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BIOMEDICAL SCIENCES

Plant phytochemicals-mediated synthesis of zinc oxide nanoparticles with antimicrobial, pharmacological, and environmental applications

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Abstract: Nanotechnology is a fast-growing field with large number of applications. Therefore, the current study, was designed to prepare Zinc Oxide nanoparticles (ZnO NPs) from A. modesta leaves extract through a cost-effective method. The prepared NPs were characterized through UV-Vis Spectroscopy (UV–Vis), Dynamic light scattering (DLS), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction analysis (XRD), scanning electron microscope (SEM), and energy dispersive X-ray (EDX). The XRD and DLS analysis revealed the hexagonal nanocrystalline nature of ZnO NPs. The FTIR results displayed multiple fictional groups and UV results confirmed its optical properties. The average size of the NPs was 68.3 nm with a band gap of 2.71 eV. The SEM images divulge a clover leaf shape of ZnO NPs. The EDX spectrum revealed the presence of zinc and oxygen. The prepared NPs showed excellent biomedical application. The highest antileishmanial activity was 68%, anti-inflammatory activity was 78%, total antioxidant capacity (TAC) was 79.1%, antibacterial potential (ZOI) 22.1 mm, and highest growth inhibition of 85 ± 2.1% against A. rabiei. The adsorption efficiency of 85.3% within 120 min was obtained. Conclusively ZnO NPs have shown potential biomedical and environmental applications and ought to be the more investigated to enhance their practical use.

Key words: ZnO Nanoparticles, green synthesis, *Acasia modesta*, biological applications, environmental application.

INTRODUCTION

Nanotechnology is a vibrant and quick-growing field having acquaintance from multiple fields like natural sciences, biological sciences and other sciences. The term “nano” refers to very small (1–100 nm). The inimitable characteristics of nanoparticles (NPs), including smaller size larger surface area, variation in structural properties, and many other physicochemical properties make them better candidates to use in fields of medicine, environment, and agriculture (Bhilkar et al. 2023). Due to their diverse nature, nanoparticles are categorized as nanotubes, nanocrystals, nanoflower, nanowires, etc (Perwez et al. 2023). The most common nanoparticles and metal oxide nanoparticles are silver (Ravindran et al. 2013), zinc oxide (Khalafi et al. 2019), titanium oxide (Chanani et al. 2023) nickel oxide (Buazar et al. 2023), and calcium oxide (Rahimi et al. 2023), and iron oxide (Safari et al. 2020). These are most stable under extreme conditions such as high temperature and pressures and some of them are considered nontoxic and even contain mineral elements essential to human health.

Hence, bio-nanocomposites have been noted as a promising alternative in food packaging market.

Different biological routes have been used for the synthesis of nanoparticles previously. The organic procedure of preparation comprises the use of bacteria (Pantidos & Horsfall 2014), fungi (Siddiqi & Husen 2016), algae (Moavi et al. 2021) and plants (Wang et al. 2023). The preparation of bio-inspired NPs has gotten huge attention in recent times in a progressive way because of the great benefits in terms of eco-friendly and cost-effective nature. The NPs prepared using plants sources have good stability and the synthesis rate is faster as compared to that of microorganisms (Khalafi et al. 2019). It is worth mention that the plant-assisted bioreduction strategy for NiO NPs fabrication has received glob attention as a renewable and sustainable supplier (Buazar et al. 2016, 2023, Koopi & Buazar 2018).

Among different types of nanoparticles, metal-source NPs are advantageous due to their non-toxic behavior to the biotic and a-biotic factors of the environment (Liaqat et al. 2024). The phyto based synthesis of ZnO nanoparticles is very useful as the biological molecules existing in the leaves extract work as effectual capping mediators thus showing a critical character in NPs preparation. The capping elements seem to stabilize the nanoparticles through multiple traditions such as steric, electrostatic stabilization, and hydration interaction. The stabilization of NPs directly related to the functions of nanoparticles in different biological assays (Pantidos & Horsfall 2014). Metal oxide nanoparticles (MO NPs) also have a vital part in multiple fields especially in nanomedicine, environment, and farming (Campaña et al. 2023, Alavi & Nokhodchi 2021).

Amongst various NPs, zinc oxide NPs are excessively used for their harmless behavior and possess excellent physio-chemical and biological characteristics (Elmaghraby et al. 2024). ZnO NPs have multiple exciting properties including electric conductivity, optical transparency, piezoelectricity, wide availability, non-toxicity, stability, and cost effectivity (Sabouri et al. 2022, Zafar & Iqbal 2024). They have applications in multiple fields, such as catalysis, solar batteries, varnishes, plastics, paints, pharmaceutical items, and optoelectronic apparatuses (Tortella et al. 2023). It commonly works as a protective agent in sunscreen products and cosmetics to the efficiency of filtering ultraviolet irradiations (Guerrini et al. 2018). ZnO NPs are also better considered for their antifungal and antibacterial activity (Ali et al. 2018). ZnO NPs can interact with phospholipids bi-layer in the cell membrane and alter the structural configuration of the cell membrane, which causes to forfeiture of membrane function, veracity, and lastly to microbial death (Nadhiya et al. 2023, Neolaka et al. 2022). Now-a-days, zinc oxide nanoparticles are also employed as exterior antimicrobial agents for microbial growth inhibition in, textile items, mouth sprays, lotion ointments, and food packages (Eleryan et al. 2023a).

Globally, environmental destruction issues are presently instigating pollution and impairing natural resources owing to the massive upsurge in the human population and the evolution of industrial activities (Raha & Ahmaruzzaman 2022). Heavy metals are the natural component of the Soil. The amount of HMs in soil, and different water sources is surpassing the tolerable range leading to a veiled threat to the biotic community (Verma et al. 2021). Among HMs Cadmium (Cd) is considered a very lethal environmental contaminant because they harmful to plants, animals, and humans. Different conventional processes are used to get rid of HMs from polluted water bodies (Theerthagiri et al. 2019, Pillai et al. 2020, Li et al. 2023). However, these traditional procedures have certain limitations such as its cots-intensive, requiring large amounts of energy and released secondary pollutants (Murali et al. 2023, El-Nemr et al. 2022). Hence, environment friendly and low-cost organic materials are required for the amputation of HMs. Currently, nano-bioremediation has been established as an auspicious technique for declining HMs pollution in the ecosystem and advanced nanoparticles have increased substantial interest. ZnO NPs have been used frequently to eliminate heavy metals from contaminated water for their small size, and biocompatible nature (Bujang et al. 2020, Nelson 2018).

