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# Phyto-assisted synthesis of zinc oxide nanoparticles using *Bauhinia variegata* buds extract and evaluation of their multi-faceted biological potentials

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**Zinc oxide nanoparticles have wide range biological, biomedical and environmental applications. However, traditional nanofabrication of ZnONPs uses various toxic chemicals and organic solvents which limit their bio-applications. To overcome this hurdle, *Bauhinia variegata* derived buds extract was utilized to fabricate ZnONPs. The greenly generated ZnONPs were successfully prepared and extensively characterized using different analytical tools and the average crystalline size was calculated as 25.47 nm. Further, bioengineered ZnONPs were explored for multiple biological activities that revealed excellent therapeutic potentials. The antibacterial potential was determined using different bacterial strains. *Pseudomonas aeruginosa* (MIC: 137.5 µg/mL) was reported to be the most resistant variant while *Bacillus subtilis* (MIC: 34.38 µg/mL) was observed to be most susceptible bacterial strain. DPPH radical scavenging potential was measured to determine the antioxidant capacity of ZnONPs and the highest scavenging potential was observed as 82% at highest of 300 µg/mL. The fungicidal effect of green ZnONPs in comparison with Amphotericin B was assessed against five selected pathogenic fungal strains. The results revealed, *Fusarium solani* (MIC: 46.875 µg/mL) was least resistant and *Aspergillus flavus* (MIC: 187.5 µg/mL) was most resistant in fungicidal examination. Cytotoxicity potential of B.V@ZnONPs was analyzed against newly hatched nauplii of brine shrimps. The results for greenly produced ZnONPs was recorded as 39.78 µg/mL while 3.006 µg/mL was reported for positive control vincristine sulphate. The results confirmed the category of general cytotoxic for greenly synthesized nano sized B.V@ZnONPs.**

**Keywords** *Bauhinia variegata*, Zinc oxide nanoparticles, Bactericidal, Antioxidant, Cytotoxicity

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Nanotechnology is a nascent field of science and engineering that concerns with the production and manipulation of nanomaterial in the size range of 1–100 nm[1]. It is research discipline that focus on the synthesis, characterization, processing and consumption of nanomaterials[2]. They consequently display new and enhanced features and are super motile when they are kept in a free state and have large area[3,4]. Different forms, sizes, and structures of nanoparticles can be found owing unique properties[5]. Nanoparticles can be amorphous or crystalline in nature[6]. Different metal and metal oxide NPs have been evaluated for different biological activities and have displayed promising antioxidant, anti-bacterial, anti-cancer, and anti-fungal potential[7–10]. In particular, ZnONPs have diverse potential and marked applications in food packaging industries, agriculture and cosmetics industry,[11]. Their capacity to penetrate microbes cell envelops make ZnONPs as a mean of killing microbes evaluating their antimicrobial potential[12]. ZnONPs have shown different biological and biomedical potentials such as antibacterial, antifungal, antioxidant, anticancer, Typhoid and asthma[13,14]. There are different approaches for the fabrication of nanoparticles including chemical, physical and biological methods. The physical method uses high energy, pressure and temperature. Further this method is expensive, use heavy equipments and complex technique[15]. Chemical methods use different chemical reagents for reducing, capping and stabilizing nanoparticles. Residue from these chemical reagents contaminate the environment and adversely affect the human health[16–18]. Keeping in mind the problems linked with physiochemical methods there is an urgent need of designing a greener, simpler and economic approach free of hazardous chemicals. As an alternative biological method utilizing natural resources including whole plant along with its root, stem, leaves, flower and fruit as well as bacteria algae and fungi can be preferred[19–21]. The adoption of a more environment-friendly approach to synthesize nanoparticles has captured the interest of numerous researchers[22]. In "green synthesis", plant parts and their extracts are used to fabricate nanoparticles[23]. Green synthesis has the ability of manufacturing wide range of metal/metal oxide nanomaterial, different hybrid materials, and biocompatible materials in an economic and environmentally friendly manner[24]. It is also eco-friendlier, energy-efficient, non-toxic, biocompatible and need no chemical reagents for reduction, stabilization and capping from outside[25]. The biofabrication of nanoparticles using plants has more potential since they are contamination-free, remarkably quick, easy to handle and can create nanoparticles of any desired form and size. Thus, utilizing plant extract as source of reducing, stabilizing and capping agents can minimize the stressful effects of other synthesis methods used at industrial scale or in laboratories[26]. *B. variegata*, member of family Fabaceae, commonly named as orchid tree, Kachnar is a medium-sized deciduous tree that grows in the Himalaya ranges[27]. The leaves are used for the cardiovascular complications. Root can act as an antidote for snake poison and also carminative, help to ease flatulence[28]. The medicinal plant *B. variegate* is a rich source of different medicinally bioactive phytochemicals namely kaempferol, imbuing, kaempferol and hesperidin together with one triterpene caffeate[29–31]. The goal of the current research was to design a novel, straightforward, quick, one-step, environmentally friendly method for creating zinc oxide nanoparticles utilizing extract of the medicinally and phytochemically important plant *B. variegate* buds.

