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BIOLOGICAL ACTIVITY OF ZINC NANOPARTICLES USING Alfalfa (Medicago Sativa) EXTRACTS

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ABSTRACT
Nanotechnology has emerged as a transformative tool in the field of biomedical and agricultural sciences. Zinc nanoparticles (ZnNPs) are particularly noted for their antimicrobial, antioxidant, and biocompatible properties. Green synthesis of ZnNPs using plant extracts provides an eco-friendly alternative to conventional chemical synthesis. Medicago sativa (alfalfa), rich in flavonoids, saponins, and polyphenols, presents an ideal biological agent for nanoparticle synthesis and stabilization. This study investigates the biological activity of ZnNPs synthesized using alfalfa extracts.
Methods:*Aqueous extracts of Medicago sativa leaves were used to reduce zinc nitrate to ZnNPs via a green synthesis approach. The biological action of the synthesized ZnNPs was evaluated through antimicrobial assays (against Gram-positive and Gram-negative bacteria), antioxidant capacity (DPPH assay), and cytotoxicity using MTT assay on selected cell lines.*
Results:*The biosynthesized ZnNPs were predominantly spherical and measured between 20–50 nm in diameter. FTIR confirmed the presence of bioactive compounds from alfalfa acting as capping agents. The ZnNPs exhibited significant antimicrobial activity, especially against Staphylococcus aureus and Escherichia coli. Antioxidant assays revealed strong free radical scavenging activity. Cytotoxicity studies demonstrated moderate biocompatibility at lower concentrations, suggesting potential for biomedical applications.*
Conclusion:*Zinc nanoparticles synthesized using Medicago sativa extracts exhibit promising biological activities, including antimicrobial and antioxidant properties. The green synthesis method is cost-effective, sustainable, and biocompatible, highlighting the potential of alfalfa-mediated ZnNPs in pharmaceutical and agricultural applications.*

INTRODUCTION:

Nanotechnology has revolutionized many scientific fields by enabling the development of materials with novel properties at the nanoscale. Among the various metal-based nanoparticles, zinc nanoparticles (ZnNPs) have gained significant attention due to their excellent antimicrobial, antioxidant, anti-inflammatory, and anticancer properties. Zinc is an essential trace element involved in numerous biological processes, and its nanoform exhibits enhanced reactivity and bioavailability (Song et al.,2025). In recent years, The environmentally friendly production of nanoparticles has evolved as an alternative to traditional chemical and physical procedures that is both sustainable and favourable to the environment. The reduction of metallic ions into nanoparticle is accomplished by the use of biological elements such as extracts from plants, bacteria, or enzymes using this technique. Among the plant-based methods, using extracts rich in phytochemicals has proven effective, safe, and scalable (Bandeira et al.,2025).

Medicago sativa, commonly known as alfalfa, is a leguminous plant widely recognized for its high nutritional content and medicinal properties. It contains a diverse array of bioactive compounds including phenolic acids, flavonoids, and saponins, making it a suitable candidate for green nanoparticle synthesis. Alfalfa has been traditionally used for its antioxidant, anti-inflammatory, and antimicrobial effects, which may be retained or even enhanced in its nanoparticle form (Álvarez-Chimal et al.,2021).

This study focuses on the biosynthesis of ZnNPs using Medicago sativa leaf extracts and investigates their biological activities. The green synthesis method not only provides an eco-friendly route for nanoparticle production but also endows the nanoparticles with additional bioactive properties derived from the plant extract. Understanding the structure, stability, and bioactivity of these biosynthesized ZnNPs can pave the way for their application in biomedicine, agriculture, and environmental science (Naseer et al.,2020),

Background of the study:

Nanotechnology, particularly in the field of nanoparticle synthesis, has created new possibilities for biomedical, pharmaceutical, and agricultural innovations. Among the various types of nanoparticles, zinc nanoparticles (ZnNPs) have attracted considerable interest due to their biocompatibility, low toxicity, and potent biological activities such as antimicrobial, antioxidant, and anti-inflammatory properties. These qualities make ZnNPs valuable for applications in drug delivery, wound healing, food preservation, and plant protection (Haddi et al.,2024).

The traditional techniques for synthesising zinc nanoparticles (ZnNPs) sometimes entail the use of hazardous by-products, excessive energy input, and toxic compounds, all of which raise questions regarding the safety of the environment and the health of humans. In order to address this issue, green synthesis techniques have been created as a safer and more environmentally friendly alternative to the manufacture of nanoparticles.These methods utilize biological materials—most commonly plant extracts which are rich in phytochemicals that can act as both reducing and stabilizing agents (Singh et al.,2025).

Medicago sativa, commonly known as alfalfa, is a nutritionally and medicinally significant legume that has been widely used in traditional medicine. Its leaves contain high levels of phenolic compounds, flavonoids, vitamins, and saponins bioactive constituents that contribute to its antioxidant, antimicrobial, and anti-inflammatory properties. These compounds also make alfalfa an excellent candidate for the green mixture of nanoparticles(Ka et al.,2025).

The useing of Medicago sativa for the biosynthesis of ZnNPs is a promising approach that integrates the plant's therapeutic potential with the functional properties of zinc at the nanoscale. This not only enhances the biological activity of the resulting nanoparticles but also reduces the environmental impact associated with conventional synthesis techniques (Abbas et al.,2025).

Studying the biological activities of alfalfa-mediated ZnNPs can provide insights into their potential applications in various sectors such as healthcare, agriculture, and environmental management. Furthermore, understanding the interactions between phytochemicals and metal ions during nanoparticle synthesis can help optimize protocols for producing more effective and targeted nanomaterials(Khajuria et al.,2021)

Purpose of the study:

The purpose of this study is to synthesize zinc nanoparticles (ZnNPs) using the aqueous extract of Medicago sativa (alfalfa) through an eco-friendly green synthesis method, and to evaluate their biological activities. This research aims to explore the potential of alfalfa-derived phytochemicals in reducing and stabilizing zinc ions to form functional nanoparticles with enhanced antimicrobial, antioxidant, and biocompatible properties.

By investigating the structural characteristics and bioactivity of these biosynthesized ZnNPs, the study seeks to demonstrate their suitability for applications in biomedical, pharmaceutical, and agricultural fields. Additionally, the study aims to contribute to the development of sustainable nanotechnology approaches that minimize environmental impact while maximizing therapeutic efficacy and safety.

LITERATURE REVIEW:

Nanotechnology has become a vital interdisciplinary field, offering promising solutions for health, agriculture, and environmental challenges. Among the many types of nanoparticles, zinc nanoparticles (ZnNPs) have emerged as particularly attractive due to their broad-spectrum biological properties. Zinc is an essential trace element involved in numerous physiological processes, and its nanoform enhances bioavailability and reactivity, allowing it to exert stronger antimicrobial and antioxidant effects compared to its bulk counterpart (Rahman et al.,2022),

Several studies have reported the successful synthesis of ZnNPs through green methods that utilize plant extracts. Plant-mediated synthesis leverages natural phytochemicals such as flavonoids, phenolic acids, terpenoids, and saponins, which serve as both reducing and stabilizing agents. This approach not only avoids the use of toxic reagents but also imparts additional bioactivity to the nanoparticles. Medicago sativa (alfalfa) is a perennial leguminous plant widely recognized for its medicinal properties. Its extract is rich in antioxidants, vitamins, and phytochemicals known for anti-inflammatory, antibacterial, and antifungal effects. Researchers have extensively explored the pharmacological properties of alfalfa, but its application in nanoparticle synthesis is relatively new and still evolving. Preliminary investigations suggest that alfalfa extract can successfully facilitate the synthesis of stable, bioactive metal nanoparticles, including silver and zinc (Malik et al.,2022).

Recent studies have demonstrated that ZnNPs synthesized using various plant extracts exhibit significant antimicrobial activity against both Gram-positive and Gram-negative bacteria. For instance, biosynthesized ZnNPs have shown effectiveness against "Escherichia coli, Staphylococcus aureus, and

Pseudomonas aeruginosa". These nanoparticles damage bacterial cell walls and disrupt metabolic functions, making them a potential alternative to conventional antibiotics (Wafaey et al.,2024).