Medicinal plants are playing a key part in the treatment of several disorders (Onyancha et al. 2022). Plants are rich in phyto-active compounds; hence they are used in the production of multiple types of medicines across the globe for the management of multiple health problems (Neolaka et al. 2023). *Acacia modesta* usually known as Phulai, is commonly applied for, fuel, medicine and wood production in Pakistan (Eldeeb et al. 2024b). Previous studies proved that *A. modesta* has pharmacological abilities to treat skeleto-muscular and stomach problems (Bairagi & Kamali 2023, Dhiman & Kondal 2021, Ozkan et al. 2016). Leaves extract have shown analgesic, anti-platelet, anti-hyperglycemic activity, and anti-oxidant potential (Koparde et al. 2017, Hashim et al. 2022, Khanum et al. 2022, Ghauri et al. 2023). The hepatoprotective properties of the *Acacia modesta* bark extracts and anti-microbial potential also investigated (Saleem et al. 2018, Arbab et al. 2015, Bukhari et al. 2010).

To the best of our knowledge, this is the first study to prepare ZnO nanoparticles from the active phytochemical constituents of the *A. modesta* extract. Additionally, our study explores the unique optical and catalytic properties of these nanoparticles, revealing novel applications in both biomedical and environmental remediation. Furthermore, we investigate the interactions between nanoparticles and biological systems at the molecular level, shedding new light on their potential toxicity and therapeutic efficacy. The scope of our work spans multiple disciplines, including materials science, chemistry, biology, and environmental science. We systematically characterize the physicochemical properties of the synthesized nanoparticles using advanced spectroscopic and microscopy techniques. Moreover, we assess their performance in various applications such as drug delivery, and pollutant removal. These advancements have the potential to revolutionize fields such as healthcare, environmental monitoring, and energy production. Overall, our work not only expands the frontiers of scientific understanding but also holds promise for addressing pressing societal challenges through innovative nanoparticle solutions.

MATERIALS AND METHODS

Chemicals

Zinc Nitrate Hexahydrate {$Zn(NO_3)_2 \cdot 6H_2O$}, sodium hydroxide (NaOH), Dimethylsulfoxide (DMSO), DPPH (2, 2-diphenyl-1-picryl hydrazyl), methanol (CH3OH), Whatman filter paper no.1, amphotericin-B, ascorbic acid, bovine serum albumin (BSA), Tris buffer, Diclofenac sodium. All the chemicals were taken from Sigma Aldrich utilized without further purification. The glass apparatus used for experiment were appropriately splashed, and autoclaved.

Collection and processing of plant samples

A. modesta was collected from the Lakki Marwat, Khyber Pakhtunkhwa (KP), Pakistan. The wild collected *A. modesta* plant was collected following standard guidelines. The collected plant was identified by Dr. Moona Nazish submitted to the herbarium with a voucher specimen (Ak-2226).

Plant extract preparation

For the synthesis of the extract, leaves of *A. modesta* were collected and splashed thoroughly in running tap water to eradicate dust and other debris attached to the leaves surface. Then all the cleaned leaves were shade dried and converted to powder form via a grinder. The prepared powder was blended in a 250 ml of double distilled water (DDW), boiled for 15 min, and then incubated for 15 min in a water bath at 80^{o} C. The obtained mixture was cooled down and filtered through muslin fabric followed by filtered via Whatman filter paper no. 1 and placed at 4^{o} C for one week.

Qualitative analysis on phytochemical constituents of plant extracts

Qualitative phytochemical analysis encompasses the process of identifying and detecting various constituents within plant samples. Its objective is to determine the existence or non-existence of specific phytochemicals or classes of compounds, including alkaloids, flavonoids, terpenoids, phenolic compounds, proteins, saponins, glycosides, steroids, anthocyanins, oils, quinones, and tannins. The Mayer's test was for alkaloids, Benedict's test for glycosides, Millon's test for protein, Alkaline reagent test for flavonoids, Salkowski's test for steroids, terpenoids, Copper acetate test for phenolic compounds were used for the identification of biomolecules. The Qualitative phytochemical analysis was performed through the previously published protocol (Ullah et al. 2018, Abid et al. 2020).

Preparation of ZnO NPs

For this purpose, 1 mM solution of Zinc Nitrate Hexahydrate {Zn $(NO_3)_2{\cdot}6H_2O$} was synthesized. Plant extract prepared in distal water and Zinc Nitrate Hexahydrate {Zn $(NO_3)_2{\cdot}6H_2O$} solution was blended in a ratio of 1:2. The obtained mixture was heat up and continuously stirred using hot plate for 90 mins at 80^{o} C. The change in color confirmed the process of reduction. The then obtained mixture was centrifuged for 35 min at 6,000 rpm and laved with DDW (Sharmila et al. 2019). To attained desiccated powder, the pallet was located in an incubator for 4 hr at 100^{o} C. Dried matter was calcinated for 2 hr at 600^{o} C to get crystalline ZnO NPs. These synthesized nanoparticles were characterized through various techniques prior to their application in various biological techniques.

Characterization of ZnO NPs

UV-Vis Spectroscopy

The optical properties of the bio-inspired ZnO NPs in a colloidal suspension was assessed through ultraviolet-visible spectroscopy. The bandgap energies of the nanocomposites were calculated from diffuse reflectance UV–Vis The bandgap of synthesized nanocomposite was calculated by the following equation 1:

$$(\alpha h\upsilon)2 = K(h\upsilon - Eg) \quad (1)$$

Where α shows absorption coefficient, hυ represents photon energy (eV), K indicates absorption index and Eg is bandgap energy.

XRD analysis

X-ray diffractometer was applied for the confirmation of the crystalline behavior of bio-fabricated NPs. The results of the XRD were explained based of their atomic structure, angles and planes where the diffraction takes place in solid sample samples. The Scherrer's formula was used to calculate the nanoparticles' sizes as follows:

$$D = 0.9\lambda / \beta\cos\theta \quad (2)$$

Where D shows average crystalline size, K denotes shape factor, ʎ represetn X-Ray wavelength, β shows full width half maximum and θ shows diffraction angle.

FTIR spectroscopy

FTIR spectroscopy of ZnO NPs was achieved for the purpose to explore structural properties and confirmation of different functional groups. For FTIR the NPs sample was synthesized using to the previous standard procedure (Kamal et al. 2022) and then measured in FTIR spectroscope (Housseiny & Gomaa 2019).

SEM and EDX analysis

SEM was performed to study the topographical characteristics of the synthesized NPs. SEM was achieved following the standard protocol Fadwa et al. 2021. The EDX spectroscopy was done to explore the elemental alignment, their purity percentage and confirmation of the existence of oxygen and zinc. EDS was performed to the analyze the qualitative analysis of the elements in a NPs in scattered pattern. The EDS was performed following the former published protocol.

DLS analysis

DLS was executed to determine the polydispersity index (PDI), zeta potential (ZP), and hydrodynamic size distribution. DLS analysis was performed using the previous protocol (Fadwa et al. 2021).

