

# Synthesis, characterization and biological investigation of zinc nanoparticles using *Acacia modesta* Wall. leaves

Hadsa Ghauri<sup>1</sup>, Qandeel Zahra<sup>2</sup>, Jamshaid Raza<sup>3</sup>, Rana Muhammad Hammad<sup>4</sup>, Mohamed Farouk Elsadek<sup>5</sup>, Alia Almoajel<sup>5</sup>, Mohamed Ragab Abdel Gawwad<sup>6</sup>, Amir Aziz<sup>1</sup>, Nida Munir<sup>7</sup>, Hafiz Ullah<sup>8</sup>, Rahat Ullah Khan<sup>9</sup>, Shubana Hayat<sup>10</sup> and Farah Shireen<sup>1\*</sup>

<sup>1</sup>Sarhad Institute of Allied Health Sciences, Sarhad University of Science & Technology, Peshawar, Pakistan

<sup>2</sup>Azra Naheed Medical College, Lahore, Pakistan

<sup>3</sup>Nawaz Sharif Medical College, Gujrat, Pakistan

<sup>4</sup>Department of Chemistry, University of Gujrat, Gujrat, Pakistan

<sup>5</sup>Community Health Sciences Department, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia

<sup>6</sup>Genetics and Bioengineering, Faculty of Engineering and Natural Sciences, International University of Sarajevo, Sarajevo, Bosnia and Herzegovina

<sup>7</sup>Centre of Biotechnology and Microbiology, University of Peshawar, Peshawar, Pakistan

<sup>8</sup>Gomal Center of Biochemistry and Biotechnology, Gomal University, Dera Ismail Khan, Pakistan

<sup>9</sup>Institute of Microbiology, Faculty of Veterinary and Animal Sciences, Gomal University, Dera Ismail Khan, Pakistan

<sup>10</sup>Department of Microbiology, Hazara Univeristy, Mansehra, Pakistan

**Abstract:** The study aimed to synthesize zinc nanoparticles (Zn-NPs) using an aqueous extract derived from *Acacia modesta* Wall. leaves. Several characterization techniques were employed to confirm the successful formation of zinc nanoparticles. UV-visible spectrophotometry indicated a peak at 374 nm, validating the bioreduction process. Scanning electron microscopy (SEM) was utilized to analyze the morphology, transmission electron microscopy (TEM) to determine particle size and shape, X-ray diffraction (XRD) for crystalline structure analysis, energy-dispersive X-ray spectroscopy (EDX) for elemental composition and Fourier-transform infrared spectroscopy (FTIR) to identify functional groups. The synthesized Zn-NPs demonstrated remarkable antibacterial activity against *Escherichia coli* (95% inhibition) and moderate antifungal activity against *Candida albicans* (70% inhibition). In phytotoxicity tests, the Zn-NPs exhibited a 55% reduction in the growth of *Lamina minor* at the highest dilution (1000 µl). Based on these findings, the study concluded that the green-synthesized Zn-NPs hold great potential as effective antibiotics against pathogenic bacteria and could be utilized in various industrial and agricultural applications.

**Keywords:** XRD, FRIT, zinc, antifungal, antibacterial.

## INTRODUCTION

Throughout human history, advancements in science and technology have greatly enhanced our way of life. In the 20th century, significant progress was made in various fields, including genomics, recombinant DNA technology, proteomics, immunology and pharmacognosy. These advancements have had a profound impact on the field of medicine, leading to improved diagnostic and therapeutic options (Sabo *et al.*, 2021). Nano-biotechnology is a field that combines the principles and techniques of nanotechnology with those of biotechnology (Shahcheraghi *et al.*, 2022). Nano-biotechnology has gained significant recognition and has found applications in various fields of science, including engineering, medicine, chemistry and physics. Its success and versatility can be attributed to the unique physicochemical properties of nanoscale materials and the ability to control their operations to achieve desired outcomes (Fang *et al.*, 2023).

While it is true that nanoparticles can be synthesized

using various metal salts, including gold, silver, platinum, zinc, copper, iron and titanium, it is important to note that the choice of materials depends on the specific application and desired properties (Hussain *et al.*, 2021). Physical and chemical methods are commonly employed for synthesizing nanoparticles, while some medicinal plants possess bioactive compounds that can serve as reducing agents and therapeutic ingredients during nanoparticle synthesis. The combination of these phyto-compounds with metal ions can potentially enhance the medicinal value of nanoparticles, but further research and understanding are necessary for their safe and effective application in various fields (Shafey, 2020).

