SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF ZnO NPS USING *AZADIRACHTA INDICA* LEAVES

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ABSTRACT

The synthesis of nanoparticles from herbal extracts is increasingly recognized for their diverse biological properties in variations of *Azadirachta indica* (family Meliaceae) from different locations. A study was conducted to synthesize zinc nitrate nanoparticles using *A. indica* leaf extract from Badin, Pakistan (ZnO NPs).Characterization techniques including UV spectroscopy, FTIR, SEM, and XRD were employed. UV spectroscopy revealed an absorption peak at 370 nm (3.3eV band gap energy). SEM analysis indicated crystal-shaped nanoparticles sized between 20 to 30 nm, while FTIR confirmed high purity with Zn–O absorption peaks above 1000 cm-1. XRD patterns demonstrated crystalline properties, estimating an average size of 27 nm. Biological evaluations showed no acute toxicological findings up to 2000 mg/kg dose. The nanoparticles exhibited significant analgesic effects 37.44% ($p < 0.001$) in acetic acid-induced writhing test, as well as anti-inflammatory activity with 326.051% inhibition in protein denaturation assays at 100 mg/kg. Antimicrobial testing revealed the highest inhibition against *E. coli* (27.33 ± 0.57 mm) and effective results against *S. aureus, E. faecalis,* and *S. pneumoniae*. In conclusion, this study confirms the potential of *A. indica* ZnO NPs from Badin, Pakistan, for various applications.

Keywords: *A. indica* ZnO NPs, FTIR, SEM, UV, XRD, analgesic. anti-inflammatory, antimicrobial

INTRODUCTION

From 1-100 nm range of nanoparticles are acknowledged for exhibiting an extensive range of the best biological, biomedical, catalytic, physical, mechanical, chemical and thermal properties (Hadi *et al.,* 2022). Currently, metal nanoparticles including aluminum, cobalt, cesium, copper, gold, silver, zinc, magnetite, nickel, palladium, platinum, silicon nanoparticles are in use. Physical, chemical, and biological methods could be used to create nanoparticles. There are greater benefits to biological synthesis of nanoparticles as compare to chemical and physical ones.Physical and chemical techniques can be harmful to the environment and living things, and they are linked to high energy demands(Farjana*et al.,* 2022). The biological synthesis of nanoparticles involves bacteria, algae, fungi, actinomycetes and plants (Um-e-Aimen*et al.,* 2021; Kanwal *et al.,* 2023). Plant derived nanoparticle synthesis is simple, eco-friendly, and having provides antimicrobial effects (Manokari and Mahipal 2017).Thus plant mediated synthesis or green synthesis has emerged as the best alternative to chemical synthesis. Plants are the richest sources of bio-active organic molecules which include polyphenols, flavanoids, alkaloids, terpenes, tannins, steroids, saponins etc. (Amrutha and Hebsur 2020; Javaid*et al.,* 2023). These phytochemicals are well reported for their multiple medicinal values (Fatemeh*et al.,* 2022; Hina*et al.,* 2022; Javaid*et al.,* 2022).

Numerous NPs of diverse transition metals have been synthesized, such as Zn, Cu, Au, Ag, and Cd, but ZnO. Among all metal oxides, zinc oxide nanoparticles (ZnO NPs) have drawn more attention for their easy, safe and inexpensive production and preparation process. ZnO has been enrolled as one of the safest metal oxides by the U.S. Food and Drug Administration (Fahadul*et al.,* 2022; Subhan*et al.,* 2022)

Azadirachtaindica(Meliaceae) recognized as neem is well known for its various biological activities (Hina*et al.,* 2022). Its different parts are reported for containing approximately 140 bioactive chemical compounds that plays a major role in disease management by modulating several biochemical, genetic pathways, and other biological processes (Akash Gupta*et al*., 2019; Subendu*et al.,* 2021; Tirtha*et al.,* 2021). *A. Indica* is well distributed throughout world, and it can grow in most tropical and subtropical countries (Ahmed*et al.,* 2017; Mohammad 2016). It is well reported that the climate changes create noticeable effects on the life cycle, moisture content, distribution and phyto-chemical composition of the world's vegetation (medicinal and aromatic plants) (Sandeep*et al.,* 2017;Inayat*et al.,* 2022;Andre*et al.,* 2022). Although several research works have already studied and published on synthesize ZnO using *A. indica* but to the best of our knowledge no scientific data available on *A. indica* grow in Badin region of Pakistan. Therefore, the objective of the present study is to demonstrate the eco-friendly synthesis of *A. indica*-ZnO

NPs using aqueous extracts of *A. indica* leaf from Badin region of Pakistan. The green synthesized nanoparticles will be characterized using modern techniques like ultraviolet (UV) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM) and X-ray diffraction (XRD), in order to obtain a new product with the better biological effects and father studied for its biological activities.