Biological Applications of the ZnO NPs

Antileishmanial assay

To assess the antiparasitic efficacy of the bioinspired ZnO nanoparticles they were applied against *Leishmania tropica* promastigotes, using former standard protocol. Each assay tubes contain 5 mL of medium with $1x10^5$ parasites/mL of *L. tropica* promastigotes. Then, 5 mL of every dose (20, 40, 80, and 160 μg/ml) of the prepared nanoparticles was transferred to each tube and incubated at 28 °C. During this activity, amphotericin-B and DMSO were used as a positive and negative control correspondingly. Parasites count was measured using hemocytometer in all treatments (NPs and control samples) at various times breaks from 24 to 96 h and the percentage (%) inhibition was calculated via the subsequent formula (3):

$$(\%)\ \text{Inhibition} = 100 \times \text{Absampl} / \text{Abcontrol} \quad (3)$$

In this, Absample shows the absorbance of the ZnO treated sample and Abcontrol mentions to the control sample.

Anti-inflammatory activity

Anti-inflammatory assay of plant extract and ZnO NPs were executed following the aforementioned procedure (Muhammad et al. 2019). Reaction cocktail bovine serum albumin (BSA) was synthesized in saline Tris buffer (PH 6.8). BSA (900 µl) was added with 100 µl of different doses of ZnO (50, 100, 200, 400 and 800 µg/ml). Diclofenac sodium (µg/ml) was applied as a standard. The reaction was performed following previous protocol and final absorbance was measured at 580 nm (Arora et al. 2014). The activity was executed thrice, and protein denaturation was calculated through the following equation (4):

$$\text{Protein inhibition} = 100 \times (\text{Abs.}(\text{control}) - \text{Abs.}(\text{sample}) / \text{Abs.}(\text{control}) \quad (4)$$

Antioxidant activities

Various antioxidant activities including total antioxidant capacity (TAC), total reducing power (TRP), and DPPH-free radical scavenging (FRSA) were performed to analyze the antioxidant potential of ZnO NPs at different doses from 50–200 mg/mL (El-Belely et al. 2021).

-TAC Determination

To determine TAC, 100 µL of each concentration of ZnO NPs was added separately with reagent using previous published protocol (Kaushik et al. 2019). AA and DMSO was used as a positive and negative control.

-TRP determination

For this purpose, we used the potassium-ferricyanide method (Bukhari et al. 2010). DMSO and AA was applied as a negative and positive control. Absorbance of each treatment was measured 580 nm.

-FRSA determination

The FRSA was determined using the previously standard protocol (Kaushik et al. 2019). % inhibition was calculated through subsequent formula (5):

$$(\%)\ \text{FRSA} = 100 \times \text{Ab NPs sample} / \text{Abs Control} \quad (5)$$

Evaluation of the Antimicrobial Activities

-Antifungal Activity

To estimate the antifungal efficacy, the prepared ZnO NPs was applied against *Ascochyta rabiei* was performed following the previous standard protocol with a little modification (Emami-Karvani & Chehrazi 2011). The preserved *A. rabiei* was freshed on potato dextrose agar (PDA) media prior to the activity for 7 days at 25 ± 1 °C. PDA media was treated with different ZnO NPs doses (0.5, 0.75, and 1 mg/mL). The 4 mm disc of *A. rabiei* was located in the middle of ZnO NPs treated PDA Petri plates. PDA lacking NPS used as a positive control. The treated plates were placed in incubator for one week at 25 ± 1, and the antifungal potential was calculated using the following formula:

$$\text{Growth Inhibition } \% = 100 \times (C - T) / C \quad (6)$$

Where C refers to the fungus growth in the control plate, and T denotes to the fungus growth in a nanoparticle amended plate.

-Antibacterial activity

The Antibacterial potential was determined against *E. coli* through agar well diffusion method (Emami-Karvani & Chehrazi 2011). The antibacterial activity was performed at different concentration (5, 10 and 20 mg/mL) according to the previous standard protocol (Emami-Karvani & Chehrazi 2011). A positive control using an antibiotic was employed, and a negative control using less than 1% Dimethylsulfoxide (DMSO). After that, plates were kept in an incubator for 24 hours at 30°C. After that, we measured the inhibition zone in millimeters (mm).

Environmental application

ZnO NPs against metals adsorption

Adsorption activity was executed consuming ZnO NPs against Cd metal. ZnO NPs (1g) was liquified in 100 ml of water having Cd (dosage of 100 mg/L) in flask (250 ml). In this study, the role of various factors like initial concentration of Cd (20-120 mg/l) and time duration (5-120 minutes) on the Cd adsorption were investigated. The prepared mixture of Cd and ZnO NPs was stirred at 120 rpm with the help of a shaker for a fixed time at 25^{o} C. The prepared mixture was filtered and Initial and final Cd concentration were measured in the filtrate through Atomic Adsorption Spectrometer. The adsorption capacity (mg/g) and the adsorption efficiency (%) of the ZnO NPs were calculated following the formula 7 and 8.

$$q_e = \frac{(C_i - C_e)}{W} \times V \quad (7)$$

$$P = \frac{(C_i - C_e)}{C_i} \times 100 \quad (8)$$

C_e refer to the equilibrium Cd concentration, C_i represent the initial concentration of Cd, W denotes the ZnO NPs weight and V denotes volume of the mixture.

Statistical Analysis

All the samples were taken in triplicates and SPSS, version 16.0 https://www.ibm.com/spss and OriginPro9 were used to assess it statistically.

RESULTS AND DISCUSSION

Qualitative analysis of phytochemical

Qualitative assessment of extracts was accepted by analyzing phytochemical compounds that were present in different parts of plants Table I. The qualitative analysis of distilled water extracts of *A. modesta* was carried out to detect the secondary metabolites like alkaloids, quinones, terpenoids, flavonoids, phenolic compounds, glycosides, saponins, steroids, anthocyanins, fats and oils, and tannins. These whole results are depicted in Table I. There is a small variation in Qualitative phytochemical constituents were identified by previous researcher worked on *A. modesta* (Niaz et al. 2023, Buazar et al. 2016), which may be due to the nature of the extract and plant physiological responses to their environment.

Characterization of NPs

UV-Visible Spectroscopy

The effective production of nanoparticles was revealed through surface plasmon resonance measurements at a wavelength of 225nm in plant extract (Figure 1a) and 350 nm in ZnO NPs (Figure 1b). The cell-free culture's color shifted from golden to creamy which gives the first indications of nanoparticles synthesis which might be due to the secondary metabolites in the cell-free filtrate reduced zinc acetate to produce ZnO NPs (Torfi-Zadegan et al. 2023). The prepared composite showed band gap values of nearly 2.71 eV calculated from the VU data. It has previously been shown that zinc oxide absorbs at a comparable peak at 340 nm (Chikkanna et al. 2019). A little difference in the peak may be due to the precursor material.