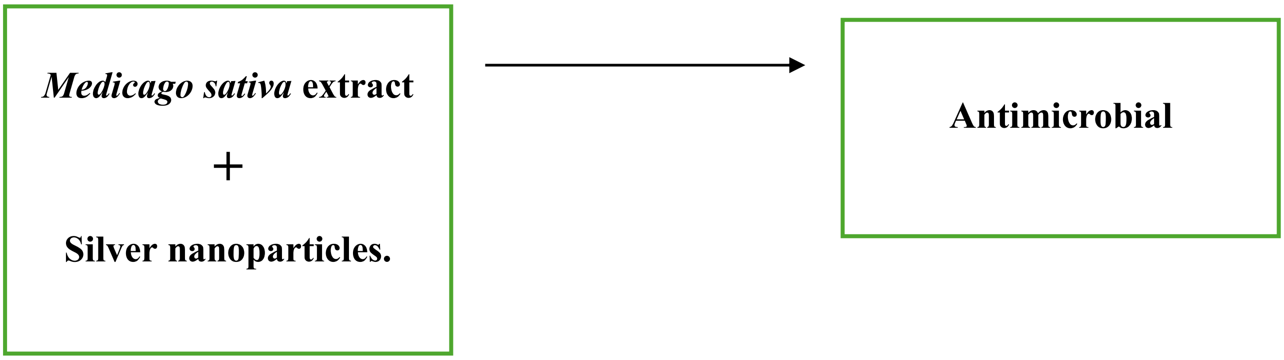
Additionally, ZnNPs have shown strong antioxidant activity by scavenging free radicals and protecting cells from oxidative stress. This is particularly relevant for biomedical applications, such as wound healing and drug delivery, where oxidative damage needs to be minimized. Furthermore, the cytotoxic effects of ZnNPs on cancer cell lines, coupled with their relatively low toxicity to normal cells, open new possibilities for anticancer therapies.While promising, much of the current literature calls for deeper exploration of the underlying mechanisms, optimal synthesis conditions, and long-term safety of green-synthesized ZnNPs. The integration of a potent plant like Medicago sativa in this process could lead to nanoparticles with enhanced biological functions. This growing body of literature underscores the potential of plant-based green synthesis in producing biologically active nanoparticles. The present study builds on this foundation by evaluating the biological activity of ZnNPs synthesized using Medicago sativa extracts, contributing to the expanding field of green nanobiotechnology (Deepak et al.,2024).

Research questions:

- How effectively can Medicago sativa (alfalfa) extract be used in the green synthesis of zinc nanoparticles (ZnNPs)?
- What are the structural and morphological characteristics of the ZnNPs synthesized using Medicago sativa extract?
- What is the antimicrobial and antioxidant potential of the biosynthesized ZnNPs against selected microbial strains and free radicals?
- How biocompatible and cytotoxic are the Medicago sativa-derived ZnNPs when tested on normal and/or cancerous cell lines?

RESEARCH METHODOLOGY:

Conceptual Framework:



Hypothesis:

The significant relationship between the antimicrobial properties and Medicago sativa extract combined with zinc nanoparticles lies in their synergistic biological activity. Medicago sativa contains a wide array of bioactive phytochemicals such as flavonoids, saponins, and phenolic compounds, which are known for their antimicrobial efficacy. When used in the green synthesis of zinc nanoparticles, these phytochemicals not only act as reducing and capping agents but also contribute additional bioactivity to the resulting nanoparticles. Zinc nanoparticles themselves possess strong antimicrobial properties due to their ability to generate reactive oxygen species (ROS), disrupt bacterial membranes, and interfere with microbial DNA and enzyme function. When synthesized using Medicago sativa extract, the combined effect results in enhanced antimicrobial potency. The phytochemicals present in the plant extract stabilize the nanoparticles and potentially facilitate better interaction with microbial cells, leading to improved inhibition of bacterial growth.Studies suggest that this combination leads to a more stable, biocompatible nanoparticle system with a broader antimicrobial spectrum and increased effectiveness even at lower concentrations. Thus, the integration of Medicago sativa extract with zinc nanoparticles results in a significant enhancement of antimicrobial properties compared to either component used alone (Alvarez-Chimal et al.,2022).

On basis of the above discussion the researcher formulated the following hypothesis, which will investigate the relationship between antimicrobial properties and Medicago sativa extract combined with Zinc nanoparticles.

H_{01}: "There is no significant relationship between Antimicrobial properties and Medicago sativa extract combined with Zinc nanoparticles."

H_1 : "There is a significant relationship between Antimicrobial properties and Medicago sativa extract combined with Zinc nanoparticles."

RESEARCH DESIGN:

Collection:

The study involved the utilization of laboratory-grade zinc nitrate and Medicago sativa extract as the substances. Various reagents and other substances of laboratory quality were employed in the experiment.

Microorganisms Used:

The study involved the utilization of laboratory-grade zinc nitrate and Medicago sativa extract as the substances. Various reagents and other substances of laboratory quality were employed in the experiment.

Microorganisms Used:

The bacteria, including Staphylococcus aureus, Escherichia coli, Bacillus subtilis, and Pseudomonas aeruginosa, were isolated from the Microbiology Postgraduate laboratory at the University. To obtain pure cultures, the bacteria were sub-cultured multiple times.

Active constituents and synthesis mechanisms of zinc nanoparticles:

The antioxidant phenolic compounds found in Alfalfa are one of many photochemicals found in the flowering plant. The synthesis of zinc nanoparticles using Medicago sativa (alfalfa) extract is facilitated by the presence of numerous active phytochemical constituents naturally found in the plant. These include flavonoids, phenolic acids, saponins, tannins, terpenoids, and proteins. "These compounds play a crucial role in reducing zinc ions (Zn^{2+}) into zinc nanoparticles (Zn^0) and simultaneously act as capping agents that stabilize the nanoparticles and prevent their aggregation."(Umamaheswari et al.,2021).

Flavonoids and phenolics serve as natural reducing agents due to their ability to donate electrons. When a zinc salt such as zinc nitrate or zinc acetate is introduced to the aqueous extract of Medicago sativa, these phytochemicals initiate the reduction process, transforming zinc ions into nanoscale metallic zinc particles. As the particles form, other constituents like saponins, tannins, and proteins bind to the nanoparticle surfaces, stabilizing them and controlling their growth and morphology. The synthesis mechanism is a green and eco-friendly approach that occurs at ambient or slightly elevated temperatures without the need for harsh chemicals. A visible color change in the reaction mixture usually indicates the formation of nanoparticles, which are then characterized using various techniques such as UV-Vis spectroscopy, FTIR, XRD, and SEM to confirm their size, shape, structure, and surface chemistry.

This biologically mediated process not only results in the formation of zinc nanoparticles but also imparts them with enhanced biological activity, including antimicrobial and antioxidant properties, due to the presence of bioactive compounds from Medicago sativa on the nanoparticle surface.

Phytochemicals like flavonoids and phenolics donate electrons to reduce Zn^{2+} ions into zero-valent zinc (Zn^0), leading to nanoparticle nucleation.

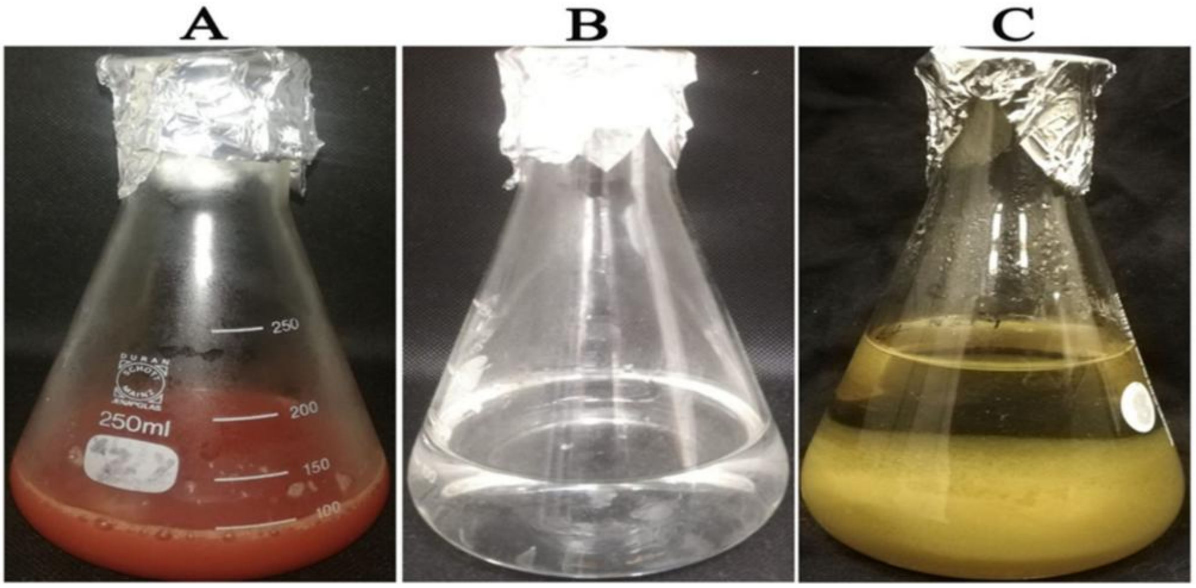
$$\mathrm{Zn}^{2+} + (\text{Reducing phytochemicals}) \rightarrow \mathrm{Zn}^{0}(\text{Nanoparticles})$$

