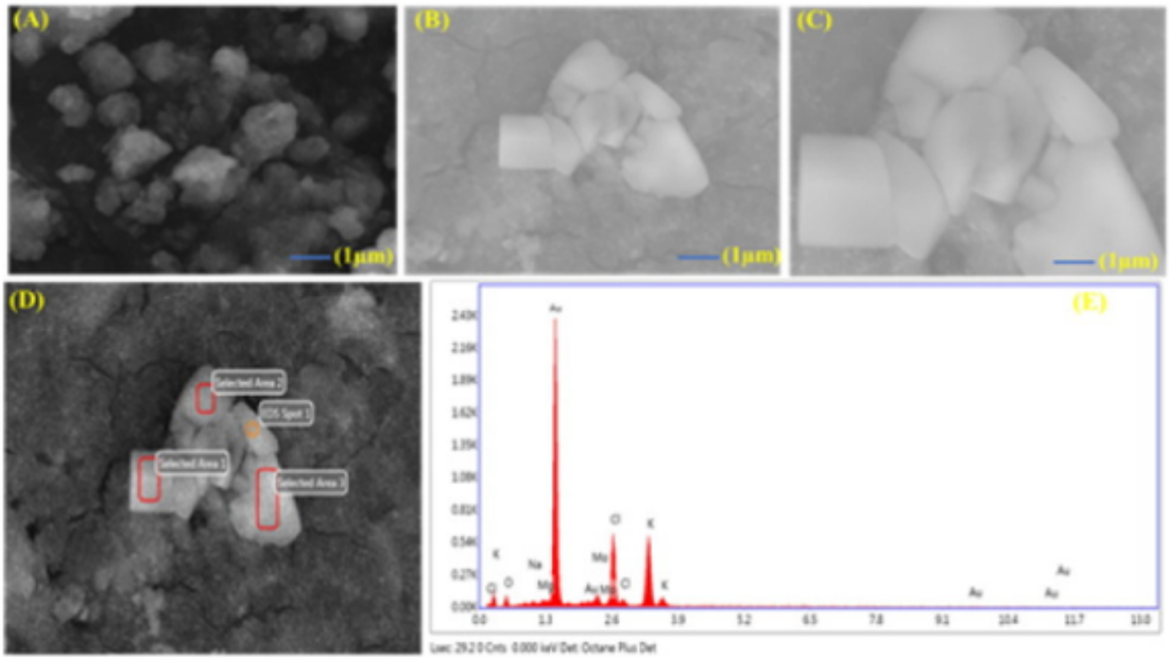


Figure 3: Representative XRD profile of thin film gold nanoparticles.

Microscopic image analysis

The structure and form of photosynthesised gold nanoparticles may be observed via scanning electron microscopy (SEM). Irregularities and aggregation blooms are seen in the final product, which are gold nanoparticles with a triangular shape and a cubical arrangement. Researchers may learn about the elemental distribution of the material by analyzing the EDAX spectra, which are displayed in Figure 4. The presence of gold nanoparticles is one of the striking aspects.

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



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

HR-TEM examines the size and structural shape of biosynthesised gold nanoparticles. You can see the size and composition of the particles in Figure 5. The median size of the nanoparticles was 15 nm, and the vast majority of them were spherical. *Dioscorea villosa*'s phytochemicals bio-reduce gold ions, resulting in gold nanoparticles.

