EVALUATION THE ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF NANOPARTICLES

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Abstract—**Nanotechnology involves the study of nanometer-sized particles known as nanoparticles, which have improved properties for use in various fields. In this study, iron oxide nanoparticles were produced using a green synthesis approach with the help of an aqueous extract of Euphorbia serpens kunth. The synthesized nanoparticles were characterized using different microscopic and spectroscopic techniques, and their antimicrobial activities were tested against various bacterial and fungal strains.**
Additionally, the antibacterial and antioxidant activities of iron nanoparticles and plant extract from Euphorbia serpens were evaluated against Staphylococcus aureus and ascorbic acid, respectively, using the well diffusion method. The results showed that the plant extract had the highest inhibition zone (9mm), followed by the nanoparticles (8mm), for antibacterial activity, while the extract was more effective than the nanoparticles for antioxidant activity.
Furthermore, the phytotoxic potential of the Euphorbia serpens extract and silver nanoparticles was assessed against Lemna aequincotialis at different concentrations. Both the plant extract and nanoparticles exhibited high effectiveness against Lemna aequincotialis. These findings suggest that silver and iron oxide nanoparticles, along with plant extracts, have potential for use in antibacterial and antioxidant therapies, as well as for weed control. Further studies are necessary to confirm their safety and efficacy in various applications.

Index Terms—Nanotechnology, Nanoparticles, Microscopic, Spectroscopic

I. INTRODUCTION

Nanotechnology refers to the design, production, and use of various materials and technologies of nanoscale size and form (Shnawa et al., 2021) [1]. Nanotechnology is a scientific field based on atomic, molecular, and supramolecular principles that aims to produce nanostructures with enhanced qualities, and the term nanoparticles refers to particulate material ranging in size from 1-100 nm. Holding a Nano scale size confers advanced properties, such as a relatively high volume-to-volume ratio surface area. Increased surface area, mixed with on the formation of nanoparticles and solution distribution, makes a significant contribution to their enhanced physicochemical characteristics, which are useful in a variety of fields, such as antimicrobial resistance (Supriya et al., 2022) [2]. Biomolecular location, diagnostics, catalysis, microelectronics, and instruments for distinguishing drug focusing of malignant growth cells are areas of study. Over the past ten years, the scientific community has shown a lot of interest in nanoparticles because of their wide range of applications and their increasing ability to alter their structure and properties. Among these areas of interest is the creation of synthetic methods that are

environmentally friendly and work. Metal nanoparticles can only be produced conventionally using chemical and physical methods if hazardous substances are used. These methods are additionally connected by their enormous energy input and exorbitant downstream interaction (Rasmussen et al., 2010) [3]. Nanotechnology is generally remembered to can possibly help medication advancement, water disinfection, data and correspondence innovation (ICT), and more grounded and lighter material handling. The creation and management of nanometer-sized materials, either by expanding from a small number of singles or by purifying or decreasing bulk resources, is the focus of nanotechnology.

When brought down to the nanoscale, conventional value materials frequently exhibit novel and one-of-a-kind properties, such as uncommon quality, chemical reactivity, electrical conductivity, super paramagnetic behavior, and other properties that the same fabric does not possess at a larger scale (Divya Rohatgi, 2020) [4].

Nanoparticles have received a lot of attention due to their outstanding performance in a variety of disciplines, which has led to their use in a variety of goods such as water filtration plants, packaging, pharmaceuticals, and surface coatings. When exposed to such materials, nanoparticles may be incorporated into these goods. Nanoparticle manufacturing is an exciting area, and several synthesis methods have been devised to make materials at the nanoscale.

However, because many of these synthesis processes use dangerous carbon-based solvents, using nanomaterials for human consumption is difficult. Despite these restrictions, nanomaterials have the potential to provide desirable properties that outperform those of current manufacturing processes. According to Gnanakumaran (2023) [5], Nanoparticles have been particularly engineered for use in plant biology and biosensors. However, the possible dangers connected with nanoparticle exposure need the development of green and sustainable nanoparticle manufacturing technologies. When implementing industrial processes with the help of nanotechnology, it is critical to use a strategy that reduces the presence of dangerous materials and successfully integrates future technologies within a pollution-free environment. To supplement these efforts, "biofabrication methods" that entail the use of natural, non-toxic, and environmentally acceptable solvents to improve the efficiency of chemical processes can be used. By understanding and using these strategies, the development and use of nanoparticles may be enhanced while keeping a focus on safety and environmental sustainability in mind (Nandipati et al., 2024) [6].