Preparation of zinc nanoparticles

The preparation of zinc nanoparticles using Medicago sativa (alfalfa) extract involves a green synthesis approach that is simple, cost-effective, and environmentally friendly. Fresh or dried leaves of Medicago sativa are first collected, methodically washed with distilled water to remove dust and impurities, and then shade-dried if necessary. The cleaned plant material is ground into fine powder and boiled or soaked in distilled water to obtain an aqueous extract rich in phytochemicals. This extract is filtered to remove solid residues, yielding a clear solution that contains the active constituents necessary for nanoparticle synthesis.

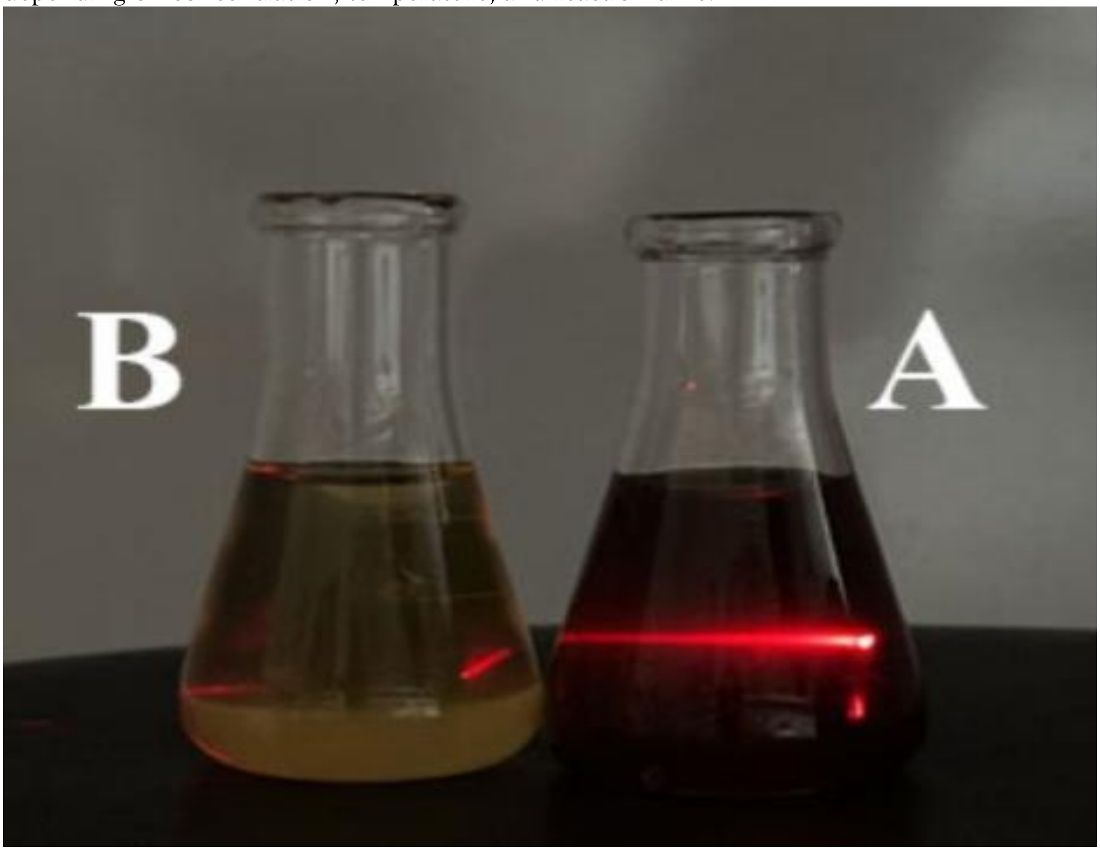
A zinc salt, commonly zinc nitrate or zinc acetate, is dissolved in distilled water to prepare a metal precursor solution. The Medicago sativa extract is slowly added to this zinc solution under continuous stirring at room temperature or at a moderately elevated temperature, depending on the desired reaction speed and nanoparticle size. During the mixing, the phytochemicals present in the extract reduce Zn^{2+} ions to metallic zinc (Zn^0), initiating the formation of nanoparticles. The reaction is typically indicated by a visible color change in the solution, confirming nanoparticle formation.

After the reaction is complete, the mixture is often incubated for several hours to ensure full reduction and stabilization. The formed zinc nanoparticles are collected by centrifugation, washed several times with distilled water and ethanol to remove any unreacted plant components or impurities, and then driedu sually in an oven or vacuum desiccator to yield a fine nanoparticle powder. These nanoparticles can be stored for further biological activity testing or physicochemical characterization.

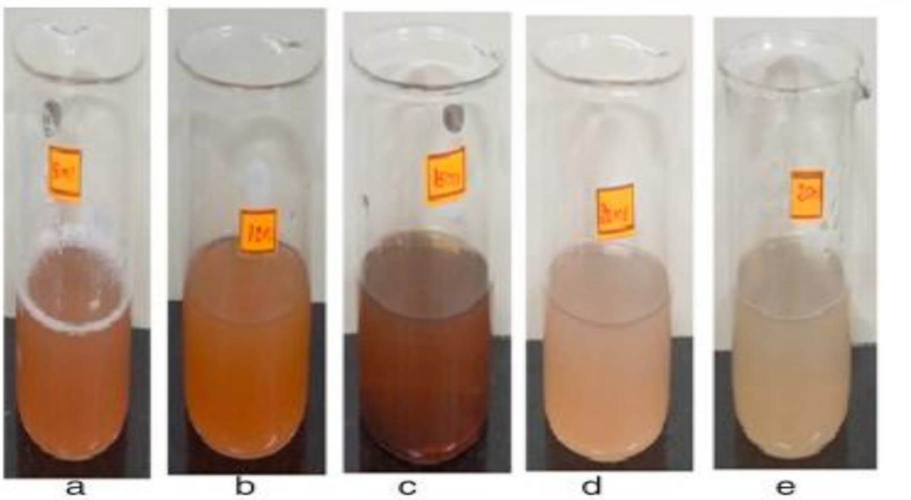


Visible observation

During the green synthesis of zinc nanoparticles using Medicago sativa (alfalfa) extract, visible observations provide an immediate indication of nanoparticle formation. One of the most noticeable changes is the shift in color of the reaction mixture. Initially, the solution containing the zinc salt (such as zinc nitrate or zinc acetate) is colorless or slightly cloudy. When the Medicago sativa extract is added and the reaction begins, a distinct color change occurs, usually turning pale yellow, brownish, or off-white, depending on concentration, temperature, and reaction time.



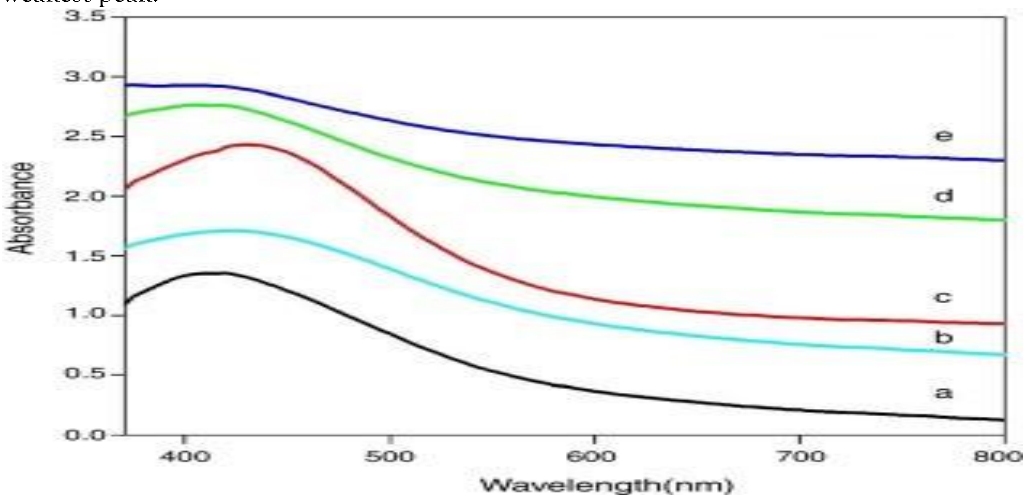
This color change is due to surface plasmon resonance (SPR), a phenomenon that occurs when light interacts with free electrons on the surface of the forming nanoparticles. The intensity and shade of the color often correlate with the size and shape, and distribution of the zinc nanoparticles. In addition to colour change, the appearance of fine turbidity or a colloidal suspension may be observed, indicating the formation of dispersed nanoparticles in the solution. Over time, if the nanoparticles are not fully stabilized, slight sedimentation may occur at the bottom of the container. These visual cues are typically followed by characterization techniques to confirm nanoparticle synthesis.