Zinc is an essential micronutrient required by the body for various physiological functions. It plays a crucial role in supporting the immune system, facilitating metabolic processes and promoting wound healing. Zinc is also involved in various enzymatic reactions and cellular processes in the body (Swart, 2018). Zinc nanoparticles (ZnNPs) have been studied for their potential benefits in various fields, including agriculture, medicine and biotechnology. Some studies have suggested that ZnNPs

\*Corresponding author: e-mail: farah.biotec@suit.edu.pk

may possess herbicidal and pesticidal properties, as well as antimicrobial, anti-inflammatory, anti-diarrheal and anti-viral activities (Shad *et al.*, 2021). Overall, the use of ZnNPs in agriculture has the potential to improve crop yield and quality, while also reducing the use of chemical fertilizers and pesticides (Nasiri *et al.*, 2022). The use of green synthesis approaches for ZnNP fabrication has the potential to provide an eco-friendly and cost-effective alternative to traditional chemical methods. To completely comprehend the characteristics and possible uses of these nanoparticles, however, more investigation is necessary (Abdelsalam *et al.*, 2019; Zou *et al.*, 2021).

Pakistan indeed boasts a rich biodiversity of plant flora, with a diverse range of medicinal plants. The country is home to an estimated 5,521 species of medicinal plants, showcasing the significant potential for traditional herbal medicine practices (Ahmed, 2016). *Acacia modesta* Wall. is a medicinal plant that is widely used in traditional herbal medicine in Pakistan. The different parts of the plant, including the leaves, bark, fruit, gum and flowers, have been reported to possess a variety of medicinal properties (Khan *et al.*, 2023). *Acacia modesta* has a long history of traditional use in various cultures and its therapeutic properties have been employed for treating a range of conditions. As you mentioned, the plant has been used as a tea for the treatment of menstrual cramps, cough, dysentery, body weakness, and constipation (Subhan *et al.*, 2018). In the study you mentioned, zinc was combined with natural phytochemicals to create ZnNPs, which were then tested for their potential to be antibacterial, antioxidant and phytotoxic.

## MATERIALS AND METHODS

### **Plant collection**

The Forest College in Peshawar provided the *Acacia modesta*'s fresh leaves. It received approval from Sarhad University of Science and Information Technology, Peshawar's Institute of Biological Sciences, Pakistan.

### **Extraction**

The process of removing dust particles from the leaves of *Acacia modesta* by detaching them from the stem and rinsing them under tap water is a common practice to ensure the cleanliness of the plant material. This step helps to remove any external contaminants, such as dust, dirt, or pollutants, that may have accumulated on the leaves (Hussain *et al.*, 2019).

### **Synthesis of ZnNPs**

The synthesis process you described involved the combination of 10 ml of aqueous extract with 90 ml of a 1 mM ZnSO<sub>4</sub> solution to synthesize green ZnNPs. The specific composition of the aqueous extract from *Acacia modesta* was likely rich in phytochemicals and served as a reducing agent or stabilizer for the formation of ZnNPs

(Shakibaie *et al.*, 2022).

### **Characterization of ZnNPs**

To determine the physical and biological properties of zinc nanoparticles (ZnNPs), several procedures can be employed (Hernández-Díaz *et al.*, 2021).

### **UV-Vis Spectroscopy**

The optical characteristics of ZnNPs made with *Acacia modesta* were evaluated at room temperature using the UV-Vis spectrophotometer. The instrument was adjusted to measure absorbance within a specific wavelength range of 350 to 500 nm with an interval of 10 nm (Trung *et al.*, 2020).

### **Scanning electron microscopy (SEM)**

SEM (Scanning Electron Microscopy) is a powerful imaging technique that allows the examination of the surface morphology of a sample at high resolution. In the current study, it was used to examine the morphology of the synthesized ZnNPs. The procedure involved placing a small amount of the ZnNPs solution on a copper grid and allowing it to dry. The grid was then examined using a SEM instrument (JSM-5910 Japan) at different magnifications of 1000X, 5000X and 10,000X. The resulting images provided information on the size, shape, and distribution of the synthesized ZnNPs (Trung *et al.*, 2020).

### **Transmission electron microscopy (TEM)**

Transmission Electron Microscopy (TEM) is a high-resolution imaging technique used to examine the size, shape, and detailed nano-morphology of nanoparticles. In the research study, TEM (Techni-G2-300kV, USA) was employed for this purpose (Jeevanandam *et al.*, 2021)

### **Fourier transform infrared spectroscopy (FT-IR)**

The FT-IR (Fourier Transform Infrared) spectroscopy technique was employed to assess the functional groups present in the synthesized ZnNPs. The instrument used for this analysis allowed for the measurement of IR spectra in a variable range between 4000 and 45000 cm<sup>-1</sup> (Jeevanandam *et al.*, 2021).