MATERIALS AND METHODS

Chemical

The chemicals used in this study, including sodium hydroxide (NaOH), zinc nitrate ($Zn(NO_3)2{\cdot}6H_2O$) (98%), were sourced from Sigma-Aldrich.

Plant collection

Fresh leaves of *Azadirachta indica* were collected from Badin Sindh, Pakistan. The leaves were identified and authenticated by the Department of Plant Sciences, University of Sindh Jamshoro. Distilled water (18.2 M.ohm.cm) was consumed in the experimental work. The chemical zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) (98%) was obtained from Sigma–Aldrich.

Plant extraction

Leaves of *A. Indica* were collected from District Badin, Sindh, Pakistan. Division of Plant Sciences, University of Sindh Jamshoro recognized and validated the specimen. The collected plant material was treated with water and sanitized using milli-Q water to remove dirt particles. After drying at room temperature ground to fine powder. The fine powder leaves (5 g) was mixed with 100 ml of milli-Q water for 20 min at 80 °C. During the way toward bubbling, yellow shaded arrangement was framed, which was frozen at room temperature. From that point onward, arrangement of yellow hued extricate was separated with channel paper (Whatman No.1) and centrifuged at 4000 rpm for 10 min to eliminate weighty biomaterials. Further the extract was then cooled down and stored in the refrigerator (4°C) for utilization in the synthesis of ZnO NPs.

Preparation of *A. indica*-ZnO

For the amalgamation of (*A. indica*-ZnO NPs) 5 ml of *A. Indica* leaves concentrate and 5 ml of mlli-Q water was added into 50 ml cone shaped jar and bubbled at 70-80°C utilizing an attractive stirrer-radiator (Fig. 1). 0.01g of Zinc nitrate [Zn $(NO_3)_2.6H_2O$] was added to the arrangement and afterward bubbled until it decreased to a profound yellow hued glue. This glue was gathered in artistic pot and warmed in a mute heater at 400°C for 2 hrs, to get a light green powder of (*A. indica*-ZnO NPs). Fine powder was used for characterization with UV–Vis, FTIR, XRD.

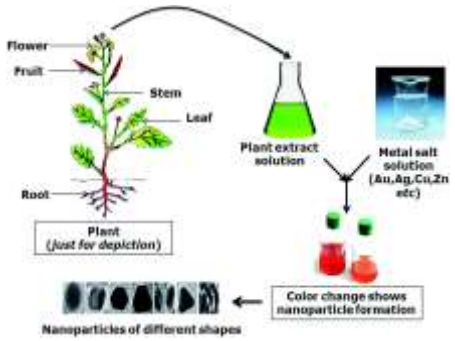

Fig. 1.Preparation method of *A. indica*ZnO

Fig. 2.Zinc oxide nanoparticles.

A. Characterization

UV-visible Spectroscopy Analysis

By employing UV-visible absorption spectroscopic analysis to measure the optical properties of the reaction mixture, the synthesis of *A. indica*-ZnO NPs was confirmed (Fig. 2).200 mg of ZnO nanoparticle powder was mixed with 1 milliliter of distilled water to perform the spectroscopy analysis. A spectrophotometer model UV-2600 (SHIMADZU) was then used to scan the mixture in the 200–800 nm range. Equation 3 was used to determine the zinc oxide's band gap energy (Ogunyemi*et al.*, 2019).

$E = \frac{hc}{\lambda}$ --- (3)

Where Planck's constant is 6.626 × 10−34 Js and c is the speed of light: $3{\times}108ms^{-1}$.

Fourier Transform Infrared (FTIR) Spectroscopy

To identify the phytochemicals in plant extract that are in charge of stabilizing and capping nanoparticles, FTIR analysis was performed. Fourier change infrared (FT-IR) spectroscopy had been performed with 3700 Thermo logical (NICOLET iSl0) FT-IR instrument checked in range 400-4000 cm-1 utilizing Potassium Bromide KBr pellets.

Scanning Electron Microscopy (SEM)

SEM was used to examine the green produced zinc oxide nanoparticles' morphology. Utilizing JOEL-JSM-6490 LV for location of pictures outside space of ZnO NPs, filtering Electron Microscope (SEM) method had been acquired.

Energy-Dispersive X-ray Analysis - (EDX)

An elemental analysis known as energy dispersive X-beam (EDX) is used to identify the primary elements contained in the produced nanoparticles. Energy Dispersive X-beams (JOEL-JSM-6490LV) acquired oxygen and different components.