The deepest, darkest shade is achieved with a 15 ml concentration of extract. When the extract concentration drops to 20–25 ml, the hue becomes hardly noticeable, almost like a faint brown. A deeper brown coloration of the reaction mixtures occurred at extract concentration of 15 ml, indicating an growth in the number of zinc nanoparticles. When the concentration of extract goes above 15 ml, the reaction mixtures become a pale brown hue, which means that the number of zinc nanoparticles has decreased. Agglomeration of the generated zinc nanoparticles occurs at higher concentrations of Alfalafa extract. Aggregating zinc nanoparticles reduces their stability. More and more zinc nanoparticles are being produced as the reaction time intensifications exponentially," as shown by the reaction mixes becoming a darker shade of brown.

UV–visible spectral analysis:

After a day of incubation at room temperature, the UV-visible preoccupation spectra of zinc nanoparticles at five changed attentions (5, 10, 15, 20, and 25 ml respectively) are shown. The absorbance (or surface plasmon resonance) band is seen at 410, 420, 435, 422, and 418 nm respectively for 5, 10, 15, 20, and 25 ml concentrations respectively of zinc nanoparticles facilitated by Alfalfa. When the Alfalfa extract concentration is 15 ml, the SPR peak is 435 nm, and when the concentration is 25 ml, it is 418 nm, the weakest peak.



UV-visible spectroscopy of zinc nanoparticles of Medicago sativa extract

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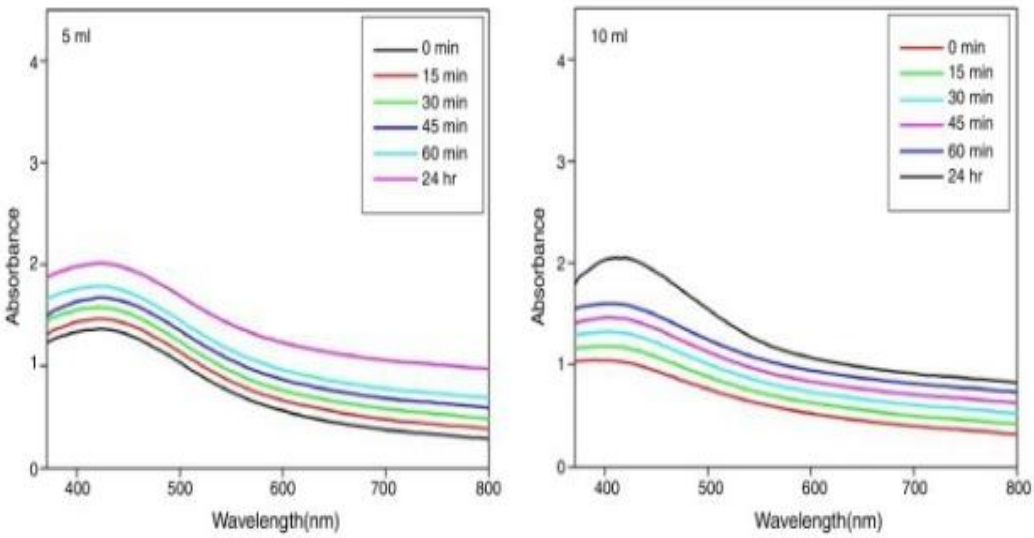
Additionally, the red shift occurs as a result of an increase in the AgNPs absorption peak wavelength, which occurs when the intensity of" Alfalfa extract rises. This instance demonstrates that the average diameter of zinc nanoparticles is increasing with time, as seen by the red shift. Twenty and twenty-five milliliter concentrations of Alfalfa extract also show a blue shift, which is associated with a reduction in the absorption wavelength of AgNPs. The steady reduction of the mean diameter of the zinc nanoparticles is emphasized by this transition to blue. At each concentration, the researcher presented the optical band gap value of the Medicago sativa extract. As the concentration of Medicago sativa extract drops from 5 to 15 ml, the energy band gap drops and particle size goes up. Results from XRD examination are in agreement with the fact that the optical band gap rises at concentrations of 20 and 25 ml of Alfalfa extract, respectively. This establishes, beyond a shadow of a doubt, that the concentrated zinc nanoparticles are much smaller.

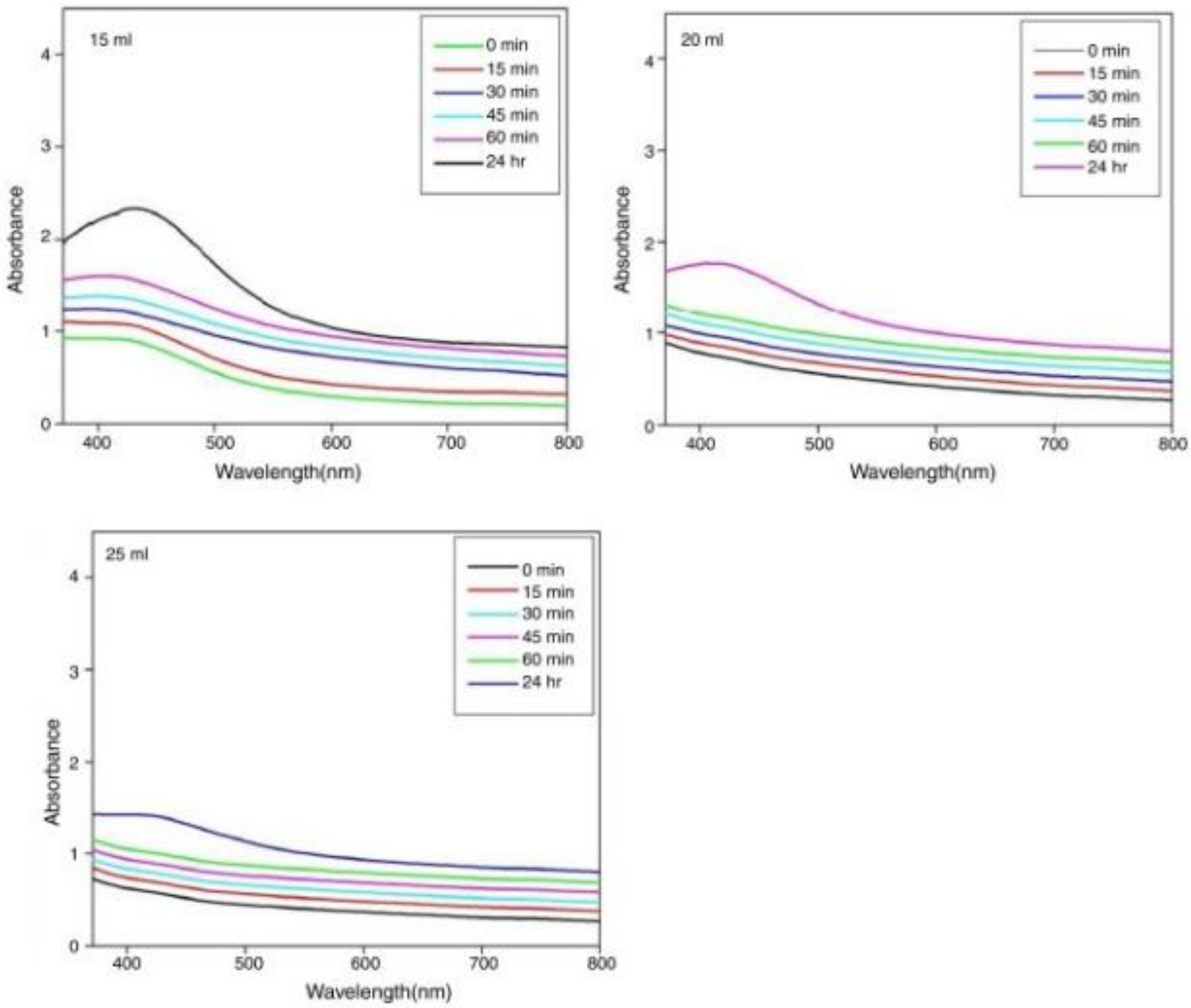
Optical band gap value of zinc nanoparticles.