Table I. Qualitative phytochemical study of extracts of *Acasia modesta*.

Sr. No	Secondary Metabolites	AMWD
1	Phenols	+ ++
2	Flavonoids	++
3	Alkaloids	++
4	Terpenoids	+
5	Saponins	-
6	Tannins	-
7	Steroids	++
	Glycosides	+ +
9	Quinones	+
10	Anthocynins	++
11	Fats and oils	+
12	Protein	++

Key: +++= refers to the abundance, ++=refers to moderate amount, +=refers to small amount, - = refers to complete absence of phytoconstituents in *A. modesta* distilled water extract.

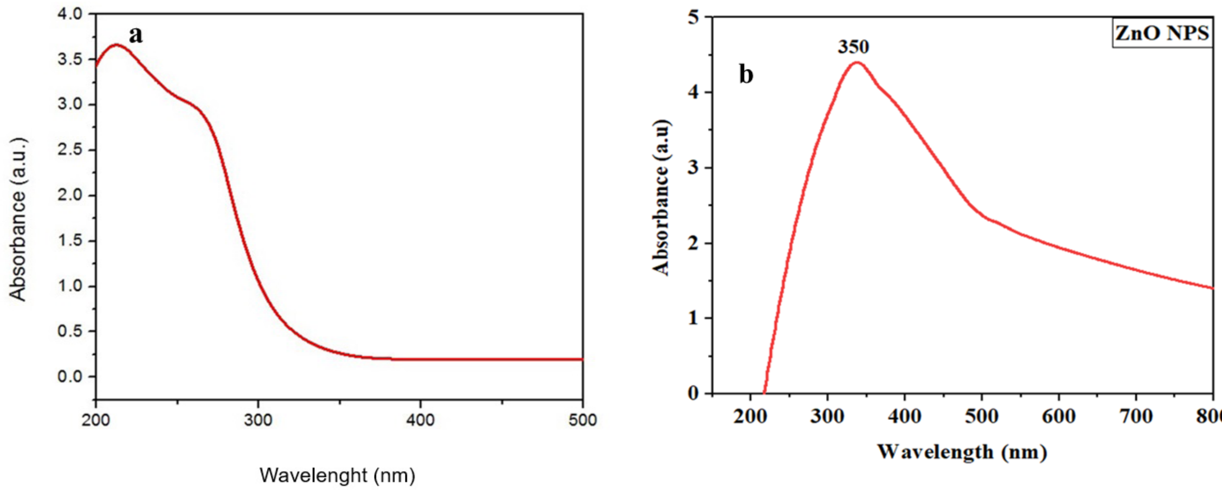

Figure 1. **UV of *A. modesta* extract (a) and ZnO nanoparticles (b).**

XRD Spectroscopy

The XRD configuration of bio-fabricated ZnO nanoparticles displayed seven visible peaks from 10° to 70°. The peak at 32.5°, 34.1°, 37.5°, 48.1°, 56.3°, 63.5°, and 67.2°, respectively (Figure 2). The different peaks indicate different planes 100, 002, 101, 102, 110, 103, 200, and 201.This illustration was indexed crystallographic properties of hexagonal structure of zinc oxide NPs, ensuing and all these peaks were according with the JCPDS no. (036–1451). By means of Debye–Scherer's equation, the average size of the particle was obtained as 68.3 nm. Similar results were reported by (Elumalai & Velmurugan 2015). The strongest peak of ZnO NPs, formed in *A. modesta* extract, confirmed of their highly crystalline nature. The XRD study for zinc oxide nanoparticles is parallel with earlier studies (Jiang et al. 2009). It was investigated previously that the crystalline nature of the NPs plays a pivotal role in their efficacy (Dubey et al. 2013). It has been also observed in earlier studies that Crystalline NPs exhibit excellent antifungal activity via the demolition of the hyphal wall (De Jesus Oliveira et al. 2019).

FTIR analysis

FTIR spectroscopy of bio-fabricated ZnO NPs is presented in Figure 3. FTIR study was assessed for the purpose to distinguish the different functional groups which participated in the development and capping of ZnO. FTIR analysis depicted seven peaks extending from 500-3500^{cm-1}. Peaks observed at 628.31 $^{cm-1}$ and 833.52 $^{cm-1}$ verified durable stretching of C-Br and medium bending of C=C, individually. The peak 1222.51 cm^{-1} refers to medium C-N stretching of amine found in the *A. modesta* extract, gives confirmation of protein in a prepared ZnO NPs, comes from the reduction process. The peaks perceived at 1417.1 cm^{-1} and 1609.9 cm^{-1} refers to medium O-H bending and strong C=O stretching correspondingly. A peak found at 2167.65 cm^{-1} denotes strong N=C=N stretching. A prominent peak detected at 3255.1 cm^{-1} indicated the occurrence of strong and broad O-H starching of carboxylic acid. Almost similar results were reported by several scientist worked on bio-fabricated NPs (Singh et al. 2020, Sumaira et al. 2018).