X-ray diffraction (XRD) analysis

Using X-ray diffraction (XRD), the crystalline size and structural characteristics of the *A. indica*-ZnO NPs are disclosed. Cu-Kα radiation (k=1.5406 A°) was used for the XRD, and 2θ varies between 20 and 80 degrees. When compared to JCPDS card No. 89-7102, the XRD peaks of the green produced ZnO NPs from *A. indica* leaf extract are verified to be in the hexagonal phase (wurtzite structure). The product's well-crystalline quality is demonstrated by the narrow and crisp diffraction peaks. Using the Scherrer equation, the mean crystalline size of the particles was ascertained from the XRD line broadening data.

$$\mathbf{D = \frac{0.89\lambda}{\beta\,(Cos\theta)}}$$

The samples' phase change and crystallite size were examined using XRD analysis. Utilizing the conventional model of X-ray diffraction, the specimen was analyzed. The X-beam Diffraction (Bruker-D8 Advanced) is done with Cu-Kα radiation (k=1.5406 A°) and 2θ territories from 20◦ to 80◦.

B. Biological Evaluation

Animal selection

Swiss albino mice (17-23g) of both sexes were chosen for acute oral toxicity study and acetic acid induced writhing reflex test. All animals were raised at animal house of PCSIR Karachi. The experimental animals were kept at 25 ± 2 °C temperature and 12 h light/dark cycle. During acclimatization and study period the animals were given water and food pellets ad libitum.

Acute oral toxicity studies

Acute oral toxicity of *A. indica*-ZnO NPs was determined in mice following OECD guidelines No 423. Animals that had been fasted for 12 hours (only given water) were divided into 5 groups at random (n=3). Groups I–IV serve as test groups received test drugs at single doses of 5, 50, 300, and 2000 mg/kg body weight orally via feeding cannula, respectively while Group V served as the control group and received a vehicle (distal water) at the same time as test groups. All animal groups were carefully observed for the initial 30 minutes, followed by a 4-hour period to assess any immediate toxic effects. Subsequently, regular intervals of observation were maintained for 72 h, spanning a total duration of 14 days. Throughout this period, mortality rates and weights of animals were noted, various laboratory findings such as body postures, movements, rearing, tremors, touch response, and any variations in skin color, mucous membrane, and pupil dilation were noted within the early4hrs and continued thereafter for the entire 14-day period of dose administration. In the last, the animals were euthanized, to examinethe organsincluding liver, spleen, kidneys, and lungs for any abnormal changes in comparison to the control group (Tehmina *et al.*, 2023).

Analgesic activity (acetic acid-induced writhing reflex)

Acetic acid-induced writhing reflex test was carried out to evaluate analgesic activity as described by Atiq-ur-Rahman *et al.*(2015). Five groups of mice (n=5) were selected for acetic acid-induced writhing. Test Groups I, II and III received *A. indica*-ZnO NPs doses of 100, 250 and 500 mg/kg body weight. Standard Group IV received diclofenac Na 5mg/kg body weight, while control Group V received distilled water only in the same volume by feeding canola. 30 minafter drug administration 1% v/v acetic acid solution (0.1 mL/10 g) were intraperitoneally

administered in all animals to induce abdominal constriction. Each animal was placed individually into a separate cage, and the numbers of writhing movements displayed during 5 to 20 min after acetic acid injection were noted and recorded. The activity was expressed as % inhibition of writhing produced by acetic acid (Tirtha*et al.,* 2021) as given by formula:

$\frac{C-D}{C} \times 100$--- (1)

C = average number of writhing for control group

D= average number of writhing of test and standard groups

Anti-Inflammatory activity (*in vitro*)

In vitro anti-inflammatory activity was carried out by inhibition of protein Denaturation method (Madhuranga and Samarakoon, 2023). The samples solutions used for this assay include:

- Control (50 mL): 2mL egg albumin, 28mL phosphate buffer and 20mL distilled water
- Standard (50 mL): 2mL egg albumin, 28mL phosphate buffer and standard drug aspirin at 100, 200, 400, 800, and 1000 µg/mL.
- Test (50 mL) 2mL egg albumin, 28mL phosphate buffer andzinc oxide nanoparticles at 100, 200,400, 800, and 1000 conc. µg/mL

All the above solutions were adjusted to pH 6.4 using a small amount of 1N HCl. The samples were incubated at 37°C for 15 min and heated at 70°C for 5 min. After cooling the absorbance of the above solutions, %age inhibition of protein denaturation was calculated using following formula.

$$Percentage\ inhibition = \frac{V_t - V_c}{V_c} \times 100$$

V_t= absorbance of test sample

V_c= absorbance of control sample

Anti-microbial activity

Test Microorganisms

Six bacteria viz., *Staphylococcus aureus, Streptococcucpneumoniae, Bacillus cereus, Bacillus pumilus*, *Echrichia coli, Enterococcus feacalis* and one fungi *Candida albican* were used to evaluate the antibacterial activity. Before testing, all of the microorganisms employed in this investigation were isolated and kept on tryptic soy agar slants at 4ºC.