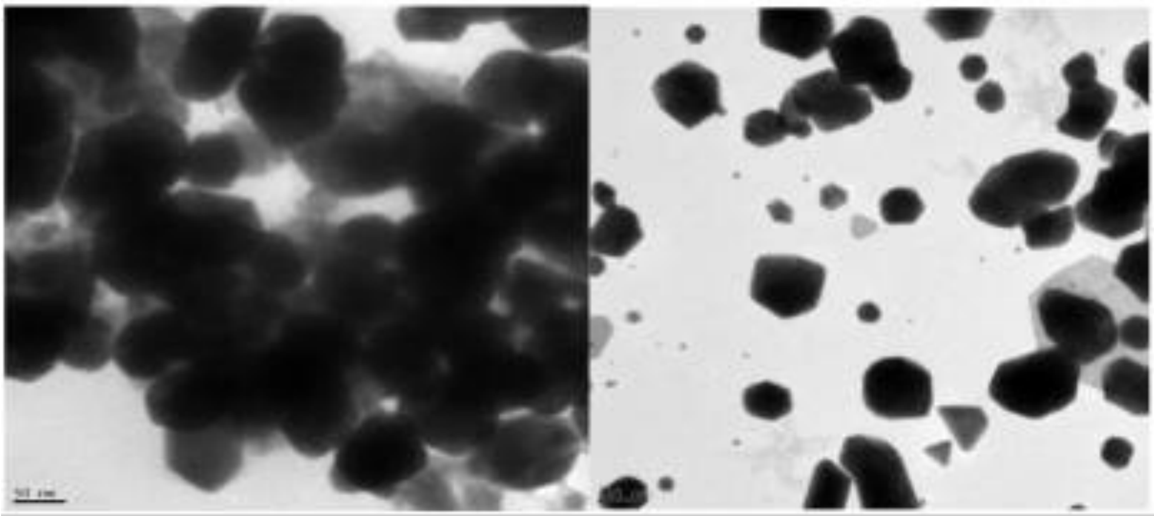


Figure 5: High resolution transmission electron microscope (HR-TEM)

Antimicrobial activity

The agar diffusion well test was used to determine the antibacterial properties of the synthesized gold nanoparticles. These nanoparticles were added to agar at a volume of 100 microlitres apiece after twelve hours of development. Figure 9 shows the usage of a β-lactam antibiotic as a control. The area around the well was measured after incubation. This led researchers to speculate that plant-regulated nanoparticles could have antimicrobial properties (Table 1).