## Materials and method

### Medicinal plant collection

The flower buds of *B. variegata* were collected from Peshawar, KPK, Pakistan following established protocol and permission was obtained. Our plant study complies with relevant institutional, national and international guidelines and legislation. Further, the plant material was identified and taxonomically validated by well-known taxonomist Dr. Syed Afzal Shah, Assistant Professor at Department of Biological Sciences, National University of Medical Sciences Rawalpindi, Pakistan. The voucher specimens were submitted to gene bank of National University of Medical Sciences Rawalpindi, Department of Biological Sciences, for allotment of voucher number (SAS-770), multiplication and to ensure availability for future use. After collection, the flowers were thoroughly cleaned using running tap water to remove all kinds of associated dust particles. The flower buds were shade dried by placing at room temperature. Once the flower buds were completely dried, they were completely grounded into fine powder to increase the surface area during flower buds extract preparation.

### Extraction process of *Bauhinia variegata*

For the preparation of *B. variegata* flower buds extracts 50 g dried and cleaned powder of *B. variegata* was carefully added into flask containing 500 mL of distilled water and was placed on magnetic stirrer (hot plate) at 80 °C for 2 h. After cooling, the plant extract was then carefully filtered three times using Whitman filter papers to obtain pure aqueous plant extract. The pH value of aqueous extract was recorded as 3.7. The pure plant extract obtained was than stored at 4 °C for further processing.

### Green synthesis of ZnONPs

The synthesis of ZnONPs was achieved by reducing Zinc nitrate precursor salt (Sigma-Aldrich) using *B. variegata* flower buds extract. To achieve this purpose, 3 g zinc salt was added to 300 mL filtered flower buds extract using Whatman's filter paper No-41 with pore size of 20–25 µm, which give radish brown color solution. The pH of the solution was measured and recorded as 4.18. With continuous stirring at 500 rpm the obtained reaction mixture was heated at 70 °C for 2 h achieving a homogeneous solution. After centrifugation at 6000 rpm for 15 min, the supernatant was discarded while the pellet was washed with deionized water twice to remove all kinds of surface impurities. The obtained ZnONPs were incubated at 100 °C until the particles were completely dried. After accomplishing room temperature, the obtained sample was stored in 2 mL eppendorf tubes, parafilmed and were stored in dry and dark place.

## Characterization of bio-assisted ZnONPs

Different characterization techniques were used to characterize the biologically synthesized ZnONPs including UV–Vis spectrophotometer, FT-IR, X-ray diffraction (XRD) analysis, X-ray spectroscopy (EDS), SEM, Zeta size and Zeta potential.

Ultraviolet–visible spectroscopy analysis was performed and ZnONPs were properly scanned and absorbance was measured in the range of 200–700 nm using UV–Vis spectrophotometer (Germany) and the adsorption band around 397 nm revealed the successful formation of ZnONPs. To determine the major functional groups, present in *B. variegata* involved in the reduction, stabilization and capping of ZnONPs, FT-IR analysis was employed using (Alpha,Bruker, Germany). The ZnONPs were scanned in the spectral array from 400 to 4000 $cm^{-1}$ at 4 $cm^{-1}$ resolutions and different spectra were obtained to determine the functional groups involved in the synthesis of ZnONPs which enhance the biological capabilities of asynthesized ZnONPs. X-ray diffraction (XRD) (PAN-alytical Empyrean Diffractometer) analysis was performed for the as-prepared ZnONPs to determine their crystalline nature and the average sizes of the crystals were determined from the X-ray diffraction spectra using the Scherrer approximation method. Surface morphology and actual particle size of the prepared ZnONPs was determined using SEM machine (EM (NOVA FEISEM-450 applied with EDX detector). The EDX function was also embedded in the SEM. The prepared ZnONPs samples were placed into SEM machine and subjected to 10 kV voltage, images at 50 kx magnification were taken. The elemental analysis of asynthesized *B. variegata* mediated NPs were employed through Energy Dispersive X-ray (EDX) spectroscopy and elemental composition was determined.

## Different biological activities of *Bauhinia variegata*@ZnONPs

After extensive characterization through different microscopic and spectroscopic analytical tools, different biological potentials of ZnONPs were investigated. Following activities were performed to investigate the biological potencies of asynthesized ZnONPs.

*In vitro anti-bacterial activity*

The anti-bacterial activity of B.V@ZnONPs was determined using disc-diffusion method against different Gram negative strains of bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and different Gram-positive bacterial strains such as *Bacillus subtilis* and *Staphylococcus aureus*. Nutrient agar media was prepared and utilized in the antibacterial test which was poured into petri plates to solidify. 100 µL of bacterial cultured cells were streaked on petri plates uniformly to grow and propagate several bacterial species over various plates. Later on different filter discs were made and autoclaved. Varied concentrations of B.V@ZnONPs ranging from 1100 to 34.38 µg/mL were used to determine the dose dependent bactericidal potentials of asynthesized NPs. 6 mm filter discs loaded with 20 µL of test samples were properly kept on uniform bacterial lawns of petri plates. 20 µL Oxytetracycline disc and DMSO served as positive and negative controls. After 24 h continuous incubation at 37 °C, ZIs were calculated in millimeter and individual MIC values were recorded for respective bacterial strains to determine their antibacterial potentials.