Culture media and Inocula preparation

For microbiological growth, Tryptic soy broth and Tryptic soy agar (Merck) were utilized. To get the cell suspension at 106 CFU/ml, 24-hour-old microbial cultures were suitably diluted in sterile normal saline.

Preparation of Solution

Dimethylsulfoxide (DMSO) was used to dissolve the A. indica-ZnO NPs, giving them a 50 mg/mL strength. DMSO served as the negative control, and chloramphenicol as the reference standard (positive control).

Antimicrobial activity assay

The antimicrobial activity was carried out by agar well diffusion method (Hina *et al.,* 2020). This procedure involved thoroughly mixing 20 milliliters of molten sterile tryptic soy agar with 0.1 milliliters of diluted inoculums (106 CFU/mL) of the test organism, then pouring the mixture into pre-sterilized petri dishes under sterile conditions. For 30 to 40 minutes, each dish was allowed to set at room temperature. Using a sterile cork borer, a well with a diameter of 6 mm was created in the middle of each seeded plate. 0.1 mL of each concentration of test solutions (crude ethanol extract) were then aseptically added to the holes. The positive control is chloramphenicol. The DMSO was used as a negative control. Assay plates for antifungal tests were incubated at 28°C ±2°C for 48 hours, whereas antibacterial plates were incubated at 37°C ±1°C for 24 hours. The zone of growth inhibition around the well was measured in order to assess the antibacterial activity. Vernier caliper was used to measure the inhibitory zone's diameter in millimeters. To reduce test error, each test was run three times.

RESULTS

UV–Visible Spectrum

The preliminary confirmation for the formation and stability of *A. indica*-ZnO NPs UV-Visible spectral analysis was carried out.Strong absorption spectra at 370 nm (Fig. 3) supported the findings and were ascribed to ZnO's intrinsic band-gap absorption brought on by electron transitions from the valence band to the conduction band. It was discovered that zinc oxide had a band gap energy of 3.3 eV.

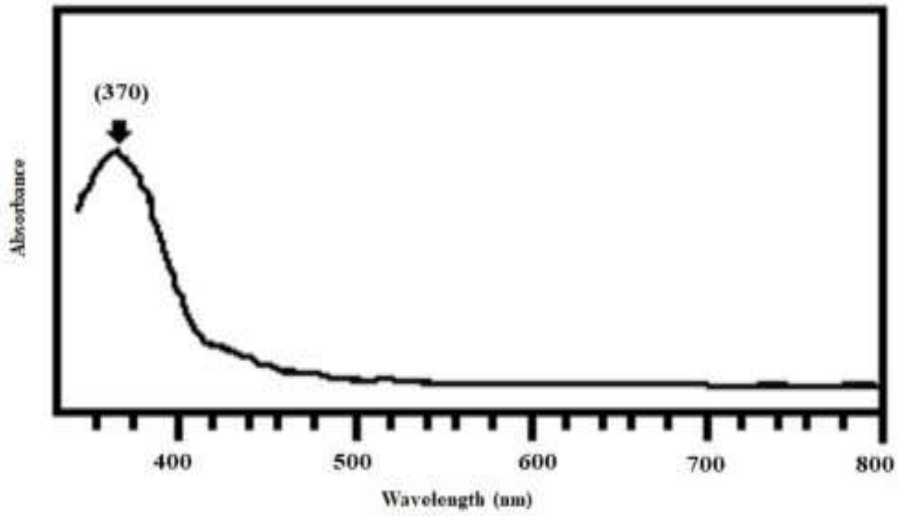

Fig 3. UV–Vis Spectrum of *A. indica*-ZnO NPs

FTIR Analysis

The characteristic stretching vibrations at 765 cm−1 correspond to the Zn-O linkage of ZnO NPs. No absorption band was observed above 1000 cm-1, which revealed the high purity of *A. indica*-ZnO NPs(Fig. 4).