The use of ecologically friendly components such as plants and microorganisms in green synthesis methods has emerged as a key strategy in nanoparticle creation (Jiang J, 2017) [7]. Green chemistry has grown in importance as an alternative to traditional chemistry, particularly in light of the energy crisis and its attendant restrictions, particularly in poor countries. As a result, considerable multidisciplinary research in a variety of domains has been conducted with the goal of understanding and leveraging the potential of green synthesis processes. These initiatives aim to solve the obstacles faced by old technologies and pave the way for ecologically responsible and sustainable nanoparticle synthesis (Hussain et al., 2018). Nanomaterials have found applications in various disciplines, including electronic instruments, photography, material science, dye removal, and bioremediation, as evidenced by studies conducted by Chaudary et al., (2018) and Saha et al., (2017).

Biological synthesis provides a practical, ecologically benign, and cost-effective method for creating nanomaterials. It permits the controlled creation of nanoparticles with specified properties, allowing them to be used as catalysts – a trait that older approaches fail to attain. Because of their environmental friendliness, biofabrication methods offer an advantage over physiochemical technology. They are readily optimized for large-scale manufacturing, as they do not require harmful chemicals, heat, energy, or high pressure. They also provide cost-effective solutions. Plants are favoured for nanomaterial synthesis over other biosynthetic processes because they can be easily tuned for experimental-scale manufacture. Plant-based synthesis, unlike other approaches, does not require cell culture upkeep or a sterile environment (Ahmed et al., 2013).

Nanoparticles (NPs)

Green nanotechnology has significant potential for transforming the planet into a more ecologically friendly one in the near future. Nanoparticles, in particular, have a wide range of uses that help to reduce

and avoid emissions. They can be used in catalytic processes to reduce large-scale waste formation, battle hazardous microbes, detect water contaminants, and aid in the cleaning of the atmosphere. Green chemistry, on the other hand, refers to a set of concepts and strategies for creating goods and innovations that remove or limit the usage of harmful compounds. We can pave the road for sustainable and eco-friendly developments in nanotechnology and beyond by embracing green chemistry methods (Lu and Ozcan 2015).

Nanomaterials are important in nanotechnology because they bridge the gap between atomic/molecular structures and bulk materials. Nanomaterials are extremely important in research because they give insights into the basic features of materials at the nanoscale. Noble metals such as iron (Fe), gold (Au), platinum (Pt), and palladium (Pd) have emerged as very effective and extensively used alternatives among the numerous types of nanomaterials. Metallic nanoparticles, particularly those formed of noble metals, have a wide range of uses, including medicine and physics. Because of their unique physicochemical features, such as catalytic activity, optical properties, electronic properties, and magnetic properties, scientists are highly interested in investigating innovative synthetic procedures for these metallic nanostructures (Ranjithum et al., 2013).

Researchers discovered that changing the size and dimensions of particles, thin films, and materials at the nanoscale results in considerable changes in their characteristics when compared to their larger-scale counterparts. Understanding these disparities and obtaining control over the formation of tiny structures offers enormous promise for breakthroughs in industries such as electronics, building, and materials research. Because of their unique features, metallic nanoparticles, in particular, are widely used in biotechnology and biomedicine. They have a wide range of surface bioconjugation properties that allow molecular samples to be attached to their surfaces. Surface functionality and optical qualities are critical in applications such as biosensors, drug delivery systems, and diagnostic imaging(Tedesco et al., 2010). Currently, there are various methods available for the production of nanomaterials, including physical, chemical, and recently discovered biomimetic approaches. However, traditional methods often lead to rapid agglomeration of nanoparticles, resulting in larger particles with poor uniformity. Biomimetic synthesis of iron oxide nanoparticles, on the other hand, utilizes biological systems such as bacteria, yeast, and plants. This approach offers several advantages over physico-chemical techniques, including improved environmental friendliness and cost-effectiveness. In light of these benefits, the concept of "greener nano synthesis" has been proposed, emphasizing the use of biomimetic processes to achieve more sustainable and eco-friendly production of nanomaterials.

