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Green Synthesis of Gold Nanoparticles Using Acacia Modesta

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Abstract**—** *Nanotechnology means any technology on a nanoscale that has application in the real world. Nanotechnology involves the production of materials with exceptional precision and dimensions on a scale as small as one billionth of a meter and implies the ability to generate, utilize structure, components and devices. Nanotechnology is the science of building very small particles. Visualizing the scale of nanotechnology can be challenging, but it is essential to understand that 'nano' refers to particles that are incredibly tiny. The ongoing exploration aimed to synthesize stable, environmentally friendly, and biocompatible gold nanoparticles (AuNPs) using Acacia modesta leaves and assess their biological activities. Prior research has underscored the effectiveness of nanotechnology in facilitating the production of faster, smaller, and more portable products and systems that are notably more efficient. Utilizing plant extracts for nanoparticle synthesis represents an alternative and more environmentally conscious approach. The green synthesis of nanoparticles aims to reduce waste generation and advocate for sustainable methodologies. In recent years, the focus has shifted towards green processes utilizing mild reaction conditions and non-toxic precursors to advance nanotechnology and foster environmental sustainability. The X-ray diffraction measurements revealed that all AuNPs possessed a polycrystalline structure, evident from the intense graphical peaks within the complete spectrum of 20 values, ranging from 10–80°, supported by data from scanning electron microscopy. Leaves of Acacia modesta were gathered, dried, and powdered, resulting in a net weight of the powdered leaves material of 25 grams. Phytochemical screening of various Acacia modesta extracts preceded the purification of gold nanoparticles. The antibacterial and antifungal activity of AuNPs and crude aqueous Acacia modesta leaves were assessed using the well diffusion method and Slant agar dilution method. The dried powder was mixed with distilled water in a 1:10 ratio and boiled for 30 minutes. Transmission electron microscopy confirmed the nano-particles' size to be within the range of 30–150 nanometers. Acacia modesta AuNPs exhibited substantial efficacy against Methicillin-resistant Staphylococcus aureus, Salmonella typhi, Escherichia coli, Strep.pyogenes, and Klebsiella Pneumoniae. In addition to these microorganisms, Acacia modesta AuNPs also demonstrated significant activity against Trichodermas, Aspergillus furfur, Penicilium and Candida albicans. Based on the findings of this current research study, it can be concluded that Acacia modesta has the potential to inhibit the growth of various pathogenic microorganisms, which could be harnessed by the medical sector for the development of effective drugs to address a range of acute to chronic infections.*

***Keywords*— Acacia modesta leaves, Antibacterial and antifungal activity, Green synthesis, Gold nanoparticles (AuNPs), Nanotechnology, Nanoscale, Phytochemical screening.**

I. INTRODUCTION

Nanoparticles (NPs), characterized by their dimensions falling within the range of 1–100 nm, when we use the term 'nano' to describe something, we are referring to its incredibly minute size. The magnitude of this smallness is evident when considering that one nanometer is equivalent to one billionth of a meter, which translates to roughly 100,000 times smaller than the width of a human hair. Exploring the intricate realm of innovation at this remarkably small scale defines the scope of nanotechnology (Khan *et al.*, 2019). These structures find diverse applications in fields such as medicine, engineering, manufacturing, and the food industry, as well as in various consumer goods, including food storage solutions, healthcare items, and personal care products. The evolving utilization of nanoparticles has precipitated an urgent need to comprehensively assess their impact on both the environment and human health, necessitating a multidisciplinary approach to ensure the safe and sustainable integration of these materials into various industries (Mateo *et al.*, 2017).

Nanotechnology is one of the most exciting and fast-moving areas of science today. Nanoparticles (NPs) are considered to exhibit heightened biological reactivity compared to their bulk counterparts, primarily attributable to their diminutive size and the resulting larger surface area to volume ratio. Several nanomaterials are naturally present, ubiquitous in volcanic ash, ocean spray, and dust particles. Additionally, natural nanostructures can be found within various plants. This heightened reactivity leads to the production of oxidative effects at the cellular level (Ijaz *et al.*, 2020). Furthermore, NPs demonstrate a remarkable ability to traverse the body, accumulate in specific organs, infiltrate cell membranes, and instigate deleterious responses such as perturbations in calcium homeostasis and gene expression, inflammatory reactions, and DNA damage. The intricate interplay between nanoparticles and biological systems underscores the critical need for a thorough understanding of their biological interactions and potential toxicity, necessitating the implementation of stringent safety assessments to ensure their responsible and sustainable integration across various applications (Zuo *et al.*, 2017).