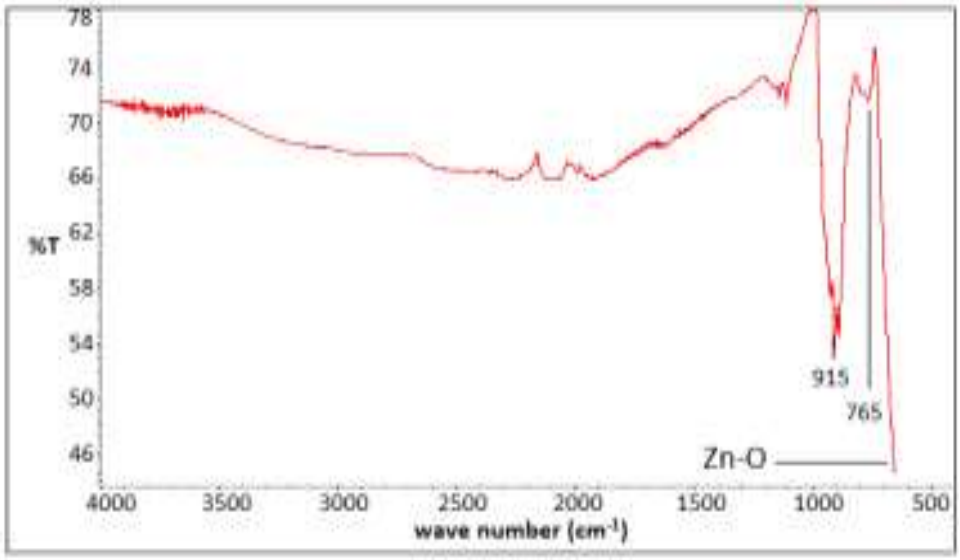

Fig 4. FT-IR Spectrum of *A. indica*-ZnO NPs

SEM-EDX Analysis

SEM-EDX is a useful technique for the formation of magnified images by utilizing electrons as a substitute for light waves. A field emission source will emit these electrons, and these electrons will help to scan an object according to zigzag patterns. The results exhibited the surface morphology of ZnO-NPs synthesized with the help of *A. indica* (Fig. 5). Most of the NPs are crystalline in shape.

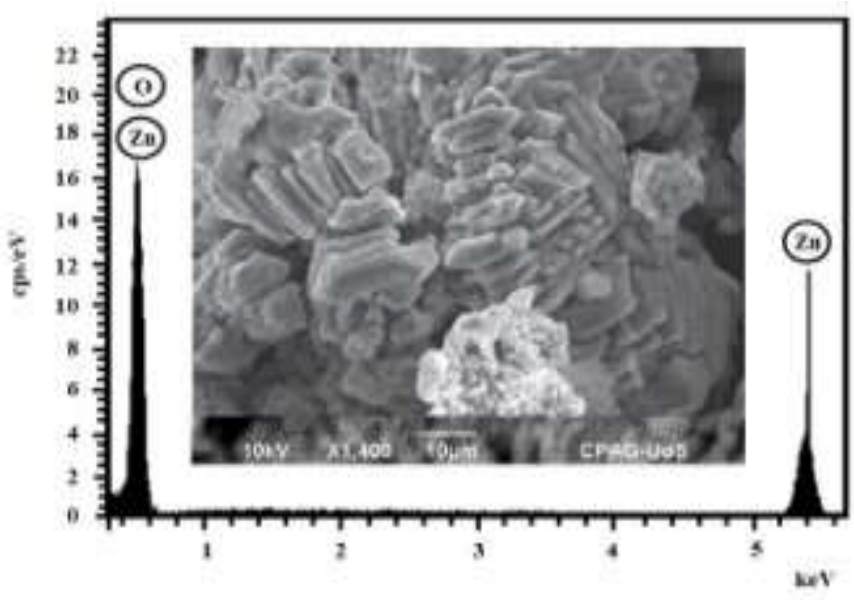

Fig. 5. Surface morphology of ZnO-NPs

XRD analysis

The XRD analysis was done to confirm the crystallinity of the synthesized *A. indica*-ZnO NPs. The crystallinity of the powder resulting from the synthesis using *A. indica*leaf extract showed the peaks at 2θ values of 34.0°, 35.57°, 36.47°, 55.19°and 65.27° correspond to the (100), (002), (101), (110), and (103) planes, respectively (Fig. 6) of hexagonal wurtizedZnO.

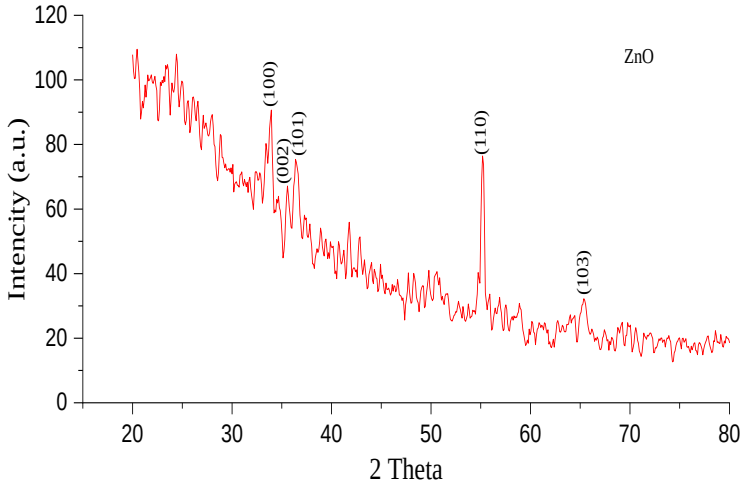

Fig 6. XRD Spectrum of *A. indica*-ZnO NPs

Acute oral toxicity studies

The results of acute oral toxicity test revealsno mortality until fourteen days of observation. All animals did not show any abnormal behavior such as alertness, restlessness, skin color, touch response, pain response, corner sitting, righting reflux, gripping, pinna reflex, corneal reflex, food and water intake in all animal groups. No signs of acute toxicity like tremors, convulsion, writhing, urination, diarrhea, salivation, lacrimation and coma were found. No significant changes in the body weight gains were detected (Table 1&2). In addition, gross necropsy findings did not show any adverse effects in all organs in treated groups as compared to control group.