### **Energy dispersive X-ray analysis (EDX)**

EDX (Energy-Dispersive X-ray) spectroscopic analysis was utilized to examine the elemental composition of the synthesized ZnNPs. The instrument used for this analysis was the INCA-200 system (Diaz *et al.*, 2020).

### **X-ray diffraction measurement (XRD)**

XRD (X-ray Diffraction) analysis is a technique used to determine the crystal structure and dimensions of materials, including nanoparticles. In the research study, XRD spectroscopy (JDX-3532, Japan) was employed to examine the crystal structure and dimensions of the synthesized ZnNPs (Hernández-Díaz *et al.*, 2021).

### Biological investigations of ZnNPs

#### Antibacterial activity

The antibacterial activity of ZnNPs against pathogenic bacteria was assessed using the well diffusion technique. The well diffusion method is a commonly used assay to evaluate the antimicrobial efficacy of nanoparticles or other antimicrobial agents (Trung *et al.*, 2020)

#### Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of ZnNPs against pathogenic bacterial strains was investigated using a protocol documented by a specific reference or research study (Trung *et al.*, 2020)

#### Antifungal activity

Agar tube dilution (Aziz *et al.*, 2017) investigating ZnNPs and aqueous extracts for their antifungal properties involved a specific technique. The pathogenic fungi used in the process included *Candida albicans*, *Trichophyton species*, *Microsporum furfur*, *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium species*, *Fusarium solani*, *Trichoderma species* and *Aspergillus fumigatus*.

#### Phytotoxic activity

To investigate the phytotoxic potentials of ZnNPs and crude leaves extract, the *Lemna minor* lethality assay can be followed (Aziz *et al.*, 2017).

#### Antioxidant activity

DPPH radical scavenging protocol (Aziz *et al.*, 2017) was used to examine the ZnNPs' and the leaf extract's aqueous antioxidant efficacy.

## STATISTICAL ANALYSIS

The statistical analysis was performed by using SPSS Software by following the SPSS survival manual. The standard means and deviations were found through the software.

## RESULTS

This study aimed to investigate the potential of using biogenic ZnNPs synthesized from aqueous leaves extract of *A. modesta*. The plant's natural phytochemicals were used to biologically reduce ionic zinc to form nanoparticles, which were then purified and characterized to evaluate their unique physical and chemical properties. Finally, the biogenic ZnNPs were evaluated for their antimicrobial, antioxidant, and phytotoxic activities and compared with the activities of the crude aqueous extract of *A. modesta* leaves.

### Characterization of biosynthesized ZnNPs

#### UV-Vis Spectroscopy

The UV-Vis spectroscopy analysis revealed that the synthesized biogenic ZnNPs exhibited a peak at a wavelength of 374nm, indicating the precise bioreduction

of zinc ions to form nanoparticles. The maximum absorbance observed at 374nm was 0.52. These results are visually presented in fig. 1, which displays the absorbance values at different wavelengths, with the peak at 374nm prominently depicted.