II. Qualitative analysis

Nanoparticles have different features such as size, shape, surface area, and dispersity, which may be investigated using a variety of approaches. These techniques, which provide insights into the properties of nanoparticles, may be roughly categorized into quantitative and qualitative methodologies. UV-vis spectroscopy, Fourier transform infrared spectroscopy, X-ray diffraction, X-ray photoelectron spectroscopy, scanning electron microscopy, surface-enhanced Raman spectroscopy, and energy-dispersive X-ray spectroscopy are among the analytical techniques used. Researchers may use these approaches to determine critical features of iron oxide nanoparticles such as particle size, shape, and purity. These analytical approaches provide a thorough understanding of the structural and compositional properties of nanoparticles, which is critical for improving their performance and adapting them to specific applications.

Fourier transforms infra-red (FT-IR)

The Fourier transform infrared spectroscopy (FT-IR) technology may be used to detect chemical functional groups in absorbance ranges ranging from 4000 to 400 cm-1. For identifying functional groups and strongly polar bonds, FT-IR spectroscopy is a rapid, simple, and effective analytical technique. It's utilized to get an infrared spectrum of a gas, liquid, or solid, as well as absorption and emission, photoconductivity, and Raman scattering. The FT-IR spectrometer obtains data throughout a wide range at the same time. Infrared Fourier transform measurements can be utilized to discover possible phytochemicals responsible for successful nanomaterial stability.

Scanning electron microscopy (SEM)

SEM (Scanning electron microscopy) is a powerful imaging technique that uses electrons to provide a detailed output image (Klein et al., 2012). SEM is often used to characterize manufactured nanoparticles, allowing for the study of important features such as size, shape, morphology, and distribution. This technology produces high-resolution images that enable researchers to inspect and assess the surface characteristics and structural aspects of nanoparticles. SEM may be beneficial in studying and improving the properties of manufactured nanoparticles.

X-rays Dispersion (XRD)

X-ray diffraction is a method for determining the atomic structure of materials, as well as quantifying mineralogical data and qualitatively identifying minerals in geological materials. It is a useful method for assessing crystallinity, clarity, purity, and polydispersity of iron oxide nanoparticles, as well as describing and validating their shape. The XRD equipment is also used to examine phase changes caused by temperature or stress, compute lattice strain, measure crystalline modules, and apply molecular modeling to quantify the size of polymer structures.

III. MATERIALS AND METHODS

A. Plant collection

The Euphorbia Serpens plant was collected from the Mardan District. At Abdul Wali Khan University, the plant was identified using readily available literature. To prepare the plant for further analysis, it was first cleaned with tap water to remove any dust particles. Subsequently, the plant material was sliced into small pieces and allowed to dry at room temperature for a period of 10 days. Once completely dried, the plant material was ground into a fine powder using a grinder (Palithya et al., 2018)

Figure 1: Plant Material

B. Preparation of ethanolic extract

To create the extract, 100g of Euphorbia Serpens powder was mixed with 500ml of ethanol in a beaker. The beaker was kept at room temperature for 7 days, with frequent shaking. After seven days, the material was filtered with filter paper. The filtrate was evaporated with a rotary evaporator at 60 0C, and the concentrated solution was completely dried on petri plates in a 600 0C water bath. Thakre N and colleagues (2024) [8]

C. 1mM iron nitrate solution preparation

Iron nitrate was accurately weighted (0.0422g) and dissolved in flask per 250ml distilled water solution using an electronic balance, providing a concentration of 1mM. HABEEB, M. S. and colleagues (2024) [9].