Table 1.Acute oral toxicity test of *A. indica*-ZnO NPs (2000 mg/kg b.w).

Sr.#	**Responses**	**Control group**	**A. indica-*ZnO NPs*treated groups (mg/kg)**			
			5	**50**	**300**	**2000**
1.	Piloerection	N	N	N	N	N
2.	Eyes	N	N	N	N	N
3.	Respiration	N	N	N	N	N
4.	Retching	N	N	N	N	N
5.	Restlessness	Nil	Nil	Nil	Nil	Nil
6.	Salivation	Nil	Nil	Nil	Nil	Nil
7.	Lethargy	Nil	Nil	Nil	Nil	Nil
8.	Fecal consistency	N	N	N	N	N
9.	Tremors	Nil	Nil	Nil	Nil	Nil
10.	Convulsion	Nil	Nil	Nil	Nil	Nil
11.	Writhing	Nil	Nil	Nil	Nil	Nil
12.	Sleep	N	N	N	N	N
13.	Coma	Nil	Nil	Nil	Nil	Nil

N= Normal

Table 2. Weight of mice treated with of *A. indica*-ZnO NPs (2000 mg/kg b.*w)*

Sr.#	**Body weight**	**Test group**	**Control Group**
1.	Day 1	18.8	18.4
2.	Day 7	21.14	20.69
3.	Day 14	23.91	22.96

Analgesic activity

The experimental data revealed *A. indica*-ZnO NPs possessed moderate peripheral analgesic activity(Table 3, Fig. 7). Among all the test groups, *A. indica*-ZnO NPs at 100 mg/kg b.w. exhibited greatest analgesic effects (37.44% writhing inhibition) compared to standard (66.38% writhing inhibition).

Fig. 7. Acetic Acid- Induced Writhing in Mice.

Table 3.Effect of *A. indica*-ZnO NPs on acetic acid-induced writhing response.

Sr.#	**Groups**	**Dose (mg/kg)**	**No. of writhing**	**Inhibition (%)**
1.	Group I	100	29.40	37.44
2.	Group II	250	32.80	30.21
3.	Group III	500	43.40	7.65
4.	Group IV	5	15.80	66.38
5.	Group V	Distilled water	47	----

Anti-inflammatory activity

As shown in Table 4, *A. indica*-ZnO NPs exhibited a significant reduction in inflammation. At concentration of 100 mg/kg*A. indica*-ZnO NPs displayed potent activity by showing 326.051% of inhibition as compared to standard drug (33.619%) inhibition.

Table 4.*In-vitro* anti-inflammatory activity of the *A. indica*-ZnO NPs

Sr.#	Concentration (µg/ml)	Inhibition (%)	
		***A. indica*-ZnO NPs**	**Aspirin (standard)**
1.	100	326.051± 0.024	33.619 ± 0.032
2.	200	303.861± 0.032	66.057 ± 0.056
3.	400	67.542 ± 0.026	135.414 ± 0.026
4.	800	16.641 ± 0.039	167.407 ± 0.034
5.	1000	6.716 ± 0.022	348.485 ± 0.046

Antimicrobial Activity

The test drugexhibited varying degrees of inhibition activity against the tested microorganisms (Table 5, Fig 8). At 50mg/mL conc. the maximum zone of inhibition found against *Escherichia coli*27.33±0.57followed by 27.016±0.28, 24.66±0.57 and 22.5±0.5 against *Staphylococcus aureus*, *Enterococcus feacalis* and *Steptococcus pneumonia*, respectively. ZnO NPs showed almost similar activity against *Bacillus cerus* and *Bacillus pumilus (*18.66±0.57 and 18.00±0.00). The least activity was observed against *Candida albican*16.53±0.83.