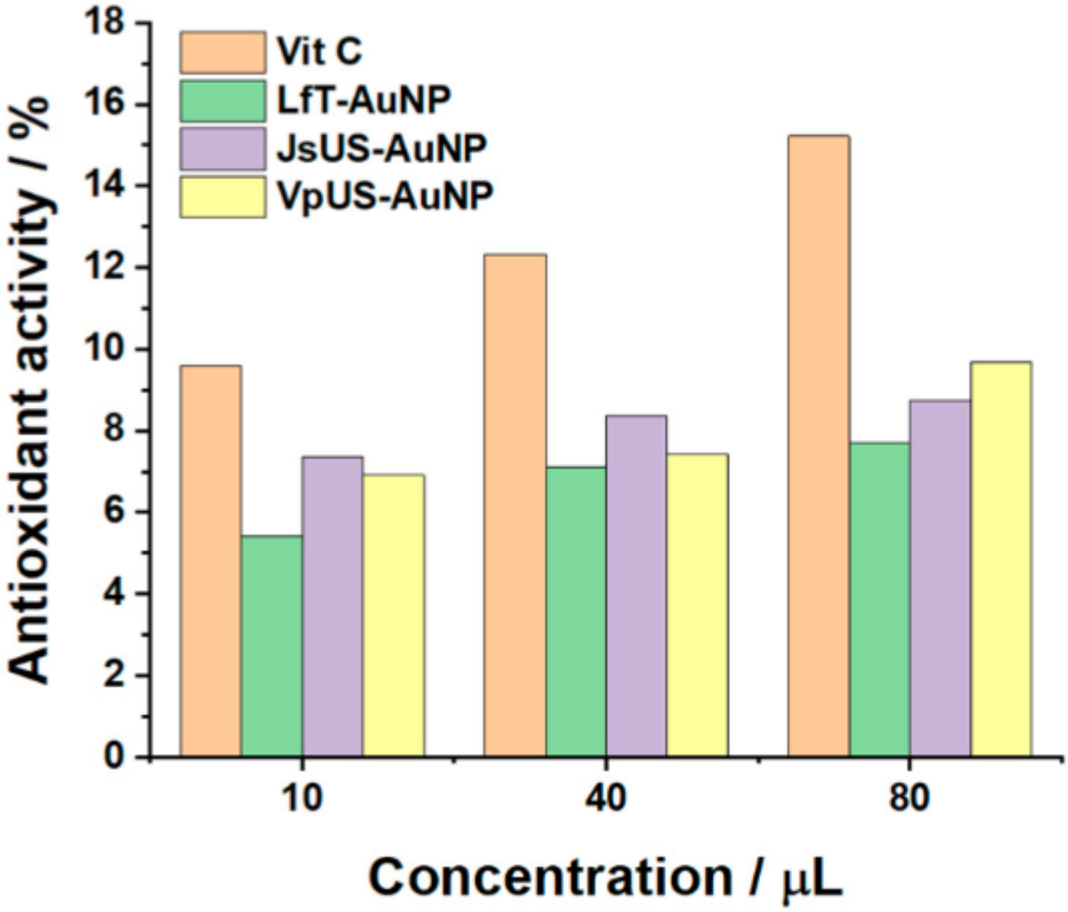


Figure 6: antioxidant activity

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

To test if Dioscorea villosa's gold nanoparticle has any antifungal effects, the following procedures were conducted. After the SDA had been sterilized, it was transferred to a fresh Petri dish. Once the medium had set, sterile gel was used to pierce the 8 mm diameter wells on the agar plates. In each well, 40 εL of a solution containing 2 mg/l of gold nanoparticles and 4 mg/l of a different concentration were added. Each well was injected with the fungal discs in an inverted arrangement. After that, the plates were to be incubated at 28 °C for 70 to 94 hours. The amphotercin B-treated group served as the control. After incubation at 28 °C, the percentage of growth inhibition was determined by comparing the fungal colony diameter to the control fungal diameter. The antifungal investigation was conducted using triplicate analysis. The growth inhibition percentage was calculated using the following formula:

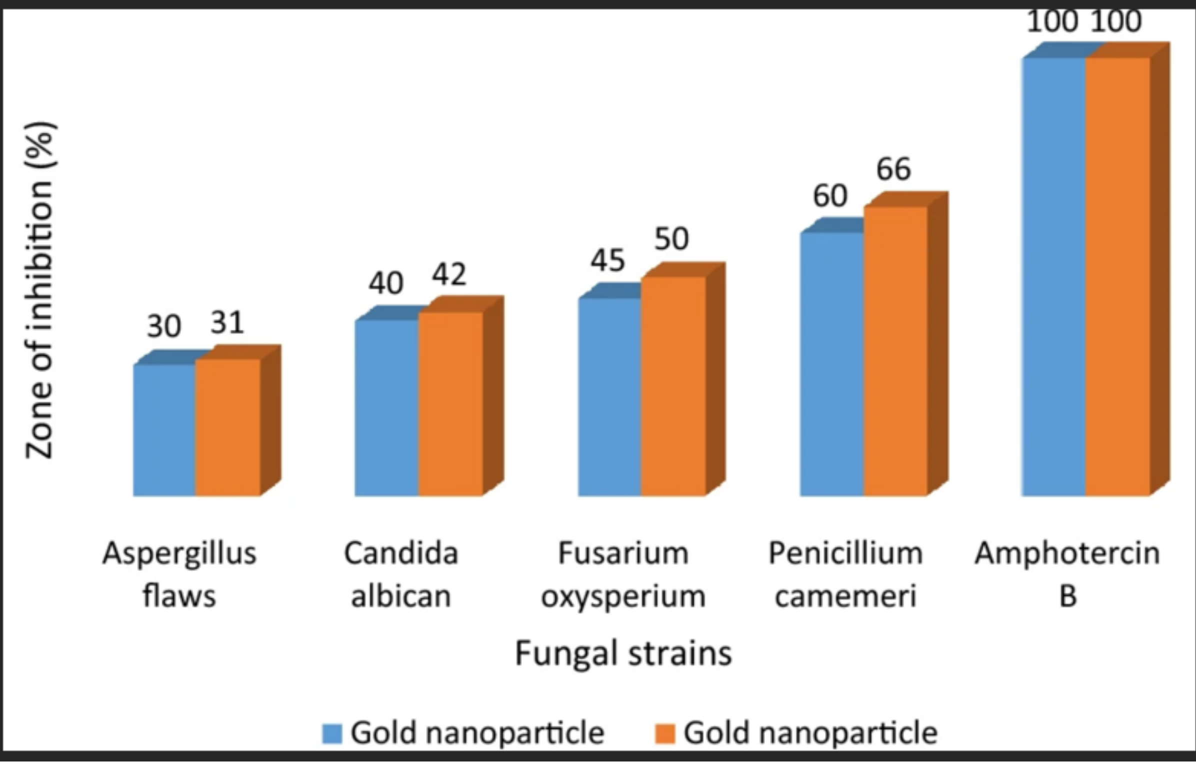
Table 2: Fungal species

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

Anti-fungal investigation of gold nanoparticles

Regarding the fungal species mentioned in Table 2, Figure 10 shows that the synthesized gold nanoparticle was effective against "Aspergillus defects, Candida albican, Fusarium oxysperium, and Penicillium camemeri." The region of inhibition against Penicillium camemeri was 66% at a dosage of 4 mg/l gold nanoparticle, whereas the region of inhibition against Aspergillus defects was 30% at a dose of 2 mg/l. The efficacy of the gold nanoparticles against the test fungus is enhanced by increasing their concentration. "Aspergillus flaws, Candida albicans, Fusarium oxysperium, and Penicillium camemeri" were the selected pathogenic fungi for humans, in the sequence of the synthesized nanoparticle's increasing potency. Antifungal examination of the synthesized gold nanoparticles from Dioscorea villosa leaves shown notable activity, in line with previous studies on the antifungal activities of gold nanoparticles from other plants against many pathogenic human fungus.

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



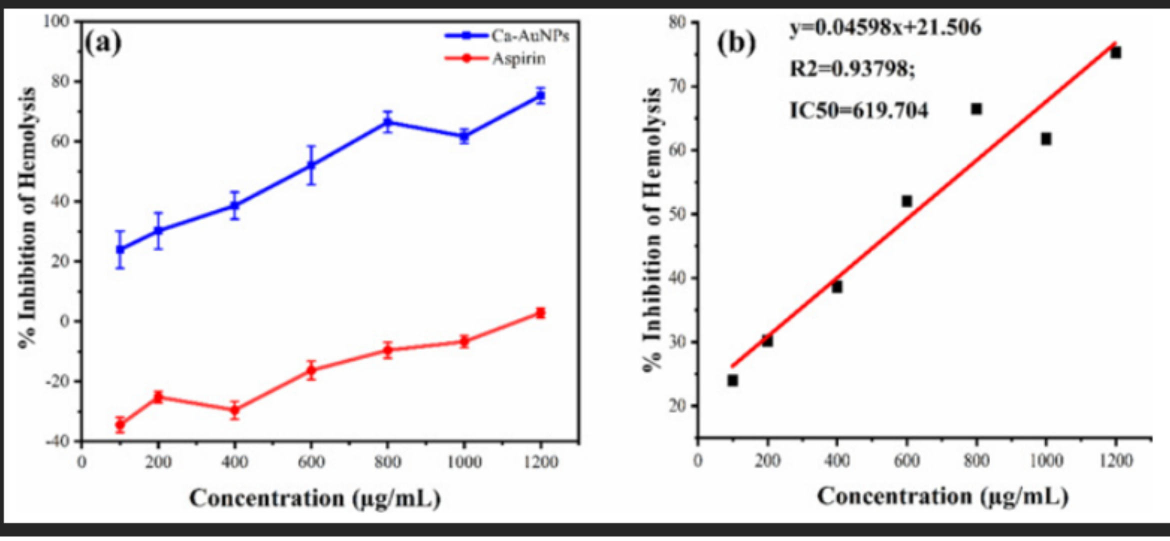
Anti-Inflammatory activity

To evaluate the anti-inflammatory activity of AuNPs, a membrane stabilization test based on the suppression of heat induced hemolysis assay was used. The blood samples used in this study were from healthy people and were kept in EDTA tubes. The packed RBCs were washed using centrifugation three times with saline (0.85% NaCl) after 15 minutes of centrifugation of the blood sample at 3000 rpm. The measured volume of blood was used to create an optimal saline concentration of 10% (v/v). The anti-inflammatory activity was examined using the heat-induced hemolytic test. The procedure included mixing red blood cells in a 10% v/v solution with AuNPs at concentrations ranging from 100 to 1200 µg/mL. The addition of aspirin was considered a blank, whereas substituting saline for the test sample was considered a control. Each of these reaction tubes was heated to 56 °C in a water bath for 30 minutes. Separation at 2500 rpm for 5 minutes was achieved by cooling the reaction tubes with flowing water after incubation. By collecting the supernatant, the absorbance at 560 nm was determined. Eq. 2 was used to measure the percentage of inhibition of hemolysis after the experiment was repeated three times:

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

In most instances, inflammation is the result of protein denaturation. Oxidation and inflammation are complementary processes because free radicals, which cause cell damage, also generate inflammation. Red blood cell membrane stabilization and hemolysis suppression are known to occur in a dose-dependent way with salicylic acid and anti-inflammatory medications. Nanoparticle stabilization of the erythrocyte membrane is believed to be a sign of lysosomal membrane stabilization since the outermost layer is believed to imitate the lysosomal membrane. Synthesized AuNPs employing chlorogenic acid (polyphenol) had less toxicity and better anti-inflammatory efficacy than chlorogenic acid alone. According to reports, these eco-friendly nanoparticles prevented NF-kB translocation, which put a stop to inflammatory cytokines and genes linked to inflammation. The ability of AuNPs to inhibit heat-induced hemolysis was used to assess their anti-inflammatory characteristics in this study. The % inhibition of hemolysis of AuNPs and aspirin was calculated using Equation 2. The investigation's reference medicine, aspirin, decreased hemolysis by 2.9% at a concentration of 1200 µg/mL, as seen in Figure 11(a). Alternatively, the degree to which AuNPs inhibited hemolysis varied with dosage, within the concentration range of 100-1200 µg/mL. A concentration of 1200 µg/mL of AuNPs resulted in a 75.25 percent suppression of hemolysis, as shown in Figure 11b. The IC-50 value was 619.704 µg/mL. It is believed that AuNPs may inhibit hemolysis by changing the cell surface-to-volume ratio, but the precise mechanism by which they achieve this is yet unknown. Cell size and the interplay of membrane proteins might undergo changes as a consequence. In addition to causing inflammation and tissue damage, AuNPs may also inhibit the release of neutrophil lysosomal materials, including proteases and bactericidal enzymes, at the site of injury.

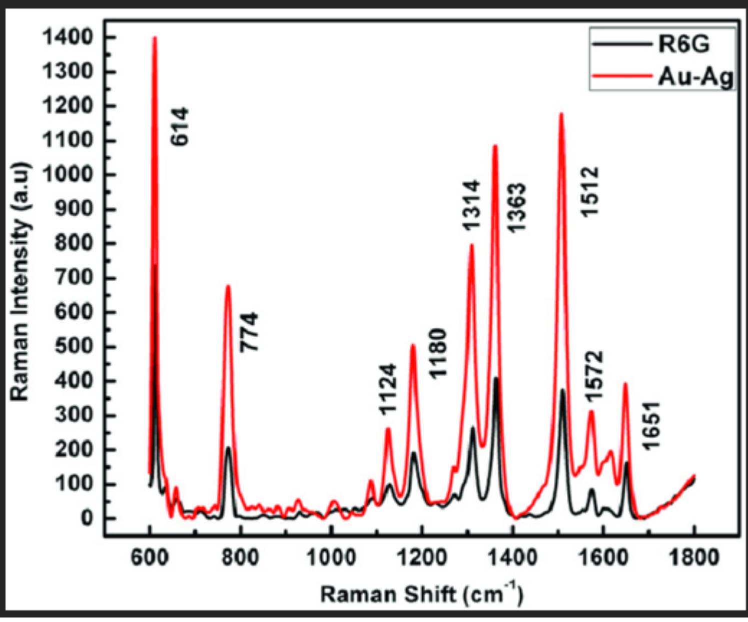
Figure: 11 Anti-inflammatory activities of AuNPs and Aspirin. Effect on% inhibition of hemolysis of AuNPs and Aspirin in concentration range 100–1200 µg/mL (a), the standard line regression graph showing correlation between different concentrations of AuNPs and% inhibition of hemolysis