Sample	Optical band gap(eV)
5ml	4.9
10ml	4.7
15ml	4.6
20ml	4.7
25ml	4.8

Optical band gap value of zinc nanoparticles.

The ultraviolet-visible of zinc nanoparticles collected from Alfalfa extract at five, ten, fifteen, twenty, and twenty-five milliliters are shown in the following table. Changes occur in the spectrum throughout time. Following the beginning of the reaction, the UV-visible spectra of zinc nanoparticles are collected at 0, 15, 30, 45, 60, and 24 hours. While the reaction mixture is being incubated, the absorbance spectra that include surface resonance show that there is a consistent increase. The band of plasmon resonance. This is because the reaction duration is shortened. The absorption peak strength rises across all dosages of Alfalfa extract as the response time increases from 0 to 60 minutes. This growth in absorption peak strength indicates that the combination contains an increasing concentration of zinc nanoparticles. All of the reaction mixtures have produced zinc nanoparticles when the absorption peak "intensity reaches its maximum after twenty-four hours of incubation" from the beginning of the reaction. This specifies that overall reaction has been successful.

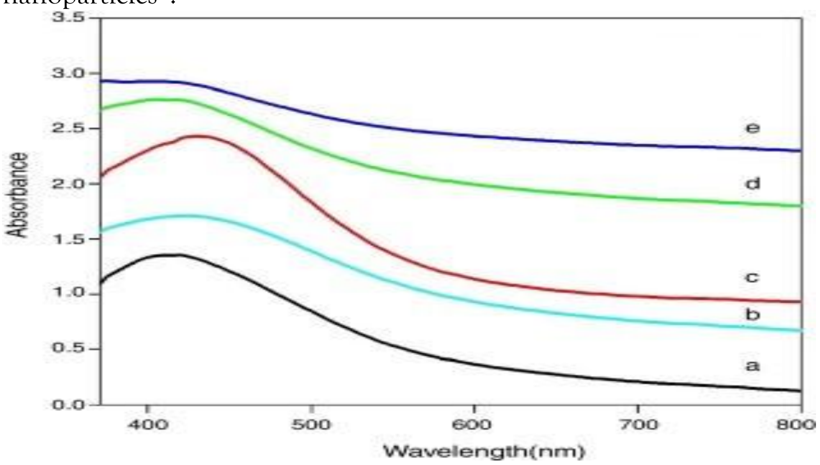




Time dependent UV-visible spectroscopy of zinc nanoparticles using concentration of Alfalfa extract.

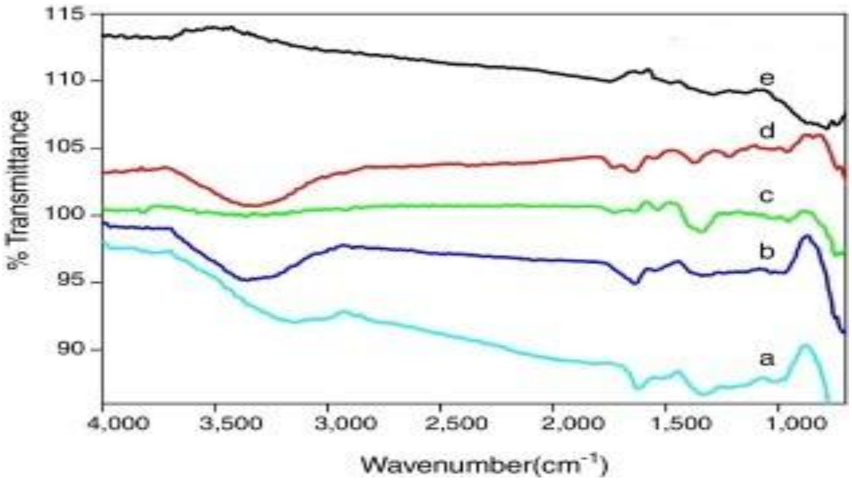
FT-IR analysis:

At five," ten, fifteen, twenty, and twenty-five milliliters, the spectra of zinc nanoparticles in Alfalfa extract are seen here, shifting over time. At 0,15,30,45,60, and 24 hours post-procedure, you may collect the UV-visible spectroscopy of the zinc nanoparticles.With time spent incubating the reaction mixture, the surface resonance absorbance spectroscopy become more pronounced. The radiation spectrum of plasma. There are less zinc nanoparticles extracted from the reaction mixture at the zero-minute mark because the absorption peak strength is lower. The absorption peak intensity for all dosages of Alfalfa extract grows as the response time rises from 0 to 60 minutes. At the end of the first day of incubation, the absorption peak intensity is at its highest, indicating that all of the reaction mixtures have successfully created zinc nanoparticles".



(FT-IR) spectra of Alfalfa (Medicago sativa) Extracts

"Displays the Fourier transform infrared (FT-IR) spectra of silver nanoparticles (AgNPs) used to mediate the extract of Medicago sativa leaves at a variety of concentrations (5 ml, 10 ml, 15 ml, 20 ml, and 25 ml). "Around 700-750 cm−1, 950-980 cm−1, 1000-1150 cm−1, 1200-1300 cm−1, 1330-1370 cm−1, 1500-1550 cm−1, 1600-1650 cm−1, and 3100-3400 cm−1 are the typical absorption bands seen in the FT-IR spectra. According to Khalil et al. (2014), the bands seen at around 700-750 cm−1 might be caused by the stretching vibrations of C-Cl alkyl halides"". It is possible that the bands seen at 950-980 cm−1 are caused by the bending vibrations of alkenes with a double bond C-H group. The stretching vibrations of carboxylic acids might be responsible for the absorption band seen between 1000 and 1150 cm−1. The N-H bend amines are linked to the bands about 1200-1300 cm−1. The bands seen between 1330 and 1370 cm−1 could be a result of the C-N stretch amine group. Nitro compounds with an N-O asymmetric stretch are shown by the bands that circle 1500–1550 cm−1. According to research the bands seen between 1600 and 1650 MHz might be associated with the stretching vibrations of both primary and secondary amines. The stretching of carboxylic acids' C-double bonds might be responsible for the bands seen at 3100-3400 cm−1(Donga et al.,2022).