*In vitro antifungal activity*

The fungicidal bioactivity of ZnONPs was tested employing five different pathogenic fungal strains such as *Mucor racemosus*, *Aspergillus niger*, *Candida albicans*, *Fusarium solani* and *Aspergillus flavus*. To achieve this purpose, Sabouraud dextrose agar media was prepared in distilled. The media was allowed to cool down and then poured in to petri plates to solidify. In the next step, different fungal strains were spread on petri plates uniformly. Filter discs loaded with 20µL of different doses of B.V@ZnONPs (1500–46.875 µg/mL) were placed on media plates and incubated at 28 °C for 48 h. 10 mg of Amphotericin B filled disc served as positive control while for negative control DMSO was used to compare and contrast the antifungal potential of ZnONPs. Finally, zones of inhibition were measured. MICs values for B.V@ZnONPs were computed at minimum concentration.

*Free radical scavenging activity (DPPH antioxidant assay)*

For the assessment of antioxidant potential of *B. variegata* mediated cobalt oxide nanoparticles, DPPH free radical scavenging assay was employed using different dosages of green B.V@ZnONPs (300–2.35 µg/mL) using microplate reader. For reagent solution, DPPH (2.4 mg) was added up to methanol (25 mL). Further, for the determination of the antioxidant capability of B.V@ZnONPs, ascorbic acid (positive control) and DMSO (negative control) were utilized to compare and contrast the antioxidant potential of biogenic ZnONPs. 20 µL of test sample (B.V@ZnONPs) added into 180 µL of reagent solution to prepare 200 µL of reaction mixture. The reaction mixture was then loaded into different wells of 96-well plate and transferred into incubator for 1 h. After wards, the incubation of reaction mixture was performed at room temperature in darkness for half an hour. Finally, the reaction mixture was scanned at 571 nm with microplate reader to determine the % scavenging potential of DPPH.

*Artemia salina cytotoxicity assay (ASCA)*

For the assessment of cytotoxicity, brine shrimps' cytotoxicity assay was performed. Artificial sea water was used for hatching eggs of *Artemia salina* (Ocean Star, UT, USA) in a bi-partitioned tray. The bipartition tray was carefully placed in incubated for 24 h. 10 hatched nauplii were collected via micro pipette and shifted to each glass vial containing sea water. Different doses of B.V@ZnONPs ranging from 1000 to 7.81 µg/mL were prepared and was used to determine dose dependent response. For positive control vincristine sulphate, mature nauplii and sea water were taken in a vial, while the vials containing nauplii, water and sea water serve as negative control respectively. After incubating the vials for 24 h, dead shrimps were carefully counted and % inhibition/ mortality

were calculated using Graph Pad software (Graph Pad Prism 6, https://graphpad-prism.software.informer.com/amp/6.0/). Detailed schematic representation showing green synthesis of *B. variegata* mediated ZnONPs is given in Fig. 1.

## Results and discussion

Green nanotechnology is a fast growing technology that uses green, simple eco-friendly and eco-sustainable approaches for the synthesis of nanoparticles. Green nanotechnology uses nontoxic, green and natural chemicals for the synthesis of nanoparticles and have replaced the approaches that utilize costly and toxic chemicals and heavy equipment's. Previously,[13, 14, 32] etc. have synthesized man-sized zinc oxide nanoparticles using different medicinal plants. Further, they have extensively characterized these asynthesized ZnONPs followed by extensive characterization and biological activities such as antibacterial, antifungal, antioxidant, anticancer, cytotoxicity and anti-diabetic activities. The current research thesis is focusing on green synthesis of ZnONPs using medicinal plant *Bauhinia variegata*. The production of ZnONPs was validated when aqueous flower buds extract of *B. variegata* changed its color from light brown to reddish brown solution upon the addition of precursor salt (Zinc nitrate hexahydrate). After the synthesis of B.V@ZnONPs, characterization was performed using different analytical tools. The synthesis of ZnONPs was confirmed by observing maximum absorption (SPR spectral analysis) utilizing Ultra Violet visible photometer. The SPR spectra revealed highest absorption of 400 nm which is in the standard range recorded for ZnONPs ranging from 200 to 700 nm. The spectral range of UV in optical analysis is from 250 to 800 nm[33, 34]. The stability, size and shape of nanoparticles/nanomaterials can directly be encountered by studying SPR spectral peak Fig. 2.

The B.V@ZnONPs were further characterized using FT-IR spectroscopic analysis to confirm the availability of bioactive functional elements adsorbed on the surface and are involved in the synthesis of greenly orchestrated ZnONPs. The FT-IR spectra of B.V@ZnONPs Fig. 3 revealed significant transmissions peaks at 3535.75 $cm^{-1}$ represent –OH stretching vibrations, 1629.13.15 $cm^{-1}$ depicts ketone, 1416.73 $cm^{-1}$ represent alkane, 978 $cm^{-1}$ C–O of carboxylic acid, 682.94 $cm^{-1}$ and 553.83 is attributed to strong bond vibrations of Zn–O. The description of FT-IR analysis is demonstrated in Table 1.