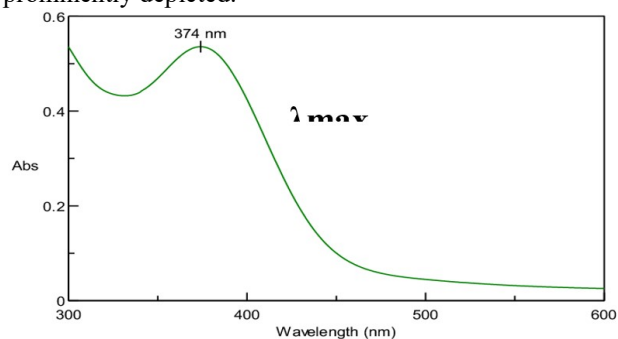


Fig. 1: UV-Vis spectra of synthesized Zn-NPs

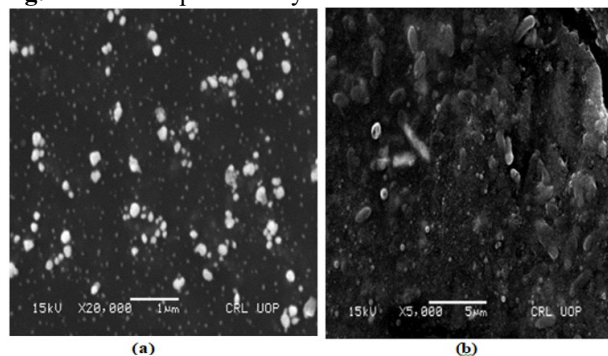


Fig. 2: (a, b). SEM micrographs of ZnNPs

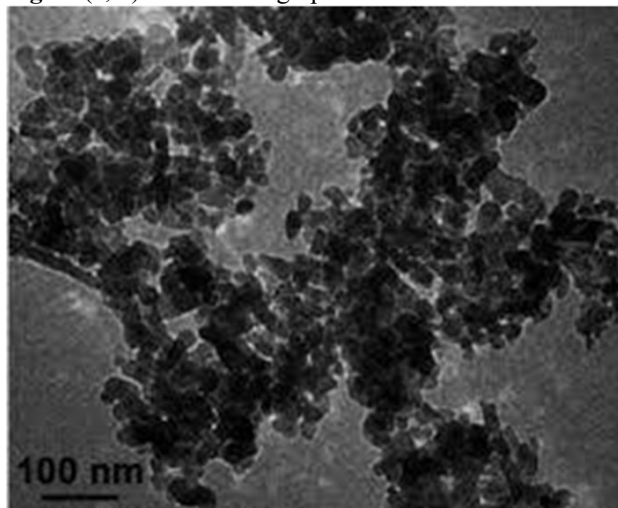


Fig. 3: TEM micrographs of ZnNPs.

#### Scanning electron microscopy

Scanning electron microscopy (SEM) analysis of the synthesized ZnNPs revealed the presence of stable nano-spherical structures. Additionally, at higher magnifications of 20,000X and 5000X, a few tubular and regular-shaped ZnNPs were also observed. The SEM micrographs depicting these different structures are displayed in fig. 2, providing a visual representation of the synthesized ZnNPs' morphology.

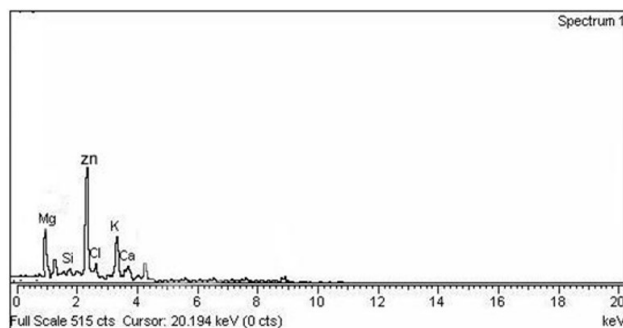


Fig. 4: EDX micrographs of ZnNPs

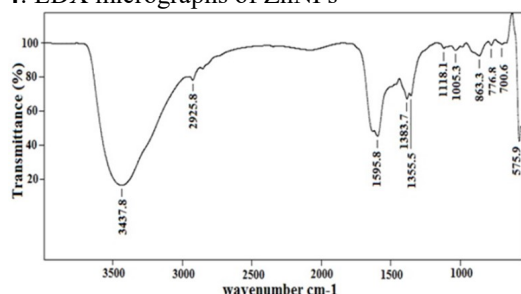


Fig. 5: FT-IR spectroscopy of Zn-NPs

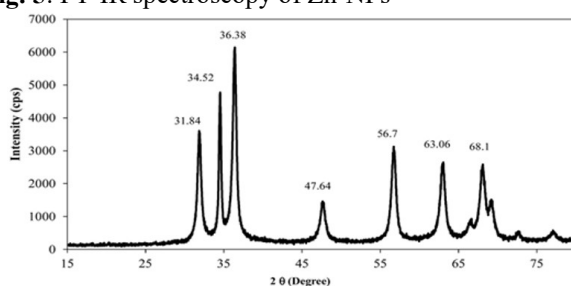


Fig. 6: XRD spectroscopy of ZnNPs

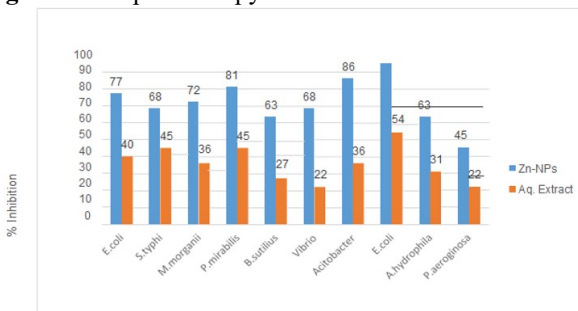


Fig. 7: Graphical representation of % inhibition against Zn-NPs.