Gold ions can be transformed, through the use of various reducing and stabilizing agents, into minute assemblies of gold atoms commonly referred to as nanoparticles. Gold nanoparticles (AuNPs) are compact structures at the nanometer scale that can be synthesized in various shapes and sizes (1–100 nm). In Nano medicine, gold nanoparticles have minimal toxicity and another highly valued property is the targeting ability, which refers to the capacity of specific nanomaterials to target particular cells with precision. Gold nano particles have various clinical applications (Boisselier *et al.*, 2009). Scientists are currently working on developing tiny gold particles that can be loaded with drugs. These nanoparticles can be modified to specifically bind to cancer cells, allowing them to be transported inside. Importantly, drug-loaded gold nanoparticles are engineered to exclusively target infected cells without affecting neighboring healthy cells. Conditions such as malignant brain tumors (glioblastoma and high-grade astrocytoma), *Alzheimer's disease*, multiple *sclerosis*, *Parkinson's disease*, and other neurological disorders necessitate prolonged-acting formulations and the controlled release of drugs in specific brain regions (Eduardo *et al*., 2021).

Nanotechnology can be used to reshape the world around us literally. Scientist can be creating nanostructure themselves by rearranging the atom of an object. They can make new nanomaterials with new property that can be more effective and used everywhere in future. In historical contexts, individuals during the 16th century were known to utilize exquisitely gold-coated materials for various applications, particularly within the medical domain (Pomerantseva *et al.*, 2019). Historical evidence indicates the use of gold particle-coated materials for oral medicine, pharmaceuticals, and the implantation of tissues and organs. Plants harbor an array of bioactive constituents, including flavonoids, alkaloids, terpenoids, phenolics, amino acids, and steroids, which serve as effective reducing agents for the synthesis of nanoparticles. Extracts derived from plants have demonstrated notable efficacy in the reduction of Au+++ ions to Au NPs (Wang *et al.*, 2019).

II. METHODOLOGY

2.1. Study Area

The research investigation was performed at Institute of Allied Health Sciences of Sarhad University of Science and Information Technology Microbiology lab.

2.2. Plant Collection

Acacia modesta (leaves) were taken from an area of the Shamshato forest, Peshawar and then shades dried and packed them in bags.

2.3. Aqueous Extract Preparation

The leaves of *Acacia modesta* were procured and subjected to desiccation. Subsequently, the desiccated leaves were pulverized, resulting in a net weight of the powdered leaf material amounting to 25 grams. The dried powder was combined with distilled water in a 1:10 ratio and subjected to boiling for duration of 30 minutes. The resulting infusion was then meticulously filtered using ten pieces of Whatman filter paper, with the purpose of eliminating insoluble components from the extract.

2.3.1. Ferric Chloride Test

The extract, quantifying 50 milligrams, was solubilized in 5 milliliters of distilled water. Following this, a small quantity of neutral 5% ferric chloride solution was introduced into the solution. The manifestation of a dark green hue signifies the existence of a phenolic compound.

2.3.2. Mayer's Test

To assess the presence of alkaloids, 5 milliliters of the plant sample extract were transferred into a test tube, followed by the addition of two drops of Mayer's reagent along the inner wall of the tube. The absence of the formation of a whitish precipitate indicates the absence of alkaloids in the sample.

2.3.3. Flavonoids Test

The addition of 3 milliliters of a 1% aluminum chloride solution to 5 milliliters of each extract led to the observation of a yellow coloration, which serves as an indicator of the presence of flavonoids.

2.3.4. Saponin Test

A 50-milligram portion of the extract was diluted with distilled water to achieve a total volume of 20 milliliters. Subsequently, the resulting suspension was vigorously agitated in a graduated cylinder for duration of 15 minutes. The formation of a 2-centimeter layer of froth indicates the presence of Saponin.

2.3.5. Salkaowsksi Test for Steroid

Transfer 5 milliliters of the aqueous extract solution into a test tube, followed by the addition of a chloroform solution. Subsequently, introduce a few drops of concentrated sulfuric acid into the test tube. The appearance of a reddish-brown color serves as evidence of the presence of steroids.

2.3.6. Benedict Test

To 0.5 milliliters of the aqueous extract, 0.5 milliliters of Benedict's reagent were added. The mixture was thoroughly shaken and subsequently boiled for duration of 1 to 2 minutes in a water bath. A color change from blue to green denotes the presence of reducing sugars.