The X-ray crystallography technique was employed to determine the crystal structure and phase purity of B.V@ZnONPs. The crystallographic spectra (Fig. 4) revealed diffraction bands observed at 32.1, 35, 35.89, 46.01, 57.74, 62.87, 67.54, 68.63 showing different crystallographic planes like 100, 002, 101, 102, 110, 103, 112 and 201, respectively. In addition, the Bragg peaks observed for B.V@ZnONPs are in accordance with the diffraction standards of pure phase ZnONPs nano sized particles (JCPD: 36-1451). The sharp peaks revealed crystalline nature and phase purity of greenly orchestrated ZnONPs. The average size 25.47 nm was calculated from peak width by utilizing quantitative approximation using the Debye–Scherrer equation, $D = K \lambda/\beta 1/2 Cos\Theta$. The XRD

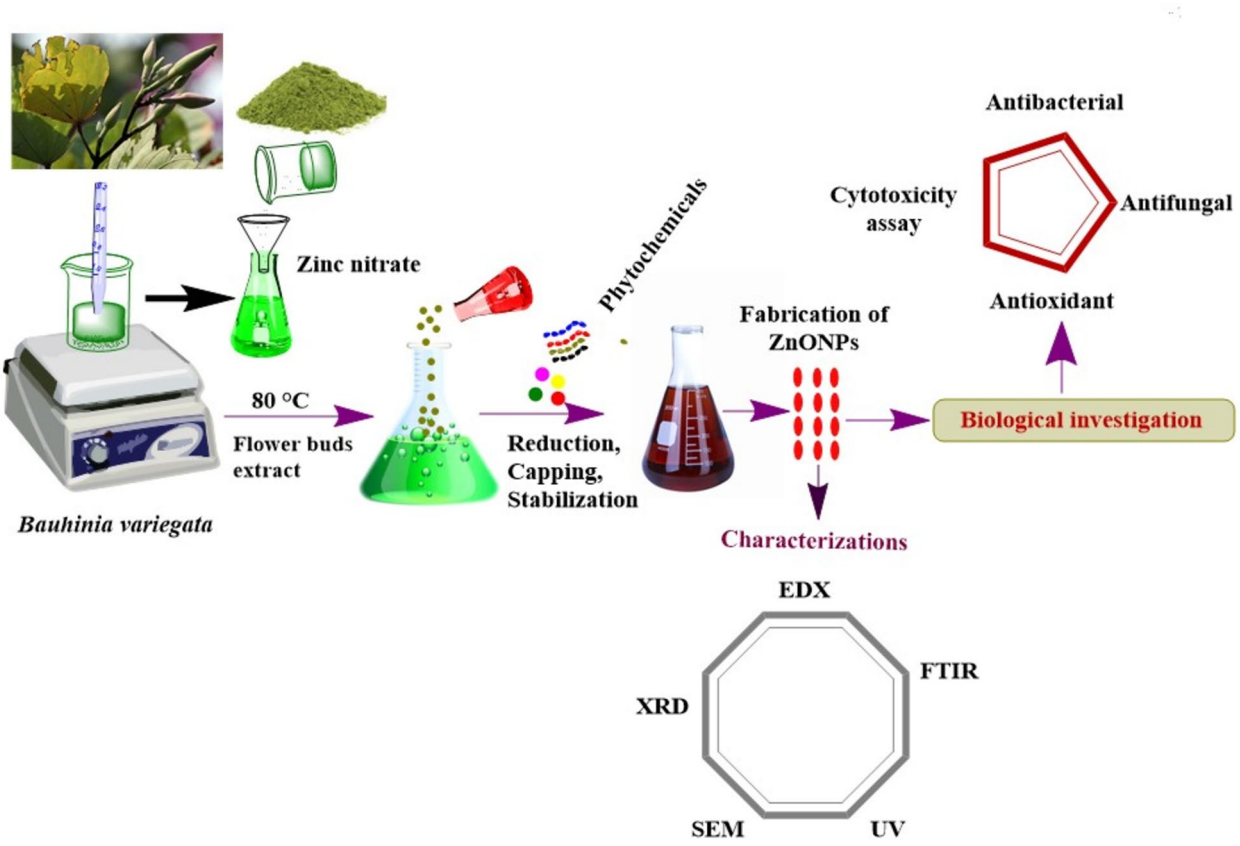


**Fig. 1.** Detailed schematic representation showing green synthesis of *B. variegata* mediated ZnONPs.

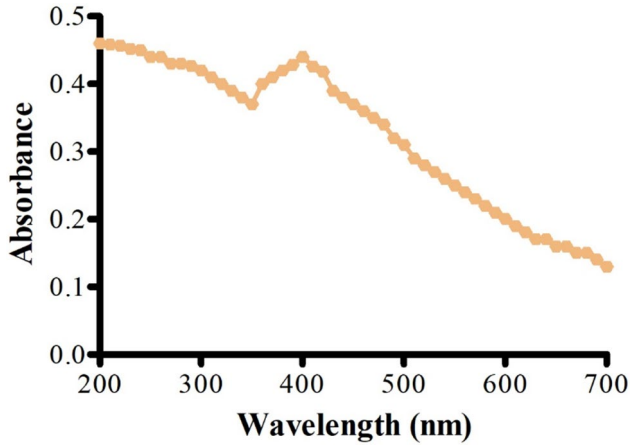


**Fig. 2.** UV–VIS spectroscopy for *B. variegata* flower buds extract mediated ZnONPs.

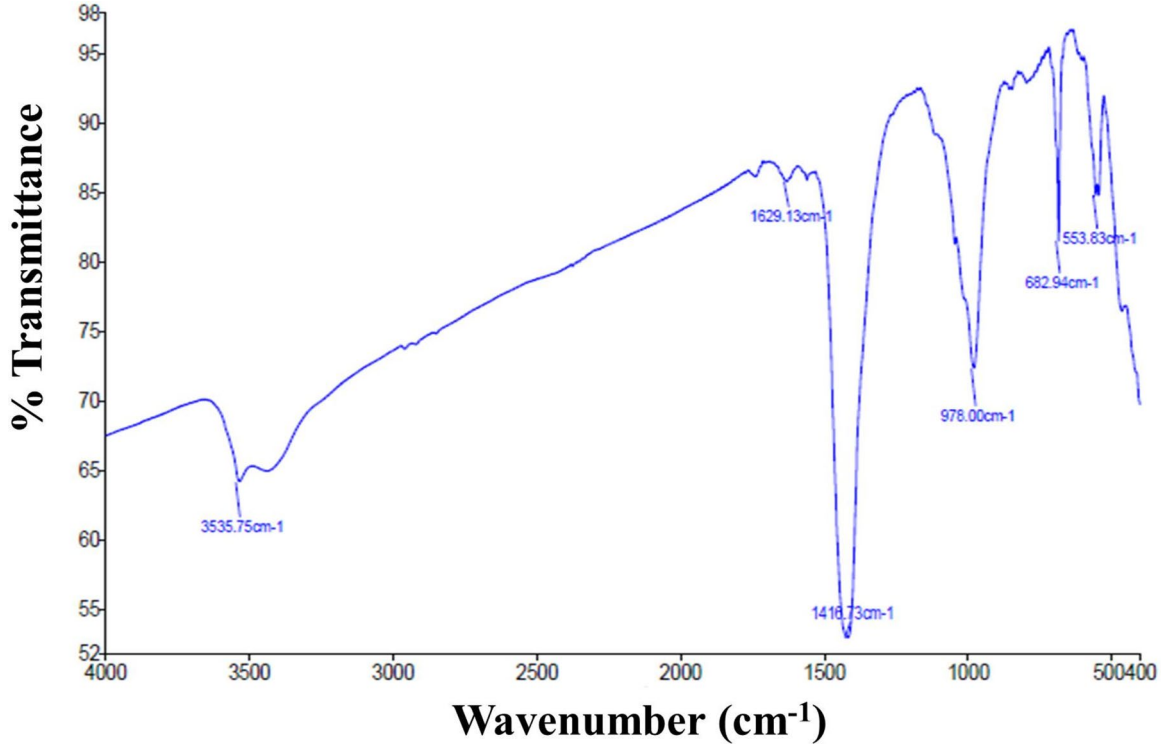