### Transmission electron microscopy

Transmission electron microscopy (TEM) analysis of the synthesized ZnNPs revealed that the nanoparticles had sizes ranging from 50 to 100nm. Additionally, a few smaller nanostructures in the range of 5 to 20nm were also observed. The detailed microscopy examination confirmed that the majority of the ZnNPs exhibited a spherical morphology. Fig. 3 displays the TEM micrograph, providing a visual representation of the synthesized ZnNPs and their size and shape characteristics.

### Energy dispersive X-ray (EDX) analysis

The EDX (Energy-Dispersive X-ray Spectroscopy) investigations revealed the presence of reduced zinc element, confirming the precise synthesis of ZnNPs. In addition to zinc, other biological elements such as magnesium, potassium, calcium, silicon and chloride were also observed. This observation suggests that the active phyto-ingredients present in the leaves of *A. modesta* possess the ability to reduce and cap the zinc ions, converting them to ZnO nanoparticles, while the phytochemicals themselves undergo oxidation. The EDX analysis graph, depicting the elemental composition of the synthesized ZnNPs and the presence of other elements, is shown in fig. 4.

### Fourier transform infrared spectroscopy

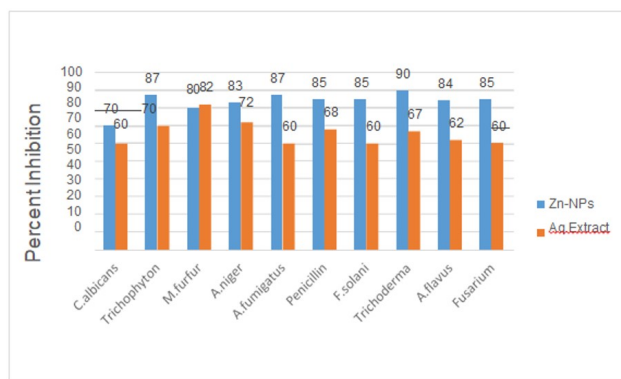
According to the results of the FT-IR spectroscopy, the functional groups -OH (alcohol) and -NH (amine) and -C-Cl (alkyl halides) had strong peaks at 3437.8cm<sup>-1</sup>, 1595.8 cm<sup>-1</sup> and 575.9cm<sup>-1</sup>, respectively. Medium peaks, or CH<sub>3</sub>CH (bending alkanes), at 1383.7cm<sup>-1</sup> and 1355.5 cm<sup>-1</sup>, may have been acquired from functional groups found in leaf extracts, acting as a bio-capping agent. The functional groups alkanes (-CH), amides (CO-NH) and aldehydes (CHO) are indicated by weak IR peaks at varying wavenumber. Fig. 5 depicts the spectroscopic depiction of FT-IR analysis.

### X-ray diffraction measurements

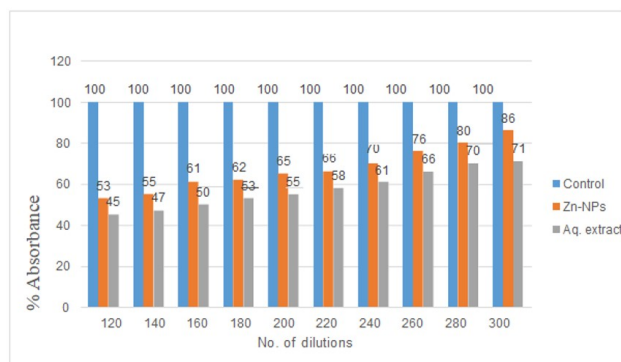
X-ray diffraction (XRD) is a technique used to analyze the crystal structure of materials. In the case of the synthesized ZnNPs, the XRD analysis showed sharp peaks at various 2θ angles, indicating a crystalline structure. The intensity of the peaks varied, indicating that the ZnNPs were not uniform in size and shape. However, the overall diffraction pattern indicated that the ZnNPs were mono-dispersed and stable in their crystal structure. The XRD analysis can provide valuable information about the crystal structure of nanomaterials and is commonly used in the characterization of nanoparticles.

### Antibacterial activity

The comparative study between the biosynthesized Zn-NPs and the crude leaf extract of *A. modesta* Wall. Revealed that the Zn-NPs exhibited efficient antibacterial activity against all ten bacterial pathogenic strains tested. The highest sensitivity was observed against *P. mirabilis* (81%) and *E. coli* (95%) among the analyzed bacterial strains. On the other hand, the crude leaf extract showed less activity compared to the zinc nanoparticles. These findings indicate that the synthesized Zn-NPs have enhanced antibacterial properties compared to the crude extract of *A. modesta*. The results of percent inhibition, representing the antibacterial activity, are summarized in fig. 7. This suggests that the biosynthesized Zn-NPs from *A. modesta* have the potential to be used as an effective antibacterial agent.