2.4. Synthesis of Gold Nanoparticles

The synthesis procedure for gold nanoparticles entailed the combination of a measured quantity of the aqueous extract of the *Acacia modesta* plant with water. Specifically, 25 grams of the aqueous extract were introduced into 500 milliliters of distilled water and subjected to a boiling temperature for 25 minutes. The occurrence of nanoparticle formation was discerned through a noticeable alteration in color, transitioning from a light yellow to a reddish wine hue. Additionally, conventional citrate-capped gold nanoparticles were synthesized using the standard procedure for the purpose of comparative analysis.

2.5. Purification of Gold Nanoparticles

The purification of water-soluble gold nanoparticles poses a significant challenge due to the closely matched solubility of the nanoparticles and impurities. Consequently, standard purification methods, such as centrifugation, often prove insufficient or ineffective. To address this, the solution containing the nanoparticles was dried in an oven maintained at 50°C. Subsequently, the dried particles were gently dislodged and transferred into Eppendorf tubes with a capacity of 1.5 milliliters, each containing distilled water. The tubes were subjected to centrifugation at a rate of 12000 revolutions per minute for duration of 15 minutes. Post-centrifugation, the resulting pellet was carefully collected and subsequently dried.

2.6. Anti-Bacterial assay

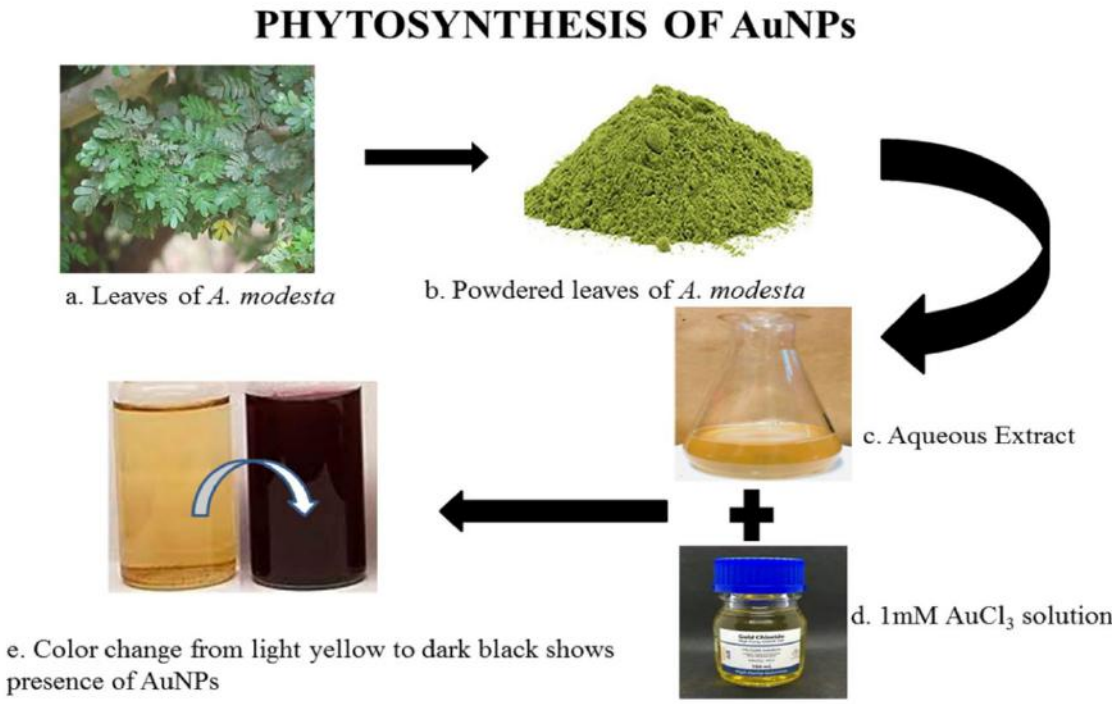
The antibacterial efficacy of AuNPs and the crude aqueous leaves of *Acacia modesta* were examined utilizing the well diffusion method. Initially, a stock solution of 3 mg/mL was prepared using sterile DMSO. Subsequently, 100 µL of the working solution was introduced into 6 mm wells carefully arranged on sterile nutrient agar plates. Following this, the test bacterial broth cultures were inoculated onto the Petri plates. The culture plates were then left undisturbed in a laminar flow hood for a minimum of 30 minutes, allowing the test samples to diffuse into the media. Subsequently, all culture plates were incubated in an upright position at 37°C for 24 hours. Sterile DMSO served as the negative control, whereas the standard drug Amoxicillin was used as the positive control. Post-incubation for 24 hours, the percentage of inhibition was determined by calculating the zone of inhibition using the following formula:

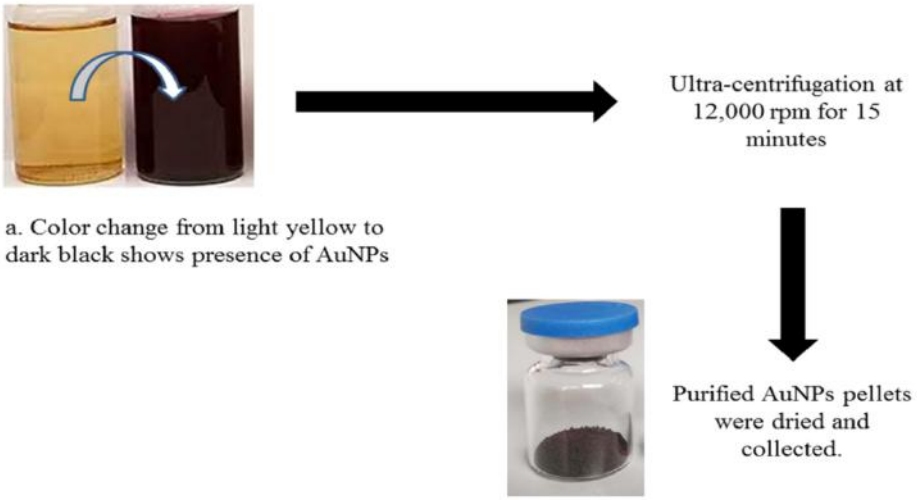
$$\textbf{\% Inhibition} = \frac{\textit{Zone of Inhibition of test Sample (mm)}}{\textit{Zone of Inhibition of Standard (mm)}} \times \mathbf{100}$$

2.7. Antifungal Assay

The evaluation of the antifungal potential of the crude aqueous extract of *Acacia modesta* and AuNPs was conducted via the Slant agar dilution method. Initially, a stock solution of 24 mg/mL was prepared for the test samples in sterile DMSO. Subsequently, the Sabouraud Dextrose Agar (SDA) medium was augmented with a 70 µL working solution of AuNPs and crude aqueous extracts. The media-containing test tubes were positioned in a slanted manner to facilitate solidification. The chosen pathogenic fungal species were then introduced onto the SDA-supplemented media. All test tubes were securely sealed and placed in a fungal incubator set at 28°C for duration of 1 week. Sterile DMSO served as the standard negative control, while the standard antifungal drug Miconazole was used as the positive control. At the culmination of the incubation period, the percentage of inhibition was computed using the provided formula below:

$$\textbf{\% Inhibition} = \frac{\textit{Linear growth of fungi in test sample (mm)}}{\textit{Linear growth of fungi in Standard (mm)}} \times \mathbf{100}$$





2.8. PHARMACOLOGICAL INVESTIGATION OF AuNPs

❖ **Antibacterial Activity**

The study explored the antibacterial effects of gold nanoparticles (AuNPs) and an aqueous leaf extract using the well diffusion method. The research focused on evaluating the impact of these substances on various pathogenic bacterial species reported by Ahmad *et al.*, (2017).

❖ **Antifungal Activity**

The study conducted thorough investigation utilizing the tube dilution method to examine the antifungal potential of both gold nanoparticles (AuNPs) and an aqueous leaf extract against various pathogenic fungal species Ahmad *et al.*, (2017).

III. RESULTS

3.1. PHYTOCHEMICAL SCREENING

Green biocompatible AuNPs were fabricated with *Acacia modesta* leaves. In order to identify the presence of bioactive phytoconstituents responsible for the reduction of Au+ to Au^0 in a cost-effective and environmentally friendly manner, an initial phytochemical analysis of the plants was conducted. Various components within the plants acted as potent bioreducers and capping ligands, facilitating the synthesis of monodispersed, stable AuNPs. These nanoparticles were subsequently purified, characterized, and optimized using established protocols from previous literature. Furthermore, the biological and pharmacological properties of the synthesized AuNPs were meticulously evaluated and compared with those of crude ethanolic, methanolic, acetonic, and aqueous leaf extracts derived from the selected plants.