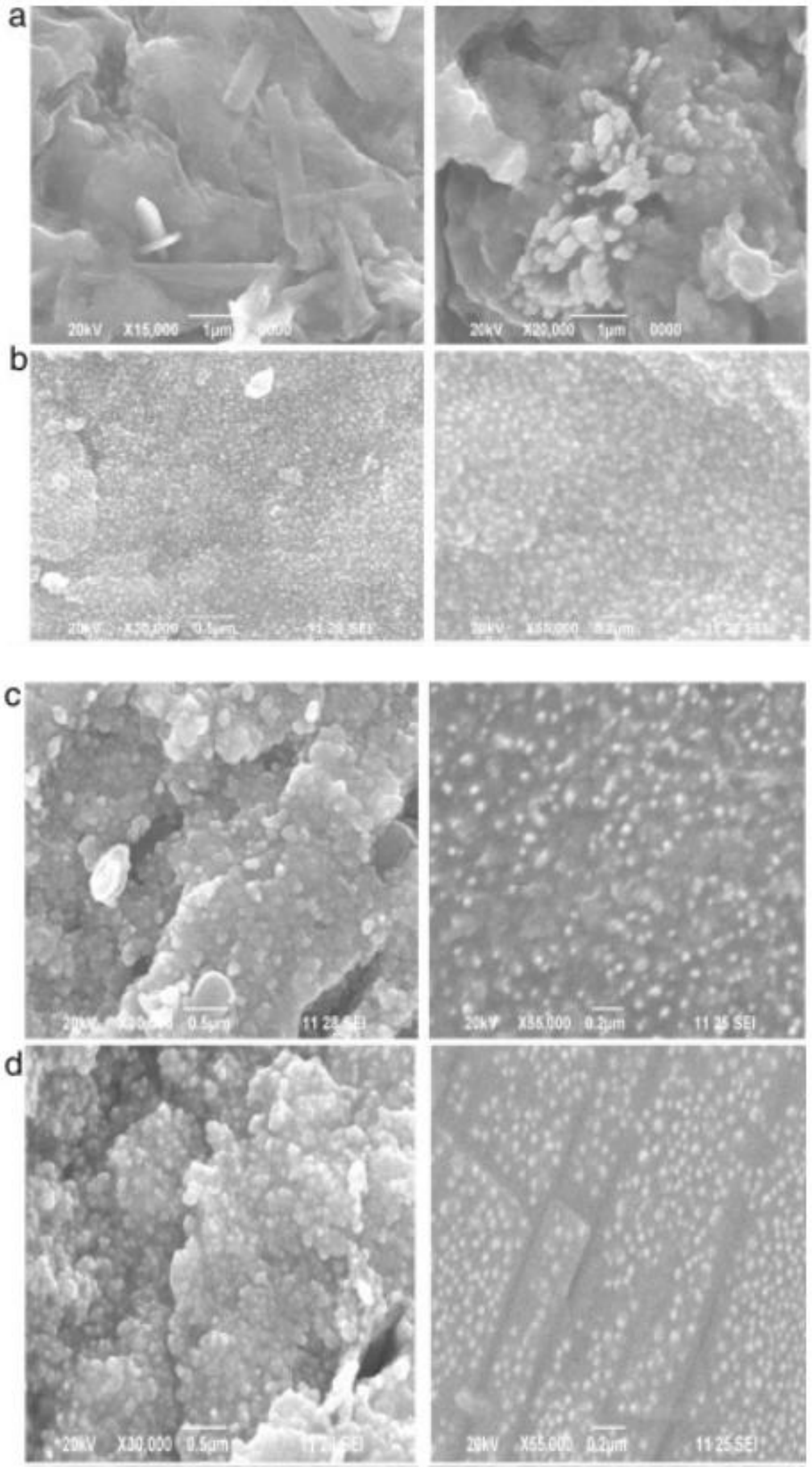


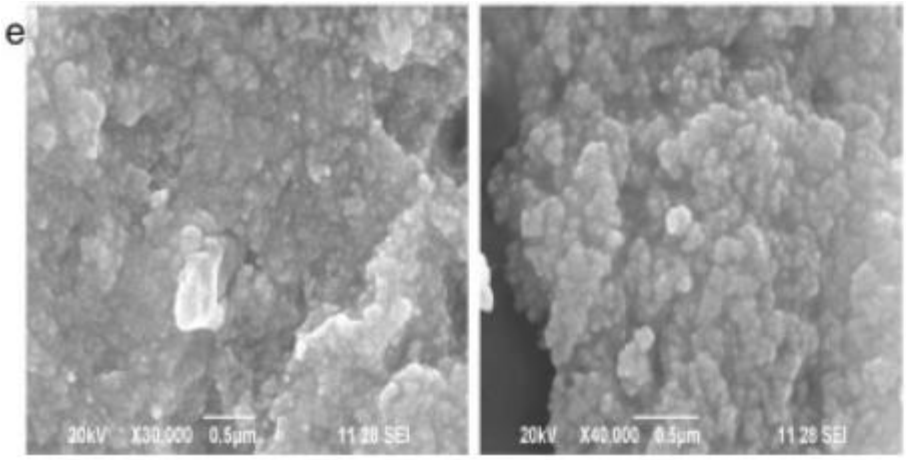
FT-IR spectra of zinc nanoparticles using of Alfalfa (Medicago sativa) Extracts
shows the zinc nanoparticles' FT-IR spectra, which, by displaying the correct bands, prove that the reaction mixture included carboxyl and amine groups. The bands are shown to shift to higher wavelengths when the concentration from Alfalfa extracts from leaves increases". As compared to the pure Medicago sativa extract, These carboxyl and amide groups demonstrate that the zinc nanoparticles were bio-fabricated by proteins or phytochemicals, as they show the presence of auxiliary amines, a protein signature. As a result, the hypothesis that these bio-molecules stabilize and cap the produced nanoparticles may be accepted. Proteins seem to have a role in the stability and capping of the produced zinc nanoparticles, according to the FT-IR study.

Structure analysis using XRD
Displays the optical reflectance spectra of bio-synthesized Ag nanoparticles at different concentrations derived from Alfalfa extract. Crystalline zinc nanoparticles were produced by bioreducing zinc ions in broth, as seen by the XRD patterns. To find out how pure, crystallinity, and phase distributed the produced zinc nanoparticles are, XRD "analysis is used.

SEM ANAYSIS
In order to investigate the surface morphology and morphologies of zinc nanoparticles, research is conducted using scanning electron microscopy (SEM). 5 millilitres, 10 millilitres, 15 millilitres, 20 millilitres, and 25 millilitres of zinc nanoparticles were produced by using Medicago sativa as the source material. The scanning electron microscope (SEM) is used to illustrate these nanoparticles at a variety of magnifications, as shown in Figure 10(a-e). It has been demonstrated that the zinc nanoparticles have a spherical shape; however, when the concentration of the extract is increased from one level to another,

the nanoparticles experience changes in both their size and their shape. Extracts with volumes ranging from 5 to 15 millilitres do not exhibit any aggregations, and the particles are distributed in a manner that is consistent throughout the extract. When the quantity of the extract is greater than 15 millilitres by volume, as shown in Figure 10(d-e), the particles exhibit a tendency to cluster together. The consolidation of particles with one another is the root cause of the instability that is observed in zinc nanoparticle solutions.