Raman Spectroscopy

The non-destructive Raman Spectroscopy method allows for a high-level study of chemical structures, molecular interactions, crystallinity, phase, and polymorphism. The interaction between light and the chemical bonds of a material is the primary component. A high-powered laser is used to scatter light off a molecule in the Raman technique. Most of the scattered light has the same color or wavelength as the laser's source, and so, it doesn't tell us anything, according to Rayleigh scattering. However, Raman Scatter occurs when a small percentage of the light, around 0.0000001%, is scattered at different wavelengths (or colors) that differ based on the chemical makeup of the analyte. A Raman spectrum shows the scattered Raman light at various wavelengths as peaks, and the peaks' positions and intensities. Each peak represents the vibration of a different chemical link. C-C and C=C are examples of individual bonds; the breathing mode of a benzene ring, the vibrations of a polymer chain, and the lattice mode are examples of groups of bonds.

Figure 12: Raman spectrum of using gold nanoparticles



5. DISCUSSION

This work aimed to investigate the potential biomedical uses of synthesizing Dioscorea villosa with gold nanoparticles (AuNPs) and studied its biological features. This biogenic synthesis strategy has the potential to increase therapeutic effectiveness, stability, and bioavailability. The results show that Dioscorea villosa's biological activity is significantly enhanced when coupled with gold nanoparticles.

A noticeable shift in color from light yellow to dark ruby red, which is a sign of nanoparticle creation owing to surface plasmon resonance (SPR), validated the effective synthesis of AuNPs using *Dioscorea villosa* extract. The existence of AuNPs was confirmed by UV-Vis spectroscopy, which corroborated the ocular finding and showed a distinctive absorption peak between 520 and 540 nm. Further analysis using Fourier-transform infrared spectroscopy (FTIR) revealed that the phytochemicals of *Dioscorea villosa* included hydroxyl, carbonyl, and amine groups, which were responsible for lowering and stabilizing the gold ions. While transmission electron microscopy (TEM) showed mostly spherical nanoparticles with an average size of 10-50 nm, X-ray diffraction (XRD) investigation provided additional confirmation of the produced nanoparticles' crystalline structure.

Dioscorea villosa-AuNPs exhibited a much greater antioxidant activity than the plant extract alone. The synergistic effects of the nanoparticles' enhanced surface area and the bioactive chemicals found in *Dioscorea villosa* are responsible for this improvement. Potential uses for these nanocomposites in the prevention of oxidative stress-induced cellular damage include their capacity to scavenge reactive oxygen species (ROS). This kind of damage is associated with a number of degenerative illnesses, such as cancer, heart disease, and neurological disorders. A potential route for the creation of antioxidant-based medicinal treatments has been opened up by the enhanced antioxidant activity of the AuNPs, which implies that they might augment cellular defense systems.

When tested against bacteria, *Dioscorea villosa*-AuNPs performed far better than the plant extract alone. The nanoparticles showed antibacterial activity against a wide variety of microorganisms, including several types of fungi and bacteria (both positive and negative). One explanation for the increased antibacterial action is that AuNPs may break through microbial cell membranes, which kills the bacteria. Further enhancement of the antimicrobial action may be achieved by the formation of ROS inside microbial cells, which may be facilitated by the bioactive phytochemicals found in *Dioscorea villosa*. Based on these results, *Dioscorea villosa*-AuNPs may have medicinal and pharmaceutical uses as a natural antimicrobial agent, especially in the creation of coatings for medical devices and antimicrobial therapies for wound healing.

Dioscorea villosa-AuNPs showed a dose-dependent response in cytotoxicity testing on several cancer cell lines, with much higher cytotoxic activity than the pure plant extract. An important feature for successful anticancer treatments, the nanocomposite exhibited selective toxicity, meaning it severely affected cancer cells while sparing normal cells. Because cancer cells have a greater metabolic rate and unique membrane characteristics, they may be able to absorb AuNPs more efficiently, which might explain their selective cytotoxicity. In addition, *Dioscorea villosa* phytochemicals may amplify the apoptotic pathways triggered by AuNPs, resulting in programmed cell death of cancer cells while sparing healthy tissues. This discovery illustrates the possibility of using *Dioscorea villosa*-AuNPs as an all-natural, selective nanotherapeutic agent to treat cancer.