Table 1. Phytochemical analysis of Acacia modesta leaves extract

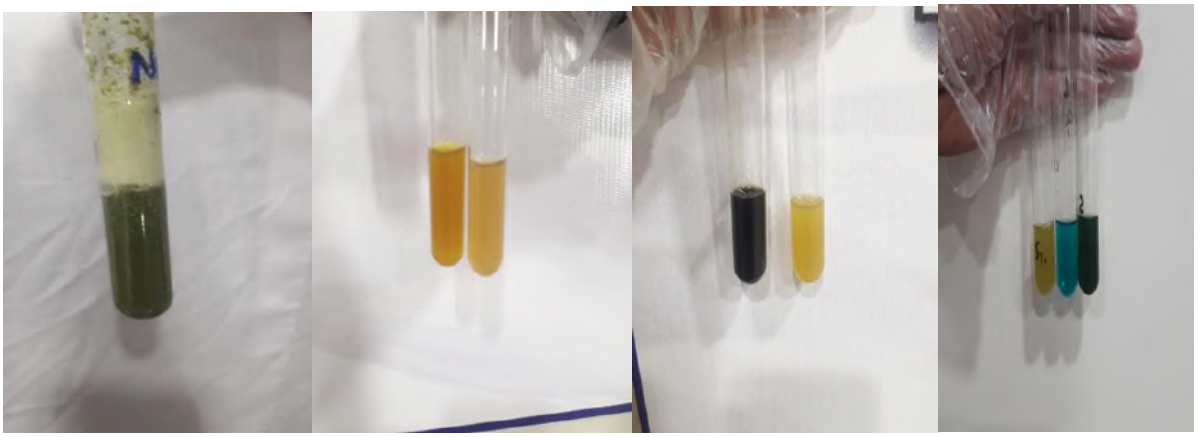
Phytochemical compounds present in plant extract	Presence of compound in *Acacia modesta* leaves
Alkaloids	+++
Tannins	+++
Phenolic compounds	++
Saponins	+
Steroids	+++
Flavonoids	+++
Reduce in sugar	-