**Fig. 3.** FT-IR pattern of B.V@ZnONPs showing different functional elements.

| S. No | Wavenumber ($cm^{-1}$) | Functional groups |
|---|---|---|
| 1 | 3535.75 | –OH |
| 2 | 1629.13 | Ketones |
| 3 | 1416.73 | Alkanes |
| 4 | 978 | C–O |
| 5 | 682.94 | Zn–O |
| 6 | 553.83 | Zn–O |

**Table 1.** FT-IR analysis of *B. variegata* mediated ZnONPs involved in reduction, stabilization and capping.

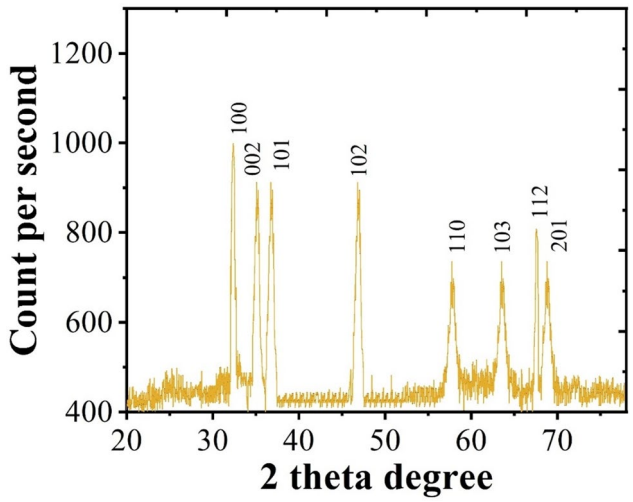


**Fig. 4.** XRD analysis of B.V@ZnONPs confirming the successful synthesis of ZnONPs.

data obtained from our current findings is in agreement to previous data reported by[32, 35, 36]. The absence of any extra peak in the X-ray crystallograph clearly indicate the pure phase synthesis of ZnONPs**.**

EDX analysis validate the elemental composition of B.V@ZnONPs. The sharp peak at 0.4 keV represent oxygen, while the peak at 0.97, 8.68, 9.59 keV revealed to the presence of zinc which is in agreement to the previous research reports[37]. The existence of carbon can be ascribed to the grid support. The EDX image indicated no impurity and any other metal were found absent which determine the purity of B.V@ZnONPs. Detail about EDX spectrum is provided in Fig. 5 and Table 2.