**Fig. 8:** Graphical description of antifungal activity by *A. modesta* Wall. leaves.



**Fig. 9:** Graphical description of antioxidant activity of *A. modesta* Wall. leaves

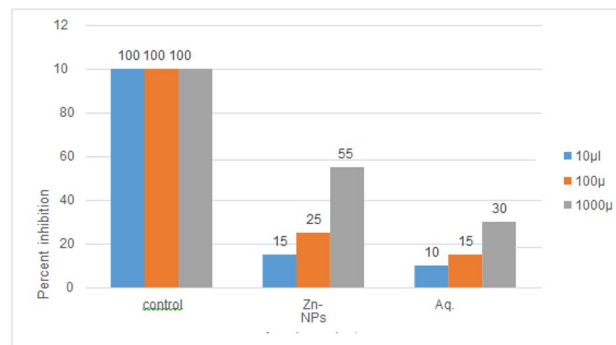
#### Minimum inhibitory concentration (MIC) assay

It is worth noting that the MIC (minimum inhibitory concentration) is the lowest concentration of a substance that can prevent visible growth of a microorganism. The MIC assay is often used to determine the efficacy of antibacterial agents against specific bacterial strains. In this case, the results suggest that biogenic Zn-NPs from *A. modesta* leaves have a stronger antibacterial activity compared to the crude leaf extract, as lower volumes of the nanoparticles were needed to inhibit bacterial growth. Additionally, the nanoparticles were effective against all ten bacterial pathogenic strains tested, with *P. mirabilis* and *E. coli* showing the highest sensitivity.

#### Antifungal activity

The antifungal activity of Zn-NPs synthesized from the leaf extract of *Acacia modesta* Wall. was investigated against various pathogenic fungal strains. The percent growth inhibition, determined by measuring the linear mycelial growth, revealed that *Trichoderma* was the most sensitive fungal strain, with a growth inhibition of 90% in the presence of the synthesized Zn-NPs. The other fungal cultures also showed significant sensitivity, indicating that the zinc nanoparticles had excellent activity in inhibiting the growth of pathogenic fungal mycelia. The results of percent inhibition, representing the antifungal activity, are summarized in fig. 8. These findings suggest

the potential of the synthesized Zn-NPs as effective antifungal agents against pathogenic fungi.



**Fig. 10:** Graphical depiction of phytotoxic activity of *A. modesta* Wall.

#### Antioxidant activity

The antioxidant activity of bio-fabricated ZnNPs was evaluated, and it was observed that at a lower sample dilution of 120µl, the ZnNPs exhibited moderate antioxidant activity of 53%. As the sample concentration increased to 300µl, the antioxidant activity of the ZnNPs significantly escalated to 86%. In comparison, the aqueous extract of *A. modesta* also showed moderate to good antioxidant activity at different sample concentrations. The results of percent absorbance, representing the antioxidant activity, are summarized in fig. 9. These findings indicate that the ZnNPs synthesized from the leaf extract of *A. modesta* possess notable antioxidant activity, which can be further enhanced with increasing sample concentration.

#### Phytotoxic activity

The phytotoxic activity of ZnNPs synthesized using *A. modesta* Wall. leaves was evaluated against *Lemna minor*, and it was observed that even at the highest dilution of 1000µg/ml, moderate phytotoxic activity of 55% was recorded. In comparison, the aqueous extracts of *A. modesta* exhibited lower phytotoxic activity of 30% at the highest dilution. The activity decreased with lower concentrations of the samples. The results, indicating the percent phytotoxic activity, are graphically depicted in fig. 10. These findings suggest that the ZnNPs synthesized from *A. modesta* leaves possess notable phytotoxic activity against *Lemna minor*, surpassing the activity of the aqueous extracts.

## DISCUSSION

Green synthesis of nanoparticles using plant extracts is a promising and eco-friendly approach for the production of nanoparticles. It involves the use of various parts of plants such as leaves, stem, roots and flowers for the reduction and stabilization of metal nanoparticles. The plant extracts contain various phytochemicals such as flavonoids, terpenoids, alkaloids, and phenolic compounds which act

as reducing and capping agents for the synthesis of nanoparticles. This method has several advantages over traditional chemical methods, including cost-effectiveness, biocompatibility, and sustainability. Moreover, the nanoparticles synthesized by green methods have shown significant antimicrobial, antioxidant, and phytotoxic activities.