Note: (+) less quantity of bioactive phytochemical

(++) moderate quantity of bioactive phytochemical

(+++) high quantity of bioactive phytochemical

(-) absence of bioactive phytochemical

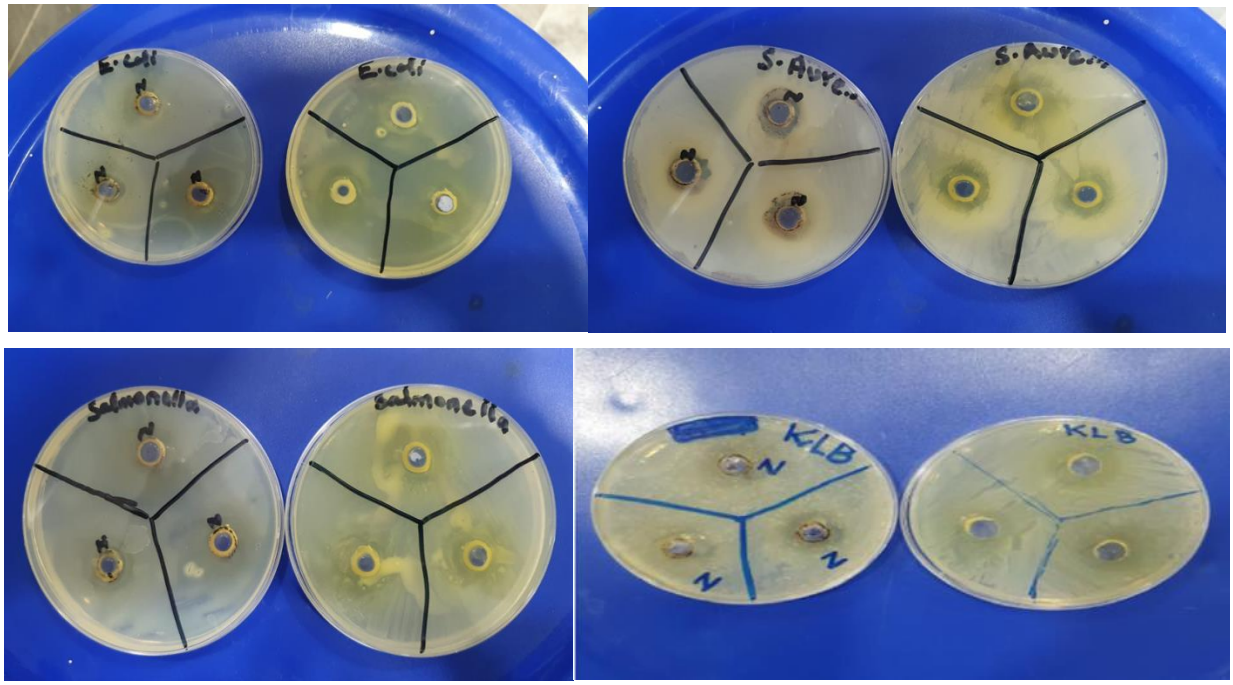


PHYTOCHEMICAL SCREENING OF ACACIA MODESTA LEAVES

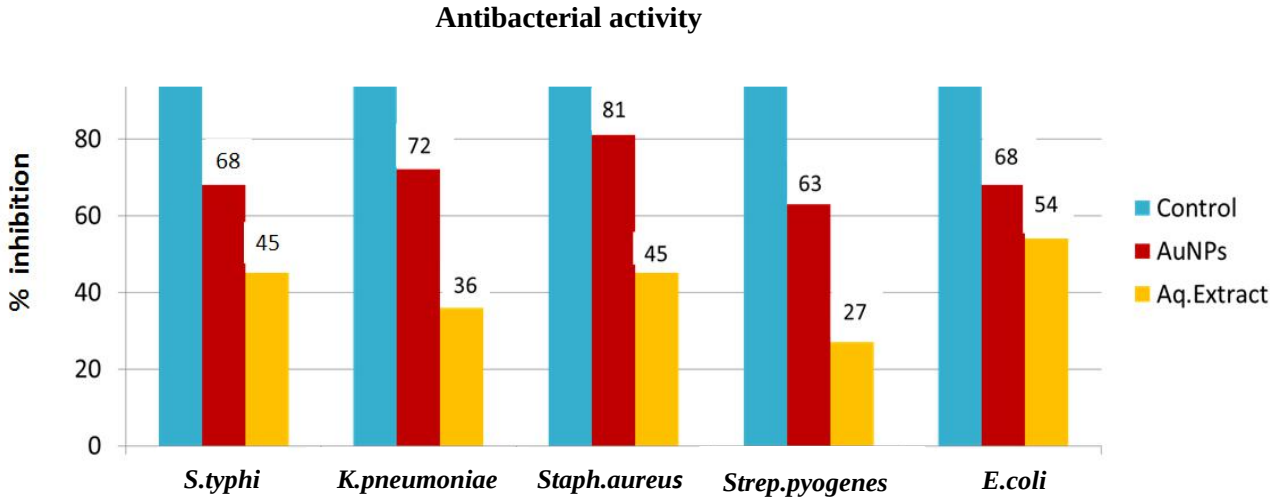
3.2. ANTIBACTERIAL ACTIVITY

Table 2. Antibacterial analysis

Extract	*Salmonella typhi*	*Klebsiella Pnuemoniae*	*Staphycoccus aureus*	*Strep. Pyogenes*	*E. coli*
Ethanolic extract of *A. modesta*	6mm	5mm	6mm	4mm	7mm
Gold nanoparticles	8mm	9mm	11mm	7mm	8mm



Antibacterial analysis of Ethanolic extracts of Acacia modesta and gold nanoparticles



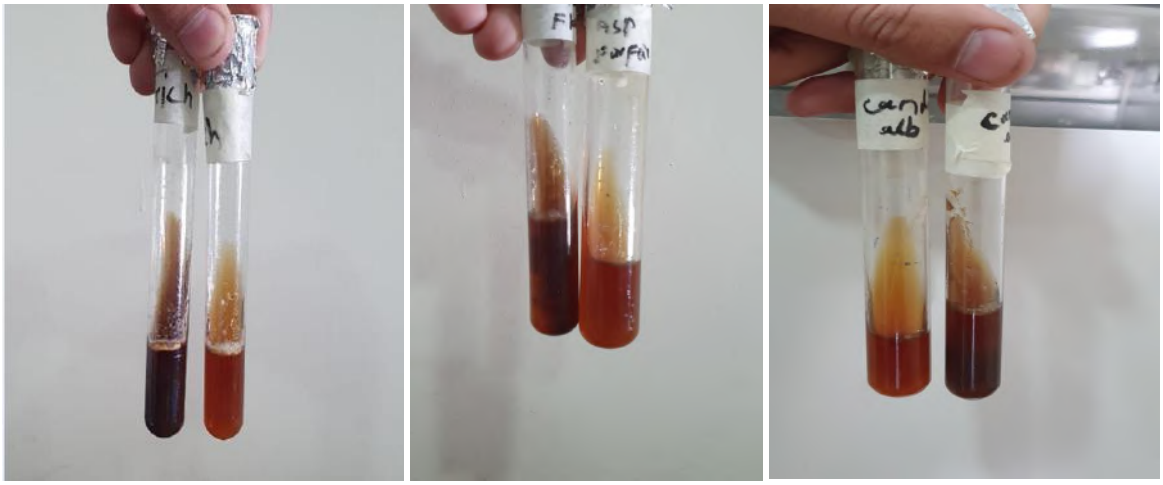
The histogram depicted above illustrates the comparative antibacterial analysis of gold nanoparticles (AuNPs) and extracts from the leaves of Acacia modesta. The blue line represents the positive control, specifically Amoxicillin. The red