Further, SEM analysis was conducted to reveal the morphological structure and topology of asynthesized NPs. The results of SEM image of B.V@ZnONPs are shown in Fig. 6. SEM images confirmed that the greenly synthesized nano sized particles are irregular in shape and agglomerated.

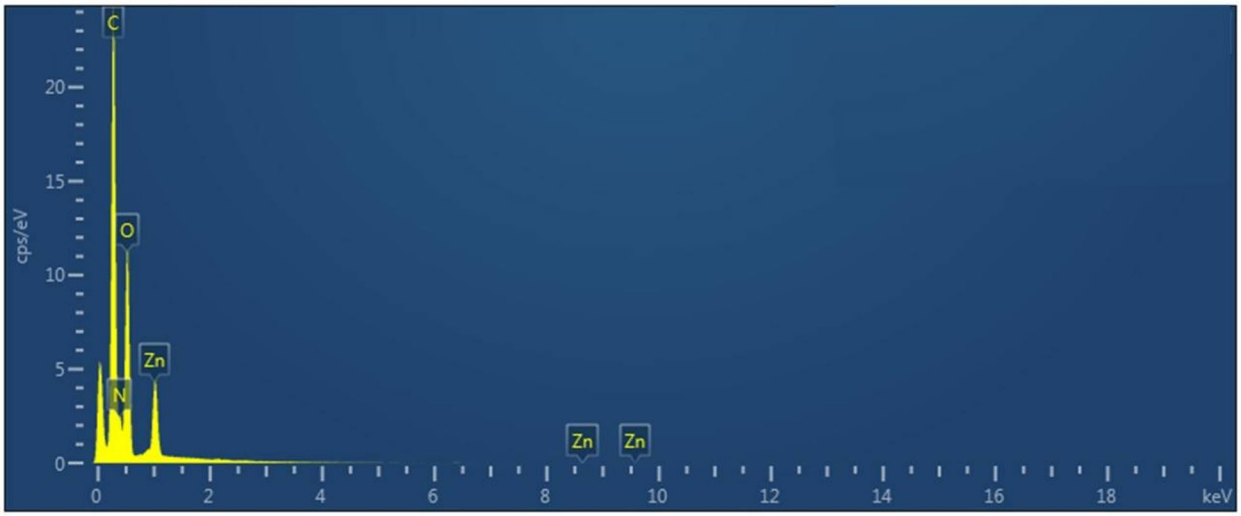


**Fig. 5.** EDX analysis of B.V@ZnONPs.

| Elements | Line type | Apparent concentration | K-ratio | Wt% | Standard label | Factory standard |
|---|---|---|---|---|---|---|
| C | K series | 39.84 | 0.39844 | 53.08 | C Vit | Yes |
| N | K series | 0 | 0 | 0 | BN | Yes |
| O | K series | 43.92 | 0.14781 | 39.14 | $SiO_2$ | Yes |
| Zn | L series | 4.97 | 0.04971 | 7.78 | Zn | Yes |

**Table 2.** Details of EDX spectra of B.V@ZnONPs.

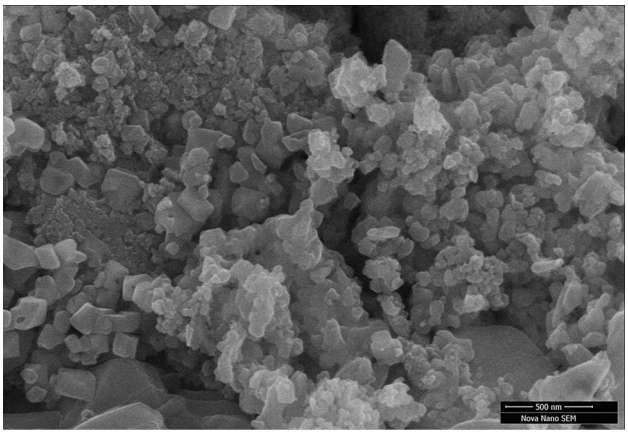


**Fig. 6.** The SEM analysis of B.V@ZnONPs.

## In vitro antimicrobial activities

The antibacterial and antifungal capabilities of green B.V@ZnONPs were investigated using different bacterial and fungal strains. For determination of the antibacterial potential, five different bacterial strains were used and were treated with different doses of B.V@ZnONPs (34.38–1100 µg/mL). The results revealed that *P. aeruginosa* was the most resistant strain (137.5 µg/mL) while *Bacillis subtilis* was observed to be most susceptible strain (34.38 µg/mL) in bactericidal studies. The MICs values are 275.5 µg/mL for *P. aeruginosa*, 137.5 for *K. pneumonia* and 34.38 µg/mL for *B. subtilis* and *S. aureus* respectively. All the different concentrations used in antibacterial study showed dose-dependent responses. The Oxytetracycline was used as positive control to reveal the highest inhibition among all different concentrations of test samples. Our results are in accordance to the previous research studies[3, 38]. Different research studies have shown that the antibacterial potentials of nanoparticles may be due to different factors including surface deficits of NPs[39, 40]. Detail results about the antibacterial activities are shown in Fig. 7.