Table 5.Antimicrobial activity of *A. indica*-ZnO NPs

Microorganisms	Concentration 50 mg/mL	Chloramphenicol Std. (10 mg)	DMSO Negative control
Staphylococcus aureus	27.016 ± 0.28	32.33 ± 0.57	7.46 ± 0.5
Escherichia coli	27.33 ± 0.57	32.16 ± 0.28	7.5 ± 0.5
Enterococcus feacalis	24.66 ± 0.57	29.33 ± 0.57	7.3 ± 0.28
Steptococcus pneumonia	22.5 ± 0.5	31.16 ± 0.28	7.76 ± 0.25
Bacillus pumilus	18.00 ± 0.00	28.66 ± 0.57	7.6 ± 0.17
Bacillus cerus	18.66 ± 0.57	29.33 ± 0.57	7.7 ± 0.26
Candida albican	16.53 ± 0.83	30.83 ± 0.76	7.4 ± 0.05

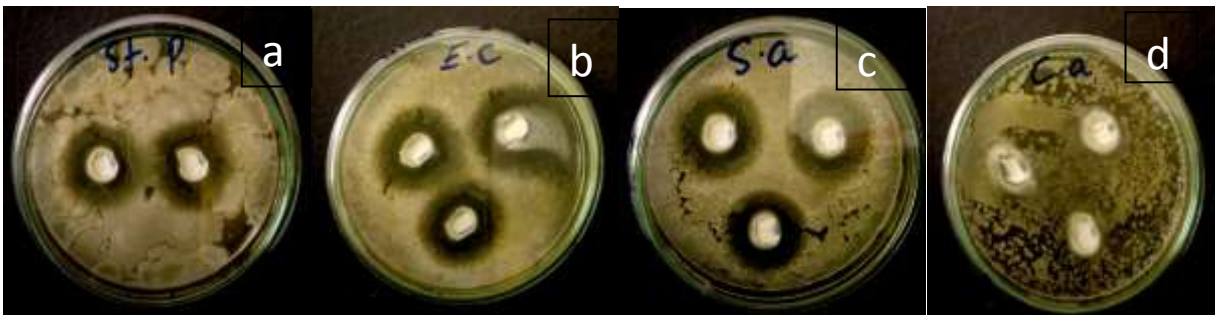

Fig.8.*A. indica*-ZnO NPsinhibition zones against various microorganisms.

DISCUSSION

Plants are gifted source for novel active substances and are a great source of new active ingredients and have been successfully used to create biocompatible nanoparticles (Manokari *et al.*, 2017). The changeability in chemical composition of same species of different origin can affect its quality and its bioactivity (Aissata *et al.*, 2021). Previously a study conducted on *A. indica* extracts from six different locations on the Colombian Caribbean coast exhibited variation on its chemical composition (Juan and Coy-Barrera 2019). Keeping

this view, a study on green synthesis of *A. indica*-ZnO NPs was planned with *A. indica* leaves from Badin region of Pakistan. The synthesized nanoparticals were further chemically and biologically was evaluated by various methods/techniques.Water is used as a solvent in the synthesis of *A. indica*ZnO NPs.Wateriswidelyusedforthesynthesis of different nanoparticles and is said to be the most affordable and abundantly available solvent on earth.

A. Indica leaf material was chosen due to the presence of potent phytochemicals and biomolecules in different plant extracts, particularly in the leaves, which serve as stabilizing and reducing agents during the creation of nanoparticles.Moreover, flavones, terpenoids, sugars, ketones, aldehydes, carboxylic acids, and amides are the primary phytochemicals found in leaves and are in charge of the bioreduction of nanoparticles (Jagpreet *et al.,* 2018). Sujata 2021 and Norul *et al.* (2020) reported the presence of these phytochemicals in leaf part of *A. indica*.

Zinc ions in the solution are reduced to zinc oxide by plants' secondary metabolites. In addition to its reducing properties, the plant extract also has stabilizing properties. By analyzing the UV-visible spectra in the 200–800 nm range, this was verified. The ZnO nanoparticle-specific peak in the spectra was located at 370 nm. It was discovered that zinc oxide had a band gap energy of 3.3 eV. (Fig. 1). Previously, a study conducted on nanoparticals synthesis with *A. indica* leaf extract reported absorbance peak between 368 - 375 nm of wavelength with band gap energy 3.0 eV (Amit*et al.,* 2019). A study conducted on same synthesis with same material showed absorbance peaks at 359 nm in same wavelength range (Amrutha and Hebsur, 2020. Another study reported absorbance peak 356 nm with ZnO nanoparticles formulated using *A. indica* leaf extract (Kartikey*et al.,* 2022). All these findings are comparable and support our result.

FTIR analyses were used to further examine the results, revealing shifts and variations in peak area. The range of the FTIR spectrum measured was 400–4000 cm-1.which clearly showed the intense absorption peak of ZnO in the range of 400-100 $cm^{-1,}$ and various other peaks provided details of the reducing and capping agents in the *Azadirachta indica* leaf extract (Fig 4). A study conducted on FTIR spectra on same nanoparticls further show that a number of functional groups, including as phenolic, carbonyl, carboxylate, and ether groups, are involved at same range (Bhumika *et al.,* 2022). The results of FTIR also correlated with the data already available in the literature for green ZnO nanoparticles.