line displays the impact of AuNPs on various bacterial species, while the yellow line demonstrates the effect of Acacia modesta leaf extracts on different bacterial species.

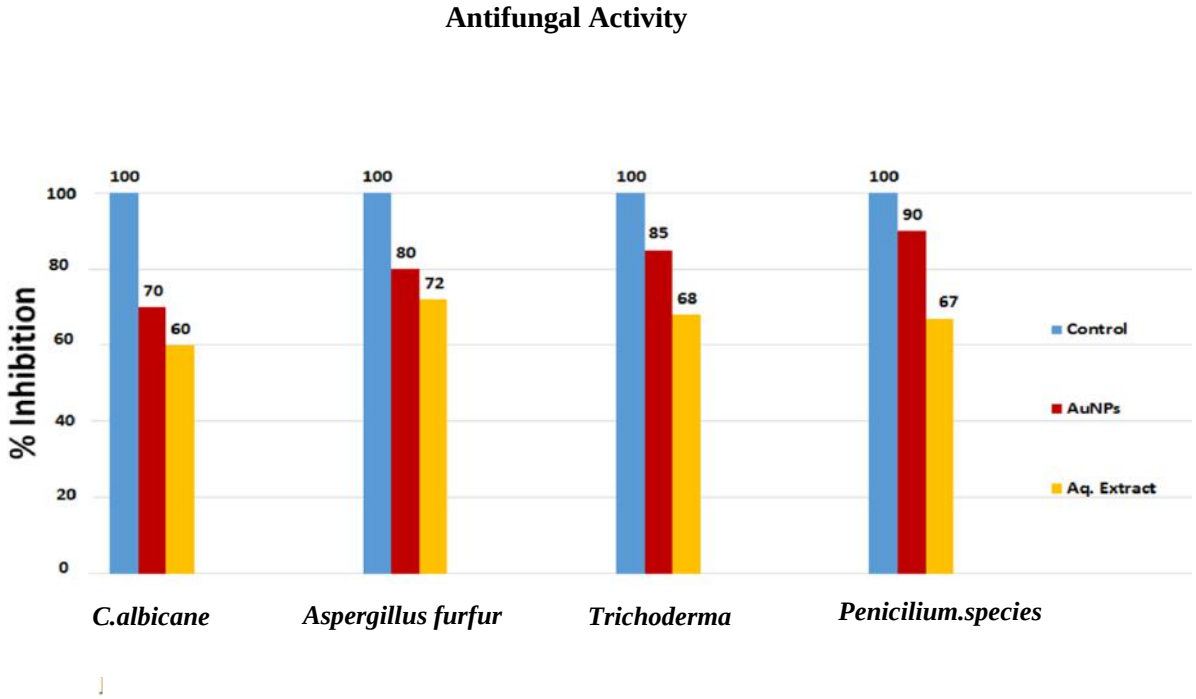
3.3. Antifungal activity

Table 3. Antifungal analysis

Extract	*Candida.albicane*	*Aspergillus furfur*	*Trichoderma*	*Penicilium.species*
Ethanolic extract of A. modesta	5mm	7 mm	8mm	6mm
Gold nanoparticles	8mm	10mm	11mm	12mm



Antifungal analysis Ethanolic extracts of Acacia modesta and gold nanoparticles



The histogram presented above delineates the comparative antifungal evaluation of gold nanoparticles (AuNPs) and the bioactive constituents extracted from the leaves of *Acacia modesta*. The blue line is indicative of the positive control, Miconazole. Meanwhile, the red line denotes the impact of AuNPs on diverse fungal species, elucidating their potential antifungal properties. Similarly, the yellow line signifies the effect of the extract obtained from *Acacia modesta* leaves on a spectrum of fungal species, thereby highlighting the potential antifungal attributes of the plant extract.