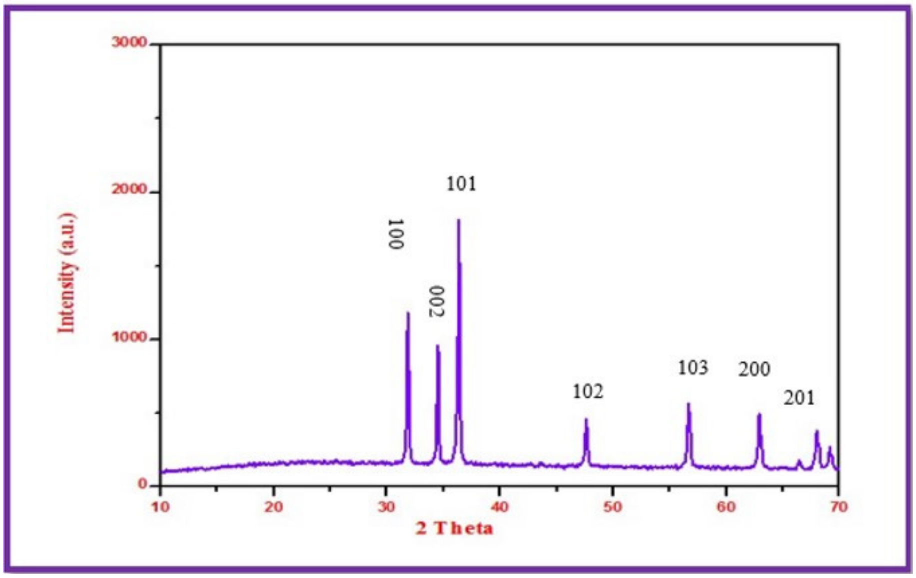

Figure 2. **XRD pattern of plant mediated zinc oxide nanoparticles.**

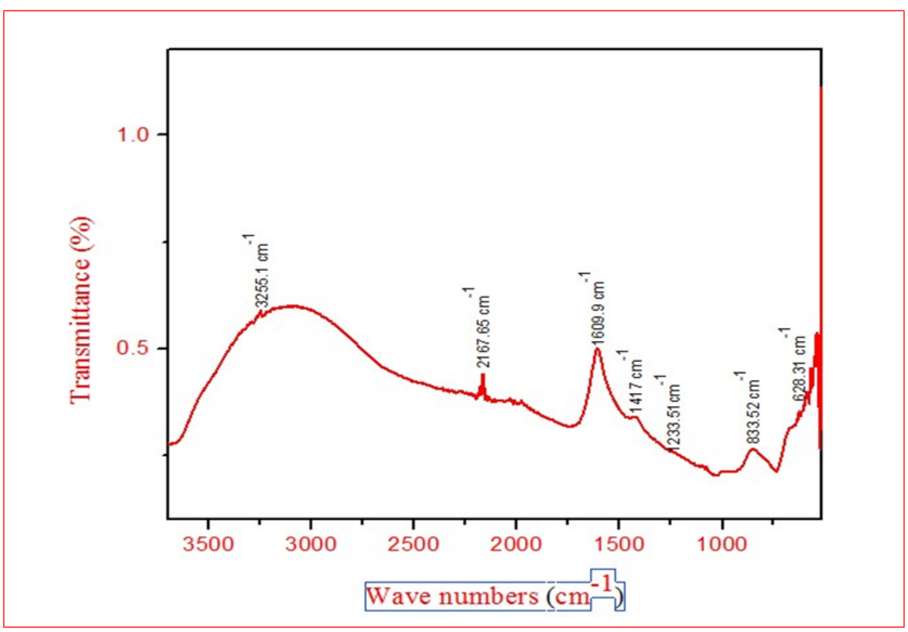

Figure 3. **FTIR spectroscopy of *A. modesta* mediated ZnO nanoparticles.**

SEM and EDX analysis

SEM was performed to examine the texture and distribution of the ZnO NPs. The morphological characteristics of was examined via SEM analysis (Figure 4a). SEM micrograph depicted that ZnO NPs are present in white cottony appearance in clover leaf shape distributed homogeneously. A uniform distribution of NPs provides us better information on a morphological analysis and estimated nanoparticles size (Sumaira et al. 2018). All these results of SEM are algin to the previous work (Abbasi et al. 2017).

The elemental composition and configuration of the prepared nanoparticles was assessed by EDX spectrum. The EDX analysis of ZnO nanoparticles confirmed the presence of zinc (Zn) and oxygen (O), which approved the confirmation of ZnO-NPs, along with some other elements including carbon (20.8%) and sodium

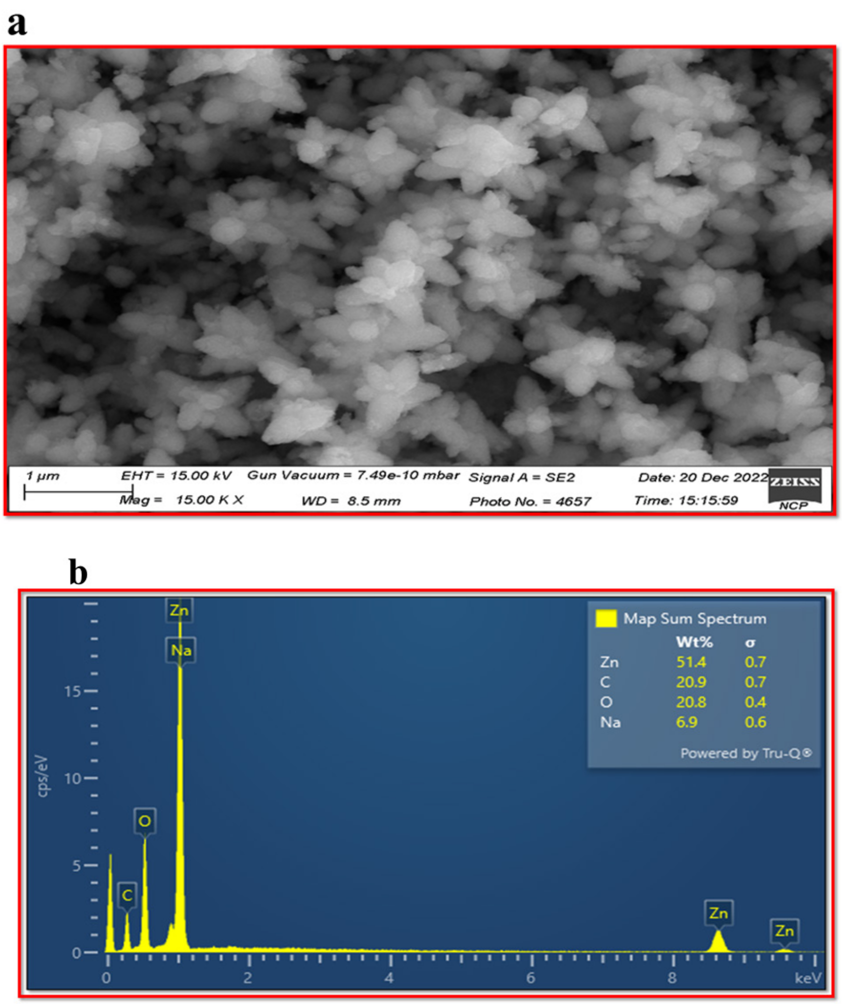

Figure 4. SEM images (a) and EDX analysis (b) of plant mediated ZnO NPs.

(6.9%) which may come from the plant extract (Figure 4b). The high percentage of carbon may be due to organic based (plant extract) synthesis of the nanoparticles. The weight percentage of Zn and O examined are 51.4, and 20.8% separately. All these findings are consistent with the former study conducted by the nanotechnologists (Agarwal & Shanmugam 2020) Through EDS element distribution was also investigated. The image (Figure 5) displays that nanoparticles are well disseminated on the carbon foil. The (Figure 5) could deliver the element distribution after two minutes. These results demonstrate the capability of the EDS detector for quick identification of the ZnO nanoparticles.