The results of SEM-EDX analysis revealed the formation of stable Zinc oxide nanoparticals that are crystalline in shape (Fig. 5). The *A. Indica* leaf extract helped to prevent agglomeration and acted as a capping agent (Ogunyemi *et al.,* 2019). EDX analysis indicates the characteristic energies of the corresponding specific atom. The atomic and weight percent values of zinc, oxygen and carbon are obtained from the EDX spectrum. Another study conducted by Gunalan *et al.*(2011) reported that the narrow width and intense peak specify that the resultant complex was crystalline in nature.

The XRD pattern confirms the presence of hexagonal ZnO with JCPDS card No. 89-7102 and reveals a crystallite size of approximately 27 nm using the Scherer equation (Fig. 6). A study conducted on ZnO synthesis with *A. indica leaf* extract reported around 22 nm and 27 nm crystalline size of ZnO Nps that supports our findings (Amit *et al.,* 2019). Another study Bhumika *et al.*(2022) conducted on ZnO-NPs with *A. indica* aqueous leaf extract reported the size range of 60–65 nm. One more document showed XRD crystallite size of ZnO with 10 and 50% and Zn1−xCux O samples to be 19.8, 23.7, 18.4 and 16.1 nm, respectively (Dawit *et al.,* 2019). One more study reported an average size of 25.51 nm in XRD studies of ZnO nanoparticls derived through *A. indica* leaf aqueous extract (Kartikey *et al.,* 2022)

In the present study acute oral administration of *A. indica*-ZnO NPs showed that, at the tested dose 2000 mg/kg no toxic sign, behavioral change or death was observed. There was no significant variation in mean body weight among treatment groups compared with the control (Table 1 & 2). The experimental data revealed *A. indica*-ZnO NPs possessed moderate peripheral analgesic activity. The 100 mg/kg body weight dose exhibited maximum peripheral analgesic activity (37.44% writhing inhibition) compared to standard 66.38% writhing inhibition. Acetic acid-induced writhing response technique is a widely used method to evaluate the peripheral analgesic activity of any plant part, where acetic acid the key inducer of pain in an animal model. When acetic acid is administered intraperitoneally, it enhances the release of some inflammatory mediators that in turns are responsible for feelings of pain (Bhuiyan *et al.,* 2020). So, it may be assumed that the mechanism of the peripheral analgesic activity of the *A. indica*-ZnO NPs of the seed may act involving the inhibition of biosynthesis or release of these mediators.

A. indica-ZnO NPs exhibited a significant reduction in inflammation. At concentration of 100 mg/kg *ZnO-NPs* displayed potent activity by showing 326.051% of inhibition as compared to standard drug (33.619%) inhibition. This substantial anti-inflammatory effect might be because of the inhibition of any inflammatory agents by the glycosides or steroids present in the leaf extract. This result validated *A.indica* as a therapeutic mediator that can be used to treat acute inflammation.

A. indica-ZnO NPs exhibited varying degrees of antimicrobial activity against the tested microorganisms (Table 5). At 20mg/mL conc. the greatest inhibition zone found against *Staphylococcus aureus* 27.016 ± 0.28 followed by

27.016 ± 0.28, 24.66 ± 0.57 and 22.5 ± 0.5 against *Escherichia coli*, *Enterococcus feacalis* and *Steptococcus pneumonia*, respectively. *A. indica*-ZnO NPs showed almost similar activity against *Bacillus cerus* and *Bacillus pumilus (*18.66 ± 0.57 and 18.00 ± 0.00). The least activity was observed against *Candida albican*16.53 ± 0.83.

On the basis of obtained results from this study and previous reported data on *A. indica*-ZnO NPs synthesis by incorporating aqueous extract of *A. indica*, I can be summarized that the geographical changes or difference in climate zone can alerts the chemistry of *A. indica.* This investigational work is also useful for the green synthesis for ZnO NPs with *A. indica* from Badin region, Pakistan. The synthesis provides aneco-friendly methods with low cost. It may be advantageous for pharmaceutical industry to explore locally available *A. indica* to develop effective drugs amended with ZnO nanoparticles. The nanoparticles formulated drugs could be used in diagnosis and treatment of various heath related issues.

Conclusion

The current study clearly shows that the green synthesis of *A. indica*-ZnO NPs is less harmful to the environment, easier to use, and more effective than the traditional approaches. Furthermore it was also observed that there is slightly changes in results as compared to published data, that proves the change in climate zone can affect the chemistry of *A. indica*. These results showed that synthesized ZnO-NPs are of great use in the pharmaceutical and biological industries.

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Conflict of Interest Statement

No conflicts of interest

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