D. Synthesis of iron oxide nanoparticals

Iron oxide nanoparticles were formed by mixing 200g/ml of aqueous extract of leaves and 200g/ml of ethanolic extract of leaves with 1mM iron nitrate aqueous solution in different ratios (1:1, 1:2, 1:3, 1:4,

1:5, 1:6). The mixture was shaken continuously at room temperature for 24 hours on a frenzied stirrer until the distinctive color of iron nanoparticles was obtained by reducing iron ions with the assistance of phytochemical signaling nanoparticles synthesis. According to Noruzia et al., (2011), the color changed quickly in the 1:5 ratio of aqueous iron nitrate solution. The iron oxide nanoparticles that were synthesized were subjected to physical characterization to validate their properties, which involved evaluating their absorbance in an iron oxide nanoparticle solution using UV-vis spectroscopy. The produced nanoparticles were then purified by repeated centrifugation at 14800 rpm for 15 minutes, followed by dispersing the pellet in distilled water to eliminate water-soluble components like protein and secondary metabolites. The final step was to dry the nanoparticles under vacuum for 24 hours. The capping agent, functional group, structure, and composition of the pure iron oxide nanoparticles were investigated using FT-IR and XRD analyses after freeze-drying, according to Shamim, M. Z. in 2022 [10].

E. Physical Characterization

1) Spectroscopy (UV-vis)

The absorbance of iron oxide nanoparticles that were produced from a combination of A. vittata bulb and leaf extracts and iron nitrate solution was measured using UV-vis spectroscopy. A double beam UV-vis spectrometer with a resolution of 1nm, UV-1602, was used to measure the spectral absorbance of the iron oxide nanoparticles produced. To observe the iron oxide nanoparticle production, distilled water was used as a blank. The spectral absorbance was measured for various ratios of leaf and bulb extracts and iron nitrate solution, including 1:1, 1:2, 3:1, 4:1, and 1:6, as reported by Nandipati Sai Akash et al., in 2024 [11].

2) FT-IR Spectroscopy

After the iron oxide nanoparticles were dried using a vacuum drier at 37 0C and mixed with Ker, they were pressed using a hydraulic pellet press to form sample pellets. FT-IR spectroscopy was carried out on these pellets using a PerkinElmer FT-IR SPECTRUM ONE spectrometer with a range of 4000cm-0cm-1 and a resolution of 4cm-1. Similarly, FT-IR spectroscopy was conducted on the bulb and leaf extracts of A. vittata, using the same spectrometer with a resolution of 4cm-1 and a range of 4000cm-0cm-1 (Ahmed MH et al., 2022) [12].

3) X-Ray Diffraction (XRD) Analysis

A gamma-ray diffractometer was used to examine the X-ray diffraction pattern of iron (Fe) nanoparticles produced from A. vittata bulb and leaf extract. The diffraction patterns were captured using a nickel monochromator with a tube voltage of 40KV and a tube current of 30mA, which filtered the Cu-K radiation wave. The line width of the most intense reflection peak was measured to calculate the average diameter of the Fe nanoparticles. The size of the Fe nanoparticles was calculated using Scherer's equation, which took into account factors like the average crystallographic domain size perpendicular to the reflecting planes, the X-ray wavelength, a shear factor denoted as K (0.89), Bragg's angle, and the full width at half maximum (FWHM) in radians.

F. Biological activities

1) Activity Feainst insects

The researchers aimed to see how iron oxide nanoparticles and plant extracts (bulb and leaf) worked against Tribolium castaneum. A number of tests were carried out to assess the insecticidal activity of iron oxide nanoparticles and plant extracts. Iron oxide nanoparticles and plant extracts were dissolved in distilled water at a concentration of 1mg/ml to make a stock solution. Three distinct concentrations (100 l/ml, 500 l/ml, and 1000 l/ml) were generated from this stock solution, with two duplicates for each concentration. Filter paper was cut to fit the size of petri dishes and placed inside them to conduct the experiment. Each produced sample concentration was applied to the filter paper, with distilled water serving as a control. The plates were then left undisturbed for 24 hours to allow the solutions to completely evaporate. Following the evaporation process, 30 healthy insects were carefully picked from the Pharmacognosy lab and transferred to each of the plates using a clean brush. The incubation times of the nanoparticles and plant extracts on the insects were recorded at 24, 48, and 72 hours.