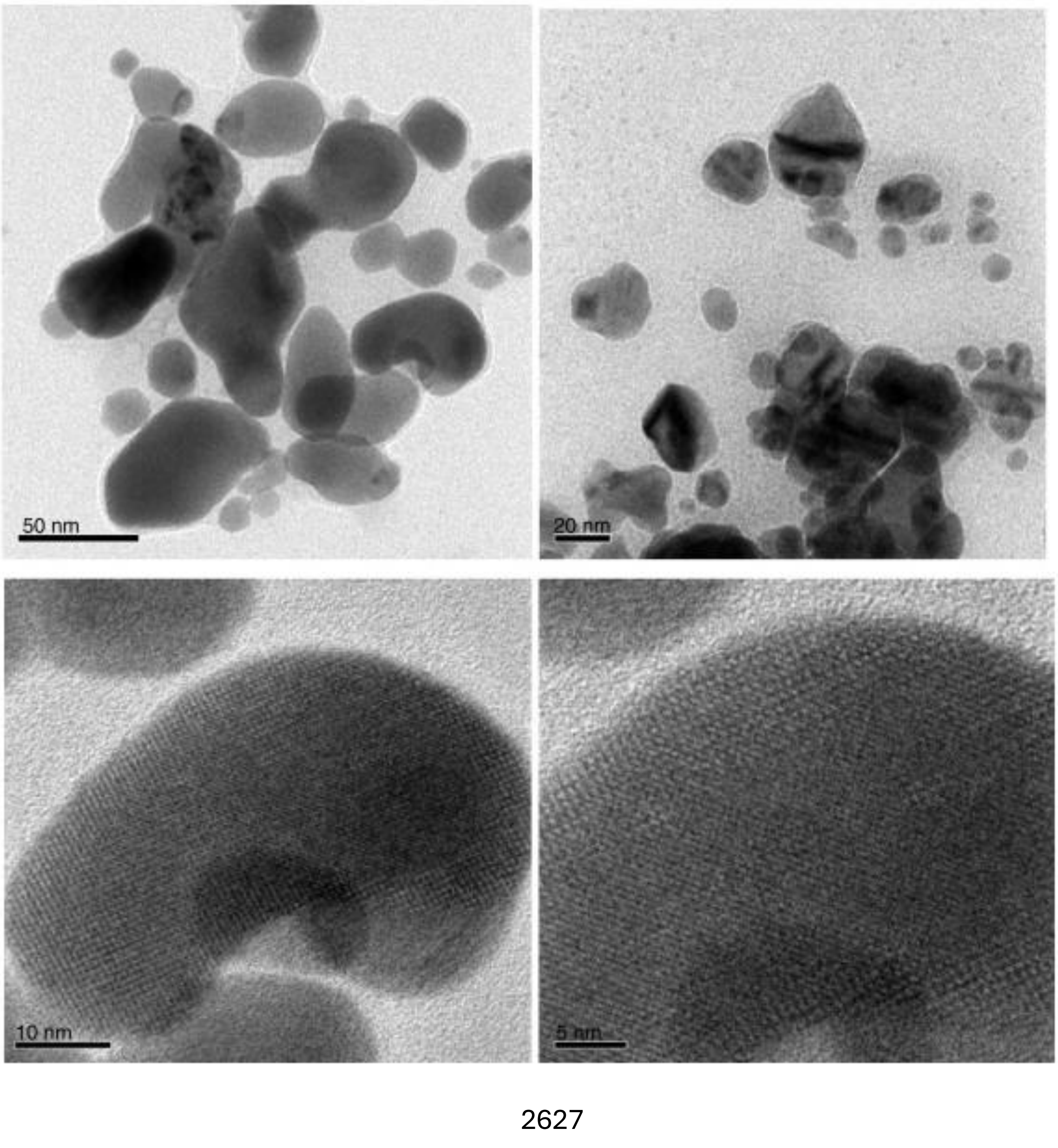




SEM image of zinc nanoparticles using of *Medicago sativa* extract

TEM ANALYSIS

Transmission electron micrographs of zinc nanoparticles made from a water-based extract of Medicago sativa is observed. The solution's zinc nanoparticles change in size and form as the extract concentration changes. Based on the pictures, it seems that the nanoparticles are mostly round. Additionally, in some areas, there are a few clumped nanoparticles, which might be signs of sedimentation that occurred later on. The typical size of zinc nanoparticles ranges from fifteen nanometers to twenty nanometers. The size of the crystallites determined by XRD analysis agrees well "with the findings.



TEM images of zinc nanoparticles using Medicago sativa Extracts

Plant Materials Collection and Processing:

"Fresh Alfalfa Medicago sativa were obtained separately from a nearby farm. The University Department of Pharmacognosy and Natural Medicine was tasked with identifying and authenticating the plant species. To eliminate any dust or debris that might have accumulated on the plant they were vigorously rinsed with tap water. After the dust removal process, the leaves were allowed to dry in the shade for an entire day. The subsequent step involved using an electric blender to pulverize" the dried.

DATA ANALYSIS:

Extraction Procedure:

Fifty grams of "powdered plant material was placed in a 500 mL conical flask, and 250 mL of distilled water was added. The flask was covered with aluminum foil and shaken continuously at 150 rpm for 24 hours on a reciprocating shaker to ensure thorough mixing. Subsequently, the extract was filtered using muslin cloth and Whatman no. 1 filter paper. The resulting solution was utilized for nanoparticle" synthesis.

Synthesis of "Nanoparticles Using Aqueous Extracts of Medicago sativa with Model Drug:

A 10 mL solution of 1% zinc nitrate ($AgNO_3$) was prepared by dissolving 0.1 g of $AgNO_3$ in 10 mL of water. Subsequently, 5 mL of the extract was added drop by drop under constant stirring using a magnetic stirrer for 5 minutes to create an $[Ag]^+$ dispersion. Following this, a 25 mL portion of freshly prepared Alfalafa extract (acting as a reducing agent) was added to the mixture, which was then maintained at 40°C for 24 hours. The resulting zinc nanoparticle" suspension was lyophilized using a Virtis 2KBTXL-75 Benchtop SLC Freeze Dryer for further analysis.

Characterization of Nano-Composites: UV-VIS spectroscopy was utilized to ascertain the Surface Plasmon Resonance (SPR) characteristic of the synthesized zinc nanoparticles.

Antimicrobial Studies of zinc -Nanocomposites: "Using the agar well diffusion technique described by Okeke et al., 2021, the antibacterial activity of zinc nanoparticles biosynthesised from Alfalfa extract was" tested.

A sterile bench hockey stick was used to aseptically disseminate 0.1 ml of each organism across the surface of the Muller-Hinton agar plate. "The plates were allowed to sit on the bench for half an hour so they could pre-disperse into the medium. To puncture the agar plates, a sterile cock borer measuring 5 mm was used. Concentrations of nan zinc oparticles were ranked from 100 mg/ml to 500 mg/ml.For each diluted silver nanoparticle, about half a milliliter was added to the agar wells created in the Muller-Hinton agar plates. After an hour of standing, the plates were given another chance to let the extract permeate the medium. Used 1% zinc nitrate as the control. Plates were kept at 37˚C for a period of 24 to 48 hours for incubation. By measuring the inhibition zone width in cm, the antimicrobial activity of the zinc nanoparticles and the control against microbiological isolates was assessed. To find the minimum inhibitory doses, various quantities of 200 mg/ml, 100 mg/ml, 50 mg/ml, and 25 mg/ml with the medium were combined. Then, streaked the organisms onto the plates and incubated them for 24 hours at 37˚C. In order to determine the minimal inhibitory concentration, the streaking line representing the lowest concentration of zinc nanoparticles that did not cause growth" was seen on the plate.

RESULT:

Table 1 displays the outcomes of the nanoparticle characterisation process. Similarly, the findings of the antibacterial activity of the produced zinc nanoparticles are presented in Tables 2 and 3.

Antimicrobial Studies :

Both "Gram-positive and Gram-negative bacteria were tested for the antibacterial activity of zinc nanoparticles and were effectively inhibited by the produced zinc nanoparticles. There is evidence that zinc can kill germs. Infections were treated with diluted zinc nitrate solutions as early as the nineteenth century. For that reason, the study's control system consisted of a "silver nitrate solution.

Distilled water(mL)	AgNO3(g)	Reducing Alfalafa extract)(mL)	Colour	SPR Peak Change (nm)
10	0.1	5	Reddish Brown	435

The table above" outlines the quantities of distilled water, AgNO3, and Alfalafa extract used in the synthesis process, along with the observed color change and the surface plasmon resonance (SPR) peak wavelength of the resulting silver nanoparticles".

The table below illustrates the zones of inhibition observed for each microorganism when treated with both the control (zinc nitrate solution) and the Medicago sativa zinc Nano-composite.

Table2. Antimicrobial activities of zinc nanoparticles synthesized

Microorganism Control (zinc-Nitrate)	Zone of Inhibition(CM) Medicago sativa Zinc Nano-composite	
Pseudomonas aeruginosa	1.0 cm	1.5 cm
Escherichia coli	1.5 cm	1.5 cm
Bacillus subtilis	1.5 cm	1.5 cm
Staphylococcus aureus	1.2 cm	1.3 cm

Table 3. Minimum inhibitory concentration of Medicago sativa zinc nano-composites

Microorganism Control (zinc Nitrate Solution)	Minimum Inhibitory Concentrations(mg/ml) Medicago sativa Zinc Nano-composite	
Pseudomonas aeruginosa	100 mg/ml	25 mg/ml
Escherichia coli	100 mg/ml	50 mg/ml
Bacillus subtilis	100 mg/ml	100 mg/ml
Staphylococcus aureus	100 mg/ml	100 mg/ml

This table illustrates "the minimum inhibitory concentrations required to inhibit the growth of each microorganism when treated with both the control (zinc nitrate solution) and the Medicago sativa zinc Nano-composite.

Agar plate inhibition zones of 1.5 cm for Pseudomonas aeruginosa, 1.5 cm for Escherichia coli, 1.5 cm for Bacillus subtilis, and 1.3 cm for Staphylococcus aureus demonstrated the antibacterial activity of the extract. Zinc nanoparticles had a minimum inhibitory concentration (MIC) ranging from 25 to 100 mg/ml. The most sensitive bacteria, Pseudomonas aeruginosa, had a MIC of 25 mg/ml, while the least sensitive, Staphylococcus aureus and Bacillus subtilis, showed the least sensitivity". Based on the research and findings, zinc nanoparticles made from Medicago sativa were effective against the four pathogen strains tested: Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis, and Staphylococcus aureus.

DISCUSSION:

The discussion surrounding the rapid biosynthesis of silver nanoparticles using Alfalfa extract encompasses several key aspects, including the synthesis process, characterization of nanoparticles, their antimicrobial properties, and potential applications.