Zeta potential (ZP)

ZP, study was applied for the confirmation of charge on the periphery of ZnO NPs that indicates the electrical nature of ZnO nanoparticles. The parameters for ZP determination as revealed in Table II. The electrostatic revulsion potential between neighbor particles in a suspension is revealed via the ZP value. Nanoparticles suspension with a Zeta potential value of +8.50 to −8.50 mV indicates the high stability of nanoparticles (Sarkar et al. 2014). The ZP analysis confirmed that the surface electrical charge of ZnO NPs was −8.95 mV (Supplementary Material - Figure S1a). Figure S1b displays the intensity of light scattering and size. The zeta average size of ZnO NPs was 663.5 d·nm with a PDI of 0.523.

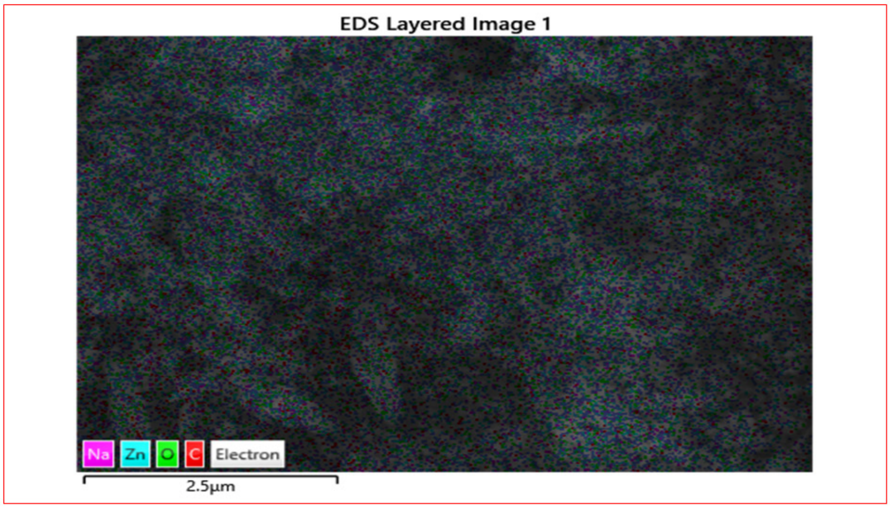

Figure 5. EDS analysis of ZnO nanoparticles agglomerates display in different colors showing elemental distributions.

Table II. Parameter for ZP measurements of ZnO NPs.

Buffer Name	Water
Dispersant RI	1.330
Temperature (°C)	25
Viscosity (cP)	0.887
Dispersant Dielectric constant	78.5
Temperature (°C)	25
Zeta Runs	12
Count Rate (kcps)	50.7
Measurement Position (mm)	4.50
Cell Description	Zeta dip cel
Attenuator	11

In green algae and plant-based NPs, ZP was examined to be negative (-) (Janaki et al. 2015). This negative value is owing to the secondary metabolite's attachment to the NPs surface (Ul-Haq et al. 2012). In this study, the negative ZP of plant mediated ZnO NPs might attributable to the chemical constituents and reducing agents along with the NPs size.

Antileishmanial Activity

To analyze the antileishmanial activity, the prepared NPs was tested as an antileishmanial agent in various concentration for a time duration of 96 hr as and their efficiency were described in Figure S2. To assess the antileishmanial potential, promastigotes numbers were counted in in NPs amended and control groups at diverse time duration including 24 h, 48 h, 72h, and 96 h. The antileishmanial potential was enhanced with the enhancing in the amount of ZnO nanoparticles. The antileishmanial potential was examined to be 20%, 30%, 32% and 37% at 20, 40, 80, and 160 µg/ml, of 24 h of incubation. Afterward 48 hr of incubation, the ZnO NPs showed antileishmanial efficacy of 27%, 32%, 39%, and 48% at at 20, 40, 80, and 160 µg/ml, correspondingly. Afterward 72 hr of incubation, the cells number was further declined in the ZnO amended samples which was 35% at 20 µg/ml, 48% at 40 µg/ml, 60 % at 80 µg/ml and 68% at 160 µg/ml. Later on, a little reduction in antileishmanial potential was observed. This decrease in the potential may be owing to the enervation of ROS from the ZnO nanoparticles. Additionally, MO-NPs have the potential of produce reactive ions, which causing pores in the pathogen wall and affects structural features of the membrane, and leakage of intracellular material and finally leads to pathogen destroying. The substantial findings of our study confirmed that ZnO might be an auspicious tool for leishmaniasis treatment which is in consistence with to earlier studies (Janaki et al. 2015). Table III shows the antileishmanial activity potential of the prepared nanoparticles to the previous work.

Anti-Inflammatory potential

The synthesized ZnO NPs and the plant extract of *A. modesta* expressed parallel anti-inflammatory efficacy in comparision to standard chemical analgesic (Diclofenac sodium). The plant mediated NPs behaved as an efficient anti-inflammatory agent (Figure S3). The ZnO NPs and *A. modesta* extract both help to reduce *in vitro* inflammation. The maximum anti-Inflammatory potential of ZnO was determined 45% at 50 µg/mL concentration, 58% at 100 µg/mL, 65% at 200 µg/mL, 70% at concentration of 400 µg/mL, and 78% at 800 µg/mL. Further increase in concentration i.e above 800 µg/mL does not show more than 78% inhibition. Comparative study exposed that the ZnO NPs is a better anti-inflammatory agent as compared to plant extract. Previous studies also that plant-based NPs has better anti-inflammatory activity as compared physically and chemically synthesized NPs and plant extract inflammatory medicine (Abdulazeem et al. 2023). Table IV shows the anti-Inflammatory potential of the prepared nanoparticles to the previous work.

Antioxidant assay

Antioxidant potential of ZnO nanoparticles were assessed at various doses (50–00 µg/ml).

Table III. Comparative Antileishmanial activity of the prepared NPs to that of previous work.

S.No	Type of NPs	Antileishmanial activity (%)	Previous work
1.	ZnO-NPs	76.5	Ul-Haq et al. 2012
2.	ZnO-NPs	65.731	Khan et al. 2023
3.	ZnO-NPs	77%	Saleh et al. 2024
4.	ZnO-NPs	77%	Abbasi et al. 2017
5.	ZnO-NPs	58%	Nazir et al. 2019
6.	ZnO-NPs	68%	Current work

Table IV. Comparative anti-inflammatory activity of the prepared NPs to that of previous work.