2) Phytotoxic *activity*

The study aimed to assess the potential phytotoxicity of Iron oxide nanoparticles produced from Euphorbia Serpens leaf extracts. The experiment was conducted using Lemna equinoctials as the test plant, and three different concentrations (50, 100, and 500) with two duplicates were used. To assess the growth inhibition of Lemna plants caused by different samples, a series of experiments was conducted. First, Petri dishes were sterilized using autoclaving. Then, a stock solution of each sample, consisting of leaf and bulb extract along with associated iron oxide nanoparticles, was prepared in distilled water at a concentration of 1mg/ml. E-medium, a specific growth medium for Lemna plants, was used to cultivate the plants. 15 ml of this E-medium was added to each sterilized Petri dish. To achieve different final concentrations (50, 100, 500g), varying quantities (50, 100, 500l) of the sample solutions were added to the Petri dishes containing the E-medium.In each sterilized plate, 10 healthy Lemna plants, each with three fronds, were carefully transplanted. For comparison, a standard herbicide (Atrazine 10%) was used as a positive control, while the E-medium alone served as the negative control.The plates were then placed in an incubator set at a temperature of 28°C, and the plants were allowed to grow for seven days. After the incubation period, the number of fronds per plate was counted.This calculation provided insights into the extent of growth inhibition caused by the different samples compared to the negative control [13].

3) Antioxidant activity

The purpose of the study was to assess the antioxidant properties of iron oxide nanoparticles made from extracts of the Euphorbia serpens plant's leaf and bulb. The antioxidant potential of the samples was determined using the DPPH method. Plant extracts and iron oxide nanoparticles were mixed with 2 milliliters of the DPPH solution to create a final volume of 3 milliliters. A DPPH solution (0.004% w/v) was prepared in 99.9 percent methanol, and the materials (plant extracts and iron oxide nanoparticles) were mixed with different concentrations (50, 100, and 500 milliliters). After vigorous mixing, the mixture was incubated for one hour in the dark at room temperature. The stock solution was made by dissolving ascorbic acid in distilled water at the same concentrations 50, 100, and 500 l/ml as a reference standard. A UV spectrophotometer was used to measure the absorbance of each sample against a blank of methanolic solution at 517 nm [14].

4) Antibacterial activity

To test the antibacterial properties of plant extracts (leaf and bulb) and iron oxide nanoparticals, the bacterium strains Staphylococcus aurous and Pseudomonas aeruginosa were obtained from the AWKUM Biotechnology department and incubated in nutrient broth for 12 hours. A series of processes were taken to evaluate the antibacterial efficacy of the test items, which comprised plant extracts and iron oxide nanoparticles. The test chemicals were first concentrated in distilled water at various concentrations (25l/ml, 50l/ml, and 100l/ml).All equipment and the Nutrient Fear medium were sterilized in an autoclave for one hour at 121°C and 17lb pressure. The media was sterilized before being put onto sanitized petri dishes and left to solidify at room temperature. Using a micropipette, a bacterial suspension was produced and poured over the hardened medium. A glass spreader was used to uniformly distribute the suspension. On each plate, four discs with varying concentrations of the test chemicals were inserted. A known antibiotic, streptomycin, was employed as a positive control, whereas distilled water was used as a negative control. The plates were then incubated for 24 hours at 25°C to allow bacteria to grow and interact with the test compounds. The antibacterial potency was measured after the incubation time by measuring the inhibition zones produced around each disc. The inhibitory zones were measured using a standard scale. This experimental setting revealed information on the antibacterial activity of the test compounds against the bacterial strains employed in the study, with inhibition zones showing how successful the chemicals were at preventing bacterial growth. (Shnawa BH et al., 2021) [15].