The produced ZnNP nanoparticles outperformed the aqueous extract of *A. modesta* Wall. plant against the tested bacterial strains. Previous research have shown that nanoparticles have greater antibacterial activity than simple plant extracts. Synthesized ZnNPs could be used to produce novel antibiotics that successfully inhibit pathogenic microorganisms that cause human diseases.

UV-Vis spectroscopy is a commonly used technique for the characterization of nanoparticles, as it provides information about their size, shape and surface properties. The absorption peak at 374nm suggests that the synthesized Zn-NPs have a relatively small size, as smaller nanoparticles tend to absorb light at shorter wavelengths. It is also a good sign that the maximum absorption peak was achieved, as it indicates that the nanoparticles are well-formed and stable (Hayat *et al.*, 2022), which is nearer to one reported by (Housseiny & Gomaa, 2019). FTIR spectra showed by study carried out by Padalia and Chanda (2017) peaks observed in the FTIR spectra of the synthesized Zn-NPs indicate the presence of various functional groups. The peak at 3437.8 cm indicates the presence of O-H stretching vibration of alcohols, phenols and carboxylic acids. The peak at 1595.8 cm indicates the presence of N-H bending vibration of amines and amides. The peak at 575.9 cm indicates the presence of Zn-O stretching vibration. The peaks at 1383.7 cm and 1355.5 cm indicate the presence of C-H bending vibration of alkanes and alkenes, respectively. These peaks provide important information about the chemical composition and structure of the synthesized Zn-NPs (Ahmad *et al.*, 2011). The present research study demonstrated that zinc nanoparticles synthesized using *A. modesta* Wall. leaves exhibited strong inhibitory effects against pathogenic fungal strains, including *A. niger* and *A. fumigatus*. The percent inhibition of fungal growth was significantly higher compared to previous studies. These findings highlight the potential of zinc nanoparticles synthesized from *A. modesta* Wall. leaves in effectively inhibiting the proliferation of pathogenic fungal strains, suggesting their potential application as antifungal agents (Sarwar, 2016).

Zinc nanoparticles can harm fungal cells by damaging their membranes and leaking their components. Zinc nanoparticles may limit fungal growth by interacting with sulfur-containing molecules in proteins and DNA. Zinc nanoparticle antifungal mechanisms need further study (Housseiny & Gomaa, 2019). This study shows that zinc

nanoparticles can effectively fight harmful fungus. Our findings confirm bioinspired ZnNPs' antibacterial properties and support other studies (Behravan *et al.*, 2019). The biosynthesized zinc nanoparticles' antibacterial efficacy against the identified bacterial strains matches earlier findings. Biosynthesized zinc nanoparticles have showed antibacterial efficacy against *E. coli*, *S. aureus*, *P. aeruginosa* and Bacillus spp. These nanoparticles kill bacteria by disrupting the cell wall and membrane, inhibiting DNA synthesis, and generating reactive oxygen species (ROS). These features make biosynthesized zinc nanoparticles interesting antibacterial agents. Khatami *et al.* (2018) believe that biogenic ZnNPs generated from plants could be used to develop novel antibacterial and antioxidant drugs. Due to its eco-friendliness, low toxicity, and cost-effectiveness compared to chemical approaches, plant extract-based green synthesis of ZnNPs appears promising.

## CONCLUSION

The research study highlights the potential of aqueous leaf extracts of *A. modesta* plant in mediating the synthesis of stable and crystalline ZnNPs. These nanoparticles exhibited favorable physicochemical properties, including mono-dispersion and a size range suitable for various applications. The antimicrobial, antioxidant, and phytotoxic activities of the ZnNPs were found to be promising, with a MIC value of 10  $\mu$ L inhibiting the growth of all tested pathogenic bacteria and fungi. The antioxidant and phytotoxic activities were observed to increase with higher sample concentrations. This suggests that the combination of medicinal properties of zinc and plant extracts can lead to the production of eco-friendly and cost-effective ZnNPs. These nanoparticles have potential applications in medicine, agriculture, and various industrial sectors, such as the development of novel antimicrobial therapies, herbicides, catalysts and biosensors.

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## REFERENCES

- Abdelsalam NR, Fouda MM, Abdel-Megeed A, Ajarem J, Allam AA and El-Naggar ME (2019). Assessment of silver nanoparticles decorated starch and commercial zinc nanoparticles with respect to their genotoxicity on onion. *Int. J. Biol. Macromol.*, **133**: 1008-1018.
- Ahmad B, Bashir S, Azam S and Ali N (2011). Screening of *Acacia modesta* for antifungal, anti-termite, nitric oxide free radical scavenging assay and brine shrimp cytotoxic activities. *J. Med. Plants Res.*, **5**(15): 3380-3386.