S.No	Type of NPs	Anti-inflammatory (%)	Reference
1	ZnO-NPs	61%	Nagajyothi et al. 2015
2	ZnO-NPs	52%	Devi et al. 2020
3	ZnO-NPs	57%	Rangeela et al. 2023
4	ZnO-NPs	64%	Minhas et al. 2019
5	ZnO-NPs	78%	Current work

Antioxidant potential including TAC, TRP, and DPPH free radical scavenging are depicted in Figure S4. The maximum result for TAC in terms of AA per mg equivalents was measured to be 79.1% for ZnO NPs at 200 µg/mL. TAC confirmed the scavenging power of the ZnO NPs to ROS. TRP was performed to study further about the presence of antioxidant species related to ZnO NPs. This activity was designed to assess the reductones that play vital role in the antioxidant activities (Siripireddy & Mandal 2017). The highest TRP (65.2%) was attained at 200 µg/mL. The maximum DPPH radical scavenging capacity was 68.7% for ZnO NPs at 2000 µg/mL. These findings are parallel to the previous reported work on bioinspired ZnO NPs (Bhosale et al. 2021, Arif et al. 2023).

In vitro antibacterial assay

In the current study, *E. coli* was utilized to gauge the effectiveness of the prepared nanoparticles. The antibacterial potential of the prepared NPs is shown Figure S5a-d. In addition, 100 µl of each nanoparticle concentration (5, 10 and 20 mg/mL) was used to treat *E. coli* and the inhibition zone (ZOI) was measured. The maximum ZOI was 22.1 mm at 20 mg/mL, followed by followed by 17.4 mm at10 mg/mL and 10 .2 mm at 5 mg/mL (Figure S5 a-d). The comparative antibacterial activity of the prepared nanoparticles with previously work is described in Table V. In antibacterial activity, membrane is the main protective impediments to bacterial fight from the outer agent. The greater surface to volume ratio ZnO nanoparticles, leading to the creation of more reactive ions. Zinc oxide nanoparticles depict better anti-microbial action as compared to soluble Zn compounds like Zinc chloride because of their active targeting potential, the ability for its generation in the cell membrane and thus, disruption of cell membrane integrity and ROS generation capacity which further aids in protein, lipid, and DNA denaturation (Muthuvel et al. 2020). Scientists have Reported that the effect of ZnO NP on *C. jejuni* bacterial culture was bactericidal and not bacteriostatic as they observed no recovery of the bacterial cell (Al Rugaie et al. 2022). Cell membrane blebbing and leakage is considered as one of the main mechanisms of inhibitory effect of nanoparticles on bacteria. Internalization of ZnO NP leads to integrity loss of phospholipid bilayer and leakage of intracellular components like lipopolysaccharide and ATP out of the cell ultimately leading to cell death. E. Brayner et al. reported cell membrane integrity loss as the major cause of bactericidal effect of ZnO NP on *E. coli* cell (Zhang et al. 2023). Nanoparticle attachment and inclusion alter the resting membrane potential of

Table V. Comparative antibacterial activity of the prepared NPs to that of previous work.

S.No	Type of NPs/Plant extract	Antibacterial activity against *E. coli* (mm)	Previous work
1	ZnO-NPs	15.8 mm	Yin et al. 2023
2	ZnO-NPs	7 mm	Summer et al. 2023
3	ZnO-NPs	20.6 ± 0.50 mm	Aldeen et al. 2022
4	ZnO-NPs	17mm	Nguyen et al. 2019
5	*Toddalia asiatica* extract	4.02 ± 0.03	Elumalai & Velmurugan 2015
6	*Aloe secundiflora* extract	12.55 ± 0.11	El-Khawaga et al. 2023
7	*Salvia officinalis* extract	13.67 ± 1.08	Odongo et al. 2023
8	ZnO-NPs	22.1 mm	Current work

the cell membrane and induces depolarization of cell membrane by blocking K^+ ion channel present in the cell membrane (Dadi et al. 2019). Zeta potential measurement i.e. measurement of charge of the bacterial surface can be used as a marker for the estimation of membrane damage (Yassin et al. 2023). A large number of proteins upregulate its expression upon exposure to zinc oxide nanoparticle leading to lipid peroxidation (Mthana et al. 2022). Nanoparticles interact with membrane proteins and inactivate them decreasing membrane permeability and causing cell death. Zn+ that is released by ZnO NPs may induce a conformational change in the enzyme and caused the distortion of the active site of enzyme and block the activity of bacterial cell which leads the main cause of bacterial inhibition (Modi et al. 2023). In our results the antibacterial activity was enhanced with ZnO concentration which are aligned to the earlier studies (Yin et al. 2023).

Antifungal assay

The antifungal potential of ZnO NPs using PDA containing petri plates were studied (Supplementary Figure S6a-d). The highest growth inhibition (85 ± 2.1%) was observed at a 1 mg/mL, followed by a 0.75 mg/mL dosage rate (66± 1.7%) and 0.50 mg/mL (56.77 ± 0.5). Additional rising in ZnO NPs dosage did not increase further inhibition, because increase in dosage leads to clump development of the NPs. Nanoparticles have a smaller size but greater surface area, makes the NPs more reactive and stable (Takci et al. 2023). The physiochemical properties, like size, Brownian movement, and surface charge, might also have an influence on the accretion. From the last decade, mycologists have efficiently used ZnO NPs to retard the growth of different fungal pathogens, including *Fusarium graminearum*, *Candida albicans*, and *Aspergillus niger* (Hosseini et al. 2023). Previous works suggest that antifungal potential of MO-NPs is because of reactive oxygen species production. It is also reported that nano material can interact powerfully to the microbial wall and thus shows antimicrobial activity (Eldeeb et al. 2024a). Metal oxide NPs creates pores in the wall and disturb the structure of the plasma membrane such as the depolarization, disturbs the fluidity and permeability of the membrane, consequently, arrive the cytoplasm without any hindrance, and leads to the DNA, RNA, and proteins disruption (Kalpana et al. 2018). This disruption causes the seepage of various materials like RNA, proteins, DNA and enzymes resulting in cell demise. The whole mechanism of antimicrobial potential is depicted in Figure S7.

Assessment of the ZnO in Cd adsorption

Effect of connection time and adsorption kinetics

Contact time is a key element in adsorption of metal by the nanosorbent in the waste water treatment. Adsorption indicates the accumulation of sorbates or solutes at the surface or interface of solid substrates (Housseiny & Gomaa 2019, Eleryan et al. 2022). Therefore, an inclusive study was conducted to explore the impact of contact time on the process of adsorption from 5 to 120 minutes. In the start the rate of adsorption was high as depicted in the Figure S8 which may be due to the free reactive places on the surface of ZnO NPs (Aigbe et al. 2022, Kumari et al. 2015). With increasing in the contact time between Cd and ZnO, the adsorption efficacy greatly enhances due to the increased interaction between Cd and active sites. Highest adsorption was 85.3 mg/g contact time of at 120 mins. These results display that ZnO NPs have efficient adsorption ability and are excellent adsorbent in the Cd removal. Results of our study parallel with the former work conducted for the elimination of pollutants

(Okpara et al. 2020, Manzoor et al. 2016, Yang et al. 2019).