The antifungal potential of V@ZnONPs was also evaluated five different pathogenic fungal strains including *F. solani, C. albicans, A. flavus*, *M. racemosus* and *A. niger*. To achieve this purpose, the fungal strains were exposed to different doses of V@ZnONPs ranging from 1500 to 46.875 µg/mL. The *F. solani* was reported the most susceptible and *A. flavus* was least susceptible strain in fungicidal examination. The MICs values are 187.5 µg/mL for *A. flavus*, 93.75 µg/mL for *C. albicans* while 46.875 µg/mL for *F. solani*, *M. racemosus* and *A. niger* respectively. All the concentrations used in antifungal study showed dose-dependent responses. The positive control Amphotericin B exhibited highest inhibitions among all different concentrations of test samples against different fungal strains. Our results are in accordance to the previous research studies[3]. Detail results about the antifungal activities are given in Fig. 8.

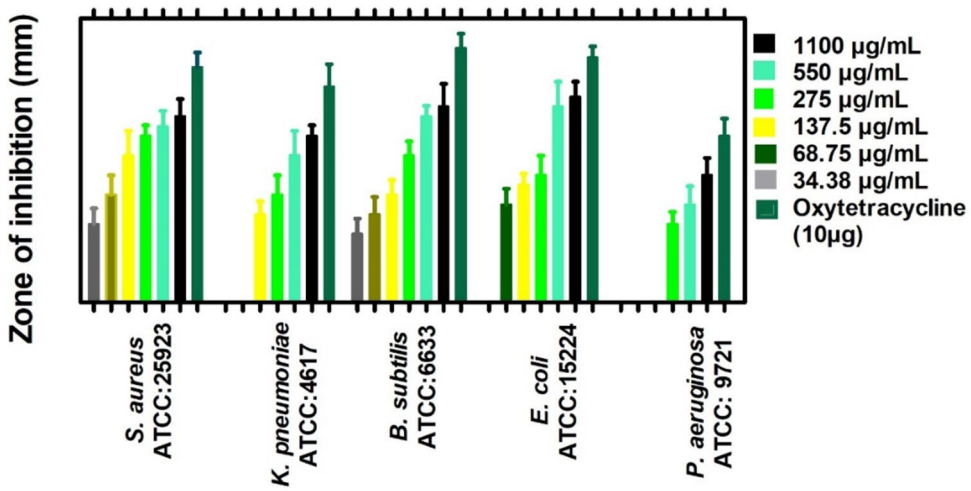


**Fig. 7.** Evaluation of antibacterial potentials of B.V@ZnONPs using different bacterial strains.

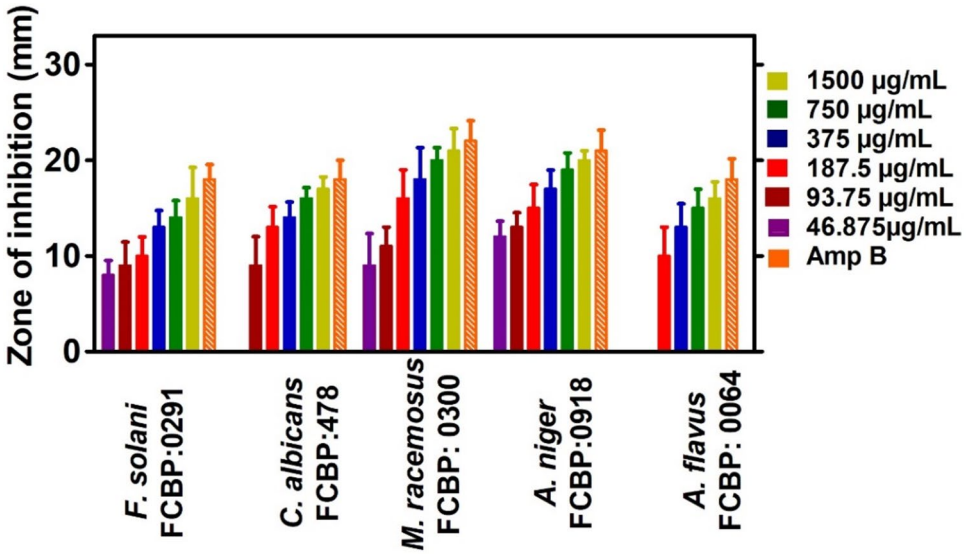


**Fig. 8.** Evaluation of antifungal potentials of B.V@ZnONPs using different pathogenic fungal strains.

### *Artemia salina* cytotoxicity assay (ASCA)

The brine shrimp lethality assay is simple cheap and rapid activity to test the nano sized crystals, which often correlates with the anti-tumor and cytotoxic activities[41,42]. The ASCA is a biological assay or screening test used to determine the cytotoxicity potential of asynthesized B.V@ZnONPs against newly hatched nauplii of brine shrimps[37,43]. Different concentrations of test samples ranging from 1000 to 7.81 µg/mL were used to unveil the dose-dependent cytotoxicity potentials of BV@ ZnONPs. To achieve this purpose, vincristine sulfate served as positive control. The percent mortality is provided in Fig. 9. The $IC_{50}$ for *Astragalus Anisacanthus* based ZnONPs was recorded as 39.78 µg/mL while 3.006 µg/mL was reported for positive control vincristine sulphate. The $IC_{50}$ of 39.78 µg/mL represents the presence of important bioactive and cytotoxic compounds upon the surface of greenly assisted ZnONPs The results confirmed the category of general cytotoxic for greenly synthesized B.V@ ZnONPs and are in line with[44,45].