- Aziz A, Khan I, Azam S, Mehnaz S and Ahmad B (2017). Comparative antimicrobial, phytotoxic and hemagglutination potential of *Eriobotrya japonica* leaf extract and its zinc nano-particles. *Pak. J. Bot.*, **49**(5): 1917-1924.
- Behravan M, Panahi AH, Naghizadeh A, Ziaee M, Mahdavi R and Mirzapour A (2019). Facile green synthesis of silver nanoparticles using *Berberis vulgaris* leaf and root aqueous extract and its antibacterial activity. *Int. J. Biol. Macromol.*, **124**: 148-154.
- Fang C, Xiao G, Wang T, Song L, Peng B, Xu B and Zhang K (2023). Emerging nano-/biotechnology drives oncolytic virus-activated and combined cancer immunotherapy. *Research*, **6**: 0108.
- Hayat S, Ashraf A, Zubair M, Aslam B, Siddique MH, Khurshid M and Naeem Z (2022). Biofabrication of ZnO nanoparticles using *Acacia arabica* leaf extract and their antibiofilm and antioxidant potential against foodborne pathogens. *Plos One*, **17**(1): e0259190.
- Hernández-Díaz JA, Garza-García JJ, Zamudio-Ojeda A, León-Morales JM, López-Velázquez JC and García-Morales S (2021). Plant-mediated synthesis of nanoparticles and their antimicrobial activity against phytopathogens. *J. Sci. Food Agric.* **101**(4): 1270-1287.
- Housseiny MM and Goma EZ (2019). Enhancement of antimicrobial and antitumor activities of zinc nanoparticles biosynthesized by *Penicillium chrysogenum* AUMC 10608 using gamma radiation. *Egypt. J. Bot.*, **59**(2): 319-337.
- Hussain F, Hadi F and Akbar F (2019). Magnesium oxide nanoparticles and thidiazuron enhance lead phytoaccumulation and antioxidative response in *Raphanus sativus* L. *Environ. Sci. Pollut. Res.* **26**: 30333-30347.
- Hussain K, Hussain T, Khan AA, Anwar F and Khan MA (2017). Nano-biotechnology for the management of pest and diseases incidence in agricultural as well as horticultural crops. *Trends in Biosci.* **10**(39): 8095-8101.
- Jeevanandam J, Pan S, Harini A and Danquah MK (2021). Challenges in the risk assessment of nanomaterial toxicity towards microbes interfaces between nanomaterials and microbes. CRC Press, pp. 58-93
- Nasiri S, Mahmoudvand H, Shakibaie M, Mousavi SAH and Sepahvand A (2022). Antifungal effects of zinc nanoparticles green synthesized by *Lavandula angustifolia* extract, alone and combined with nystatin against *Candida albicans*, a major cause of oral candidiasis *J. Herbmed. Pharmacol.* **11**(4): 540-545.
- Sarwar W (2016). Pharmacological and phytochemical studies on *Acacia modesta* Wall; A review. *J. phytopharm.*, **5**(4): 160-166.
- Shad S, Bashir N, Nault MFBD and Lynch I (2021). Incorporation of biogenic zinc nanoparticles into a polymeric membrane: Impact on the capture of organic herbicides. *Clean. Technol.*, **5**: 100339.
- Shafey AME (2020). Green synthesis of metal and metal oxide nanoparticles from plant leaf extracts and their applications: A review. *Green Process. Synth.*, **9**(1): 304-339.
- Shahcheraghi N, Golchin H, Sadri Z, Tabari Y, Borhanifar F and Makani S (2022). Nano-biotechnology an applicable approach for sustainable future. *3 Biotech.*, **12**(3): 65.
- Subhan N, Burrows GE, Kerr PG and Obied HK (2018). Phytochemistry, ethnomedicine and pharmacology of *Acacia*. *Stud. Nat. Prod. Chem.*, **57**: 247-326.
- Trung ND, Nhuan T, Van L, Minh NV, Anh NP and Tri N (2020). Biofabrication of silver nanoparticles using *Curcuma longa* extract: Effects of extraction and synthesis conditions, characteristics, and its antibacterial activity. *J. Biochem. Technol.*, **11**: 57-66.
- Zou M, Zhong Z and Wen C (2021). Characterization and anti-acute myeloid leukemia and anti-acute T cell leukemia properties of zinc nanoparticles synthesized by a green approach for bioremediation applications. *Arch. Med. Sci.*, 140295.