Kinetic study is basic approach to assess the efficacy against the environmental pollutants A detailed kinetics study was conducted on the adsorption Cd ions by applying different kinetic models, including the pseudo-first-order ($P^{1st}OK$), and pseudo-second-order kinetic models ($P^{2nd}OK$). As presented in Figure S9a, b.

Pseudo firs order kinetics depends upon the weak forces between the ZnO NPs and Cd mainly ruled by physisorption forces (Eleryan et al. 2023b). The linear form of ($P^{1st}OK$) is presented in formula (9)

$$\ln(qe - qt) = \ln qe - K1t \quad (9)$$

Where qt refers to the amount of Cd at a particular time, qe is the amount of Cd onto the adsorbent at equilibrium. The rate constant linked with $P^{1st}OK$ is designated by the parameter K_1 (1/min). This study comprises plotting log(qe-qt) against time (t) to obtained the rate constant and correlation coefficient for the $P^{1st}OK$.

$P^{2nd}OK$ model mainly controls chemisorption interaction (Hoseinzadeh et al. 2016). Following formula was applied mathematical representation of $P^{2nd}OK$.

$$\frac{t}{q_t} = \frac{1}{K_{2q_e^2}} + \frac{1}{q_e}t \quad (10)$$

The $P^{2nd}OK$ rate constant, represented as K_2, is calculated by drawing a plot t/qt versus (t).

Significantly greater correlation coefficient (R^2) was attained from the $P^{2nd}OK$ model to that of the other kinetic model gives confirmation the adsorption kinetics was administered via the $P^{2nd}OK$ model (Table VI). This designates that the rate-regulating phase in the Cd adsorption onto the ZnO NPs was chemisorption mechanisms. All these findings are consistent with previously published studies (Zhang 2014, Nguyen et al. 2019, Jain 2018).

Impact of initial metals concentrations on adsorption

Figure S10 shows the impact of the primary concentrations of metals ranging from 20 to 120 mg/L on the Cd adsorption onto ZnO NPs. It was concluded that Cd adsorption enhanced with rising initial concentration of Cd. The maximum Cd absorption (80.3 mg/g) was perceived at highest Cd concentration (120 mg/L). The results of the, increase in metals concentration, the adsorption efficiency of nanoparticles was enhanced all exactly parallel to previous study conducted on the adsorption by using organic based substances (Ameh 2023, Dubey al. 2016).

One of the aims of the current study was to apply Freundlich, and Langmuir, isotherm models on adsorption data. When the solid and liquid phases are in equilibrium, the Langmuir model depicts the distribution of metal ions and measures the creation of an adsorbate monolayer on the adsorbent's surface (Ho & McKay 1998). Equations designated below applied to determine the parameters required to elucidate these isotherm models:

Langmuir isotherm model

Table VI. Parameters of the $P^{1st}OK$, and $P^{2nd}OK$ kinetic models.

	$P^{1st}OK$			$P^{2nd}OK$		
Metals	**$qe_{(cal)}$ (mg/g)**	**K_1 (min^{-1})**	**R^2**	**$qe_{(cal)}$ (mg/g)**	**K_2 ($gmg^{-1}min^{-1}$)**	**R^2**
Cd	110.1375	-0.00117	0.9294	91.3242	0.000994	0.99668

$$\frac{1}{q_e} = \frac{1}{K_L \quad q_{max}} \cdot \frac{1}{C_e} + \frac{1}{q_{max}} \quad (11)$$

$$R_L = \frac{1}{1 + C_i \times K_L}$$

K_L denotes Langmuir constant, R_L shows separation factor, and qmax is the highest adsorption potential

Freundlich isotherm is appropriate for elaborating multilayer adsorption surfaces that show heterogeneity (Kaewsarn 2002). The equation 10 shows the Freundlich adsorption isotherm:

$$\text{Log}\, q_e = \text{Log}\, K_f + \frac{1}{n} \log C_e \quad (12)$$

K_f shows Freundlich's constant, and 1/n refers to intensity adsorption.

Plots of isotherm study are shown in Figure S11a, b and the conforming isotherm constants and correlation coefficients are described in Table VII. Attained findings show that the Langmuir model exhibit a remarkably greater R^2 value in comparison to that of isotherm model, suggesting its more suitable to the data of adsorption Cd and supporting the adsorbent surface homogeneity. Adsorption isotherm revealed a well-fitted Langmuir isotherm at high residual concentrations of Cd in the solution phase, indicating a declination of adsorbents active sites (Aigbe et al. 2022, Kumari et al. 2015). Highest adsorption potential of ZnO NPs was assessed with the Langmuir isotherm as 85.6898 mg/g. Moreover, the Freundlich model borne 1/n between 0 and 1 (0. 0.3013 for Cd). The Values of 1/n less unity gives confirmation of the efficient adsorption was done (Chanani et al 2023, Ho & McKay 1998). Results of the current study recommend that the adsorption process closely align to the Langmuir model.

CONCLUSIONS

Interest in green technology has increased because of the of non-toxic substances and development of effective, economical, and eco-friendly materials. This study intended on preparation of ZnO nanoparticles through the eco-friendly procedure using *A. modesta* leaf extract. The inspired ZnO NPs were efficaciously characterized through different microscopic and spectroscopic techniques and used in biomedical and environmental assays. The prepared NPs were assessed antioxidant activities, antibacterial, antifungal activity, anti-inflammatory and anti-leishmanial activity. Lastly, the ZnO NPs were assessed for the and environmental application against the Cd adsorption. ZnO NPs displayed the efficient potential to adsorb Cd. Inclusive, our study show that the bio-fabricated ZnO NPs may work as a true candidate for biological and environmental applications for the removal of chemicals from contaminated water and metals sewage treatment. Conspicuously, the applied procedure is green, clean and natural without the consumption of any risky materials. However, despite the promising advancements, several challenges remain to be addressed. Future research efforts should focus on developing sustainable synthesis routes for nanoparticles, improving their stability, enhancing their targeted delivery to specific

Table VII. **Conditions of Isotherm models.**

	Langmuir				Freundlich		
Metals	$q_{max\ (mg/g)}$	$K_{L\ (L/mg)}$	R_L	R^2	$K_{F\ (mg/g)}$	1/n	R^2
Cd	85.6898	0.74189	0.01110	0.9988	33.6077	0.3013	0.8514

sites in the body, and minimizing adverse effects on human health. Furthermore, in the coming years, interdisciplinary collaborations between scientists, and policymakers will be crucial for unlocking the full potential of nanoparticles and addressing societal challenges. By addressing the existing limitations and harnessing the opportunities offered by nanoparticles, we can pave the way for a more sustainable, efficient, and technologically advanced future.

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SUPPLEMENTARY MATERIAL

Figures S1-S11.

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