IV. DISCUSSION

Nano-biotechnology offers an eco-friendly way to make tiny gold nanoparticles using *Acacia modesta* plant extract. This extract contains natural compounds from the plant's leaves that help both in making the gold particles and keeping them stable. The study aimed to explore the presence of potential phytochemicals, specifically polyphenols, in the leaves of Acacia modesta, and to employ a green synthesis approach to create gold nanoparticles (AuNPs) for subsequent investigation of their pharmacological activities (Timoszyk *et al.*, 2022). The research study outlined the following specific objectives. The objective involved utilizing the aqueous extract of Acacia modesta to synthesize gold nanoparticles via a green synthesis approach. The second objective centered on the purification of the synthesized gold nanoparticles. The third objective emphasized the biological assessment of the synthesized gold nanoparticles (Rodriguez-Luis *et al.*, 2016).

In recent studies, scientists are finding easier, cheaper, and eco-friendly ways to make tiny particles. They use things like *Bacteria*, *Fungi*, and plant extracts to do this. These ways are popular because they work well with living things. They help turn metals into tiny particles (Lee *et al*., 2020). In one study, gold particles made with *Acacia modesta* leaves stopped the growth of some fungi and bacteria, including *E. coli* and *Staphylococcus aureus*. Another study showed that these gold particles also worked against other *Bacteria* like *Staphylococcus aureus*, *Klebsiella pneumonia*, and *E. coli* (Nadeem et *al.*, 2017)

Past studies have shown that making gold particles using natural methods is good for the environment and easy for people to do. made with *Acacia nilotica* and *Olea europaea* leaves didn't harm normal cells, even at higher amounts. From the *Acacia modesta* antimicrobial assay, it is estimated that crude extracts It's also been found to have great potential for medicine with very few side effects (Latif *et al*., 2020) Found that gold particles of Acacia *modesta* and gold nanoparticles extract inhibits the growth of various types bacteria at different levels of inhibition, such as *klebsiella, salmonella, Staphylococcus auerus* and *Escheria coli.* In addition, the results showed that *Acacia modesta* ethanolic extract inhibits the growth of selected *Fungi* at different percentages of inhibition, including *Trichodermas, Aspergillus furfur, Candida albicane* (Awad *et al*., 2019).

V. CONCLUSIONS

The leaves of *Acacia modesta* harbor phytochemicals with the capacity to facilitate the active synthesis of gold nanoparticles (AuNPs) through their natural functions as reducing and capping agents. Examination of the resulting AuNPs revealed their crystalline nano-spherical structure, with a size distribution of less than 100 nanometers. When contrasted with the crude aqueous leaf extract of *Acacia modesta*, the AuNPs demonstrated notable efficacy in combating microbial and leishmanial infections. They exhibited strong activity against various tested bacterial and fungal strains, while also displaying anti-leishmanial properties on par with the effectiveness of Miltefosine. Remarkably, the cytotoxic effects were minimal, even at high concentrations of 1000 µg/ml. These findings suggest potential applications in the medical domain for the development of innovative therapeutic strategies.

Based on the outcomes of the present research investigation, it can be inferred that *Acacia modesta* possesses the ability to hinder the proliferation of diverse pathogenic microorganisms. This attribute could be harnessed by the medical sector to develop a range of effective drugs for mitigating various forms of acute and chronic infections. Moreover, these extracts hold promise for effective integration into medicinal formulations targeting a wide spectrum of microbial and neurological ailments.

RECOMMENDATION

Investigation into a diverse array of phytochemicals suitable for precise utilization in the production of gold nanoparticles (AuNPs) is warranted. *Acacia modesta* holds promise for future exploitation as a source of valuable antimicrobial compounds for the pharmaceutical industry. The potent AuNPs generated can serve as a foundation for the development of novel antimicrobial agents, with potential applications as community medicine. Furthermore, the exploration of AuNPs for application in agricultural and engineering domains is crucial for the development of biosensors, biocatalysts, and nano-devices. Finally, assessment of the

toxicity on human and animal cell lines is essential to ascertain the safety and potential risks associated with these nanoparticles.

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