1. **Synthesis Process:** The utilization "of Medicago sativa extract for the biosynthesis of zinc nanoparticles offers a green and sustainable approach. The bioactive compounds present in the extract act as reducing and capping agents, facilitating the reduction of zinc ions to nanoparticles. This method presents several advantages, including simplicity, cost-effectiveness, and eco-friendliness compared to conventional chemical synthesis methods".
2. **Characterization of Nanoparticles:** The synthesized "zinanoparticles undergo rigorous characterization to confirm their formation and assess their properties. Techniques such as UV-Vis spectroscopy provide valuable insights into the optical properties of nanoparticles, with a characteristic absorption peak observed at 435 nm due to the Surface Plasmon Resonance (SPR) phenomenon. Additional characterization methods, including TEM imaging, XRD analysis, and FTIR spectroscopy, can further elucidate the size, shape, crystallinity, and chemical composition of nanoparticles".
3. **Antimicrobial Properties:** One of the most "significant aspects of the discussion revolves around the antimicrobial efficacy of the synthesized zinc nanoparticles. The nanoparticles exhibit potent antimicrobial activity against a variety of pathogenic microorganisms, including bacteria such as Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis, and Staphylococcus aureus. The mechanism of antimicrobial action involves the interaction of nanoparticles with microbial cells, leading to cell membrane disruption, interference with cellular processes," and induction of oxidative stress.
4. **Comparative Analysis:** A comparative analysis "may be conducted to evaluate the antimicrobial efficacy of the Medicago sativa nanoparticles against conventional antimicrobial agents. This comparison can highlight the potential advantages of using nanoparticles, such as broader spectrum activity, lower toxicity, and reduced likelihood" of resistance development.
5. **Potential Applications:** The discussion may also explore potential applications of Medicago sativa zinc nanoparticles beyond antimicrobial therapy. These nanoparticles have promising potential in various fields, including biomedical applications such as wound healing, drug delivery, and medical device coatings. Additionally, they may find utility in environmental remediation, catalysis, and food preservation.
6. **Challenges and Future Directions:** Despite the "promising results, challenges such as nanoparticle stability, scalability of synthesis, and regulatory considerations should be addressed. Future research directions may include optimizing synthesis parameters, elucidating the mechanisms of antimicrobial action, exploring synergistic effects with other natural extracts, and conducting in vivo studies to assess biocompatibility" and therapeutic efficacy.

Overall, the discussion on the" synthesis, characterization, antimicrobial properties, and potential applications of Medicago sativa nanoparticles underscores their significance as a promising antimicrobial agent with diverse biomedical and environmental applications".

CONCLUSION:

The swift and efficient "biosynthesis of silver nanoparticles using Medicago sativa extract presents a compelling avenue for synthesis, offering a method that is both cost-effective and environmentally friendly. A notable hallmark of this synthesis process is the distinct change in color observed, transitioning from a colorless solution to a reddish-brown hue, indicative of the presence of silver nanoparticles. This alteration in color is attributed to the Surface Plasmon Resonance (SPR) phenomenon, a characteristic feature of zinc nanoparticles. The confirmation of zinc nanoparticle formation was further substantiated through UV-Vis spectroscopy analysis," revealing a prominent absorption peak at 435 nm, characteristic of the entire nanoparticle ensemble.

Moreover, the "synthesized nanoparticles were subjected to rigorous evaluation for their antimicrobial efficacy against a spectrum of microbial strains, including Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis, and Staphylococcus aureus. Encouragingly, the synthesized nanoparticles exhibited substantial inhibitory effects against all four mentioned microorganisms, signifying their potential as antimicrobial agents. Among the tested strains, Pseudomonas aeruginosa displayed the highest sensitivity to the nanoparticles," suggesting their promising antimicrobial activity across a range of pathogens (Al-Momani et al.,2023).

REFERENCES :

1. Song, K., Zhao, D., Sun, H., Gao, J., Li, S., Hu, T., & He, X. (2022). Green nanopriming: responses of alfalfa (Medicago sativa L.) seedlings to alfalfa extracts capped and light-induced silver nanoparticles. **BMC Plant Biology**, 22(1), 323.

2. Bandeira, M., Giovanela, M., Roesch-Ely, M., Devine, D. M., & da Silva Crespo, J. (2020). Green synthesis of zinc oxide nanoparticles: A review of synthesis methodology and mechanism of formation. **Sustainable Chemistry and Pharmacy**, 15, 100223.

3. Álvarez-Chimal, R., García-Pérez, V. I., Álvarez-Pérez, M. A., & Arenas-Alatorre, J. Á. (2021). Green synthesis of ZnO nanoparticles using a Dysphania ambrosioides extract: structural characterization and antibacterial properties. **Materials Science and Engineering C**, 118, 111540.

4. Naseer, M., Aslam, U., Khalid, B., & Chen, B. (2020). Green route to synthesize zinc oxide nanoparticles using leaf extracts of Cassia fistula and Melia azadarach and their antibacterial potential. **Scientific Reports**, 10(1), 65949.

5. Haddi, R., El Kharraz, A. M., & Kerroumi, M. I. (2024). Green synthesis of zinc oxide nanoparticles using Pistacia lentiscus L. leaf extract and evaluating their antioxidant and antibacterial properties. **Nano Biomedicine and Engineering**, 16(2), 232–247.

6. Singh, K., & Yadav, S. (2025). Biosynthesis of a range of ZnO nanoparticles utilizing Salvia hispanica L. seed extract and evaluation of their bioactivity. **Scientific Reports**, 15, 4043.

7. Ka, F. N. N., Tiwari, A., Shaik, A. H., Pa, I., Brianna, B., Anwar, A., & Chandan, M. R. (2025). Anti-cancer and antimicrobial efficacy of ZnO nanoparticles synthesized via green route using Amaranthus dubius leaves extract. **Green Chemistry Letters and Reviews**, 18(1).

8. Abbas, M., Hanif, U., Nasir, A., Zahid, F., Liaqat, I., Ali, S., Bhanot, D., Ataya, F. S., & Anwar, A. (2025). Green synthesis and application of zinc oxide (ZnO) nanoparticles from Cenchrus setigerus Vahl in herbal handwash as antimicrobial reducer. **ChemistrySelect**, 10(2), e202404733.

9. Khajuria, A. K., Kumari, M., Kandwal, A., Singh, A., & Bisht, N. S. (2021). Biofabrication of zinc oxide nanoparticles from two different zinc sources and their antimicrobial activity. **BioNanoScience**, 11(3), 793–809.

10. Rahman, T. U., Anwar, M. R., Zeb, M. A., & Liaqat, W. (2022). Green synthesis, characterization and antibacterial activity of metal nanoparticles and composite oxides using leaves extract of Lamiaceae species. **Microscopy Research and Technique**, 85(8), 2857–2865.

11. Malik, A. R., Sharif, S., Shaheen, F., Khalid, M., Iqbal, Y., Faisal, A., Aziz, H., Atif, M., Ahmad, S., Fakhar-e-Alam, M., Hossain, N., Ahmad, H., & Botmart, T. (2022). Green synthesis of RGO-ZnO nanocomposite mediated by Ocimum basilicum leaf extract: antioxidant, antibacterial, antidiabetic and photocatalytic activity. **Journal of Saudi Chemical Society**, 26(2), 101438

12. Wafaey, A. A., El-Hawary, S. S., Abdelhameed, M. F., El Raey, M. A., Abdelrahman, S. S., Ali, A. M., & Kirollos, F. N. (2024). Green synthesis of zinc oxide nanoparticles using ethanolic extract of Gliricidia sepium stem: characterizations and their gastroprotective effect. **Bioorganic Chemistry**, 145, 107225.

13. Deepak, S., Anilkumar, P., Jasmin, J., & Kumar, E. R. (2024). A facile synthesis of ZnO nanoparticles and their structural, optical, morphological, biological, and photocatalytic properties. **Journal of Drug Delivery Science and Technology**, 101, 106123.

14. Alvarez-Chimal, R., et al. (2022). Influence of particle size on the antibacterial activity of green synthesized ZnO nanoparticles using Dysphania ambrosioides extract, supported by molecular docking analysis. **Arabian Journal of Chemistry**, 15(6), 103804.

15. Umamaheswari, A., Prabu, S. L., John, S. A., & Puratchikody, A. (2021). Green synthesis of zinc oxide nanoparticles using Raphanus sativus var. longipinnatus leaf extract and evaluation of anticancer potential. **Biotechnology Reports**, 29, e00595.

16. Donga, S., & Chanda, S. (2022). Biological synthesis of ZnO nanoparticles using Caesalpinia crista seed extract and evaluation of their antioxidant and antimicrobial activities. **BioNanoScience**, 12(2), 451–462.

17. Al-Momani, H., et al. (2023). Green synthesis of zinc oxide nanoparticles using Olea europaea leaf extract and their antimicrobial and antibiofilm activities. **Scientific Reports**, 13(1), 5096.