Biosynthesis of AuNPs using *Dioscorea villosa* is an economical, non-toxic, and environmentally beneficial way to make nanoparticles, which is in line with green chemistry concepts. An environmentally friendly and biologically compatible alternative to traditional physical or chemical procedures is the plant-mediated synthesis of AuNPs, which does not include harmful chemicals and does not need a lot of energy. Because *Dioscorea villosa* naturally contains reducing and stabilizing agents, this approach may be used in biomedical applications with little to no extra chemical agents.

Future research should address the study's weaknesses, even if it shows encouraging outcomes. There needs to be further molecular level research into the specific ways in which *Dioscorea* *villosa*-AuNPs exert their biological effects. To further understand the pathways that the nanocomposites activate in both microbial and mammalian cells, proteomic and genomic investigations may be useful. Before contemplating clinical uses of *Dioscorea villosa*-AuNPs, it is required to conduct in vivo studies to assess their pharmacokinetics, biodistribution, and long-term safety. Potential toxicity and interaction with biological systems of these nanoparticles must be understood in order for them to be developed as therapeutic agents.

The uniformity and effectiveness of the end product might be enhanced by refining the synthesis process to regulate the size, shape, and stability of the nanoparticles. To optimize yield and biological activity, one might experiment with different synthesis settings, such as pH, temperature, and plant extract concentration. For commercial uses, it is also vital to scale up production while keeping quality and biological efficacy in mind.

Ultimately, this study's results show that *Dioscorea villosa*-synthesized gold nanoparticles are far more biologically active than the plant extract alone. Potentially useful in nanomedicine, pharmaceuticals, and other biomedical fields, the nanocomposites have strong antioxidant, antibacterial, and anticancer effects. To completely understand the potential of *Dioscorea villosa*-AuNPs, further study is needed. This research is especially important for understanding their action mechanisms, in vivo effects, and clinical application. The research lends credence to the idea that plant-based materials may be used to create sustainable, biocompatible nanomaterials that might be used in the future for medicinal purposes, and it also adds to the expanding area of green nanotechnology.

6. CONCLUSION

This study concludes that *Dioscorea villosa*, often known as wild yam, has biological features that may be used to synthesize and apply gold nanoparticles (AuNPs). The research proved that *Dioscorea villosa* extract may stabilize and reduce AuNPs during green synthesis, providing a sustainable and eco-friendly alternative to traditional physical and chemical procedures. By using the plant's intrinsic phytochemicals, this strategy not only reduces the usage of harmful chemicals but also enhances the biological activities of the generated nanoparticles.

The stability, homogeneous size distribution, and favorable physicochemical features of the produced AuNPs were validated by characterisation, and they are necessary for biological applications. In addition, the research found that *Dioscorea villosa*'s bioactive chemicals, when combined with gold nanoparticles, improve a range of biological functions. The antimicrobial investigation revealed that these nanoparticles had potent inhibitory effects on a variety of harmful bacteria and fungi, which bodes well for their potential as new antimicrobial agents. The antioxidant tests also showed promising results in free radical scavenging, which may be useful in the treatment of disorders caused by oxidative stress.

At suitable doses, the cytotoxicity evaluation of *Dioscorea villosa*-mediated AuNPs showed encouraging anticancer characteristics, especially against certain cancer cell lines. The potential use of these nanoparticles in cancer treatment is highlighted by their selective cytotoxic impact; this focused approach has the ability to reduce the adverse effects often seen with conventional chemotherapy.

Because these biosynthesized nanoparticles are biocompatible and can increase the bioavailability of medicinal chemicals, the research also reveals how they may be used in drug delivery systems. In particular, this may allow for the creation of more effective treatment techniques for long-term illnesses including cancer, heart disease, and neurological problems.

All things considered, this study shows how great *Dioscorea villosa*-mediated AuNPs may be for biological uses. To fully understand their therapeutic potential, further research is needed to investigate their pharmacokinetics, long-term safety, and effectiveness in living organisms. To put these results into practice in nanomedicine, it will be necessary to scale up the production process and perform clinical studies.

In conclusion, this research adds to what is already known about green nanotechnology and highlights the significance of combining natural resources with cutting-edge scientific methods to create novel, long-term, and successful medical treatments. This study lays a solid groundwork for further research that might lead to revolutionary developments in environmental science, biotechnology, and medicine.