IV. RESULTS

1) Antioxidant Activity

Iron nanoparticles and Euphorbia serpens extract's ability to scavenge DPPH radicals was compared to that of ascorbic acid, a well-known antioxidant drug. A total of three distinct concentrations—50 g/ml, 100 g/ml, and 200 g/ml—were used in triplicate for each experiment. The results showed that at a

concentration of 50g/1ml, the Euphorbia serpens extract demonstrated the highest percentage inhibition (97%) of the DPPH radical. When compared to ascorbic acid at the same dose, which demonstrated a 71% inhibition percentage, this inhibition was substantially higher (p 0.0001). The rhizome NPs and extract also demonstrated high percentage inhibition (95.5%) at 200µg/1ml and 100µg/1ml concentrations. These findings suggest that the Euphorbia serpens extract and silver nanoparticles have strong antioxidant properties and may be effective in scavenging free radicals. However, further studies are needed to evaluate their safety and efficacy in vivo before considering their potential use in therapeutic applications.
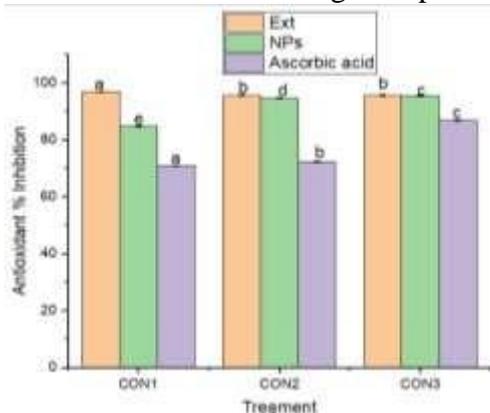

Figure 2: Antioxidant activity of leaf-Iron oxide nanoparticals, Euphorbia Serpens extracts.

2) Antibacterial Activity

The disc diffusion technique was used to test the antibacterial effectiveness of iron oxide nanoparticles produced from *Euphorbia Serpens* plant leaf and bulb extracts against Staphylococcus aureus and Pseudomonas aeruginosa. The presence or absence of inhibitory zones was evaluated in millimeters to determine potential antibacterial activity. The biofabricated iron oxide nanoparticles demonstrated a considerable zone of inhibition, indicating antibacterial activity. At a concentration of 2 extract, the leaf-derived iron oxide nanoparticles revealed a substantially greater (p0.05) zone of inhibition against Staphylococcus aureus, but a significantly reduced (p0.05) zone of inhibition against *Pseudomonas aeruginosa.*
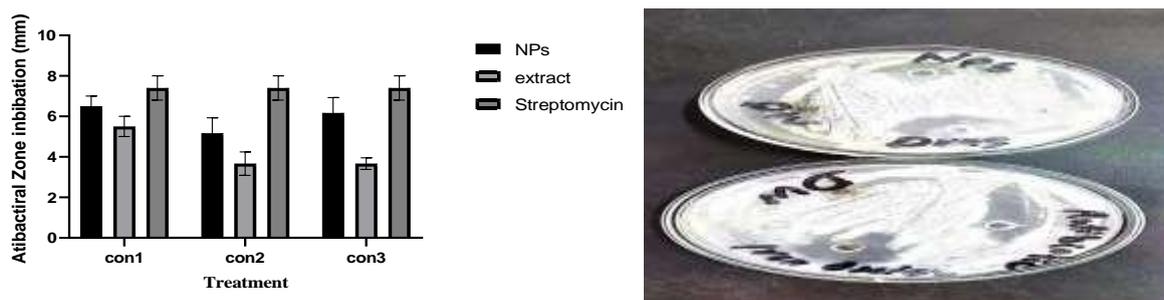

Figure 3: Antibacterial activity of leaf-Iron oxide nanoparticals, Euphorbia Serpens extracts.

V. CONCLUSION

In this work, we created FeNPs from fresh Euphorbia Serpens extract and investigated their range of biological activities, including antibacterial, antioxidant, phytotoxic, and insecticidal traits. It was an amazing observation that the solution's color changed from bright green to blackish following effective bio production of green-mediated silver nanoparticles. The researchers discovered that the maximum absorption of rhizome extract nanoparticles. The results revealed that the extract's nanoparticles had excellent antioxidant activity, making it an appropriate source for further study.

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