### Free radical scavenging activity (DPPH antioxidant assay)

The free-radical scavenging assay was performed for the B.V@ZnONPs to investigate the anti-radicle potentials of bioengineered test samples[46,47]. For this purpose, different concentration of ZnONPs were prepared (300–2.35 µg/mL). DPPH-radical scavenging test was exploited to measure the reduce ions adsorbed upon the surface of *B. variegata* mediated ZnONPs. The highest scavenging potential was observed as 82% at highest of 300 µg/mL. The results of our study concluded excellent anti-radicle potency of greenly orchestrated ZnONPs which are in agreement to earlier literature reports[48]. The detailed DPPH activity is illustrated in Fig. 10.

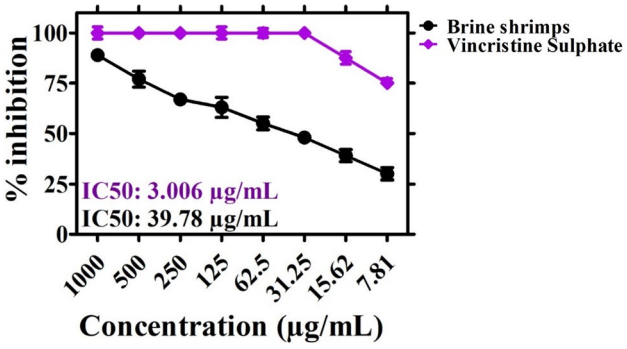


**Fig. 9.** Cytotoxicity potential of BV@ ZnONPs against brine shrimps nauplii at different concentrations of test samples.

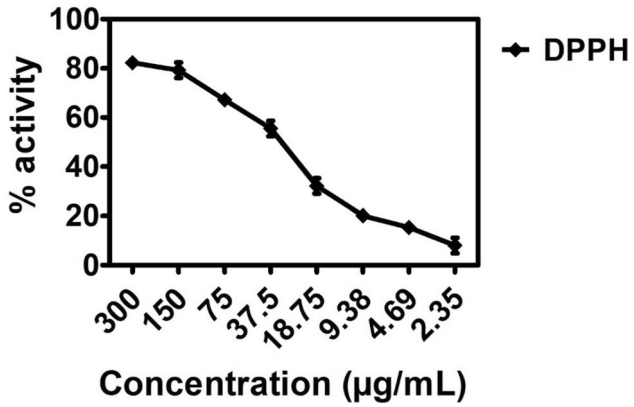


**Fig. 10.** Free radical scavenging activity (DPPH antioxidant) potential of B.V@ ZnONPs.

## Conclusion

The current research study is the first report on the green, environmentally safe, sustainable and cost-effective protocol for the synthesis of ZnONPs using flower buds extract of *Bauhinia variegata* as a biocatalyst for reduction, stabilization and capping of ZnONPs. The synthesis and different other physical and chemical properties of B.V@ZnONPs were verified using different characterization tools (UV, XRD, FT-IR, SEM, EDX). Different biological activities such as cytotoxicity (brine shrimps cytotoxicity assay), antibacterial (using different bacterial strains), antifungal (using different fungal strains) and antiradical assays were evaluated and have shown exceptional biological activities due to their diverse phytochemicals on their surfaces. In future, we suggest different other in vitro and in vivo biological applications of ZnONPs as an eco-friendly catalyst to further unveil the biocompatible and bio-safe nature of ZnONPs. In future, we also recommend different mechanistic studies to further explore the mechanism of action of as-prepared ZnONPs. In nutshell, ZnONPs possess significant promise for applications in pharmacological and biological fields, making them a promising subject of investigation.

## Data availability

All the raw data in this research can be obtained from the corresponding authors upon reasonable request.

Received: 13 May 2024; Accepted: 5 September 2024
Published online: 11 September 2024

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## Acknowledgements

The authors extend their appreciation to the Researchers supporting project number (RSP2024R470), King Saud University, Riyadh, Saudi Arabia.

## Author contributions

S.A., J.I. designed and conceived the study. S.A., B.A.A. and J.I., completed the experiments. S.I., T.Y., R.U., F.Z., and Z.U. analysed and reviewed the data. G.M., R.I., Z.A.S., S.A.M., M.S.E., S.A., and M.R., performed visualizations and statistical data analysis. S.A., and J.I., wrote the original draft. J.I. and B.A.A., provided resources. All authors made valuable revisions and edited the manuscript and approved the last version.

## Funding

Open Access funding enabled and organized by Projekt DEAL. Researchers Supporting Project number (RSP2024R470) King Saud University, Riyadh, Saudi Arabia.

## Competing interests
The authors declare no competing interests.

## Statement on guidelines
All experimental studies and experimental materials involved in this research are in full compliance with relevant institutional, national and international guidelines and legislation.

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