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Green Synthesis and Characterization of Zinc OxideNanoparticles using *Iphiona scabra* DC. ex Decne Leaf Extract

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Abstract:

Yemeni *Iphiona scabra* (Y*IS*) is widely employed in traditional medicine for the treatment of various diseases and is abundant available in Yemen. In this study, the aqueous leaf extract of *Iphiona scabra* (*IS*.L) was utilized for the green synthesis of zinc oxide nanoparticles (IS-ZnONPs), following a sustainable and environmentally friendly approach. The plant extract acted as both a stabilizing agent and a reducing agent. During synthesis, noticeable color changes were observed in the reaction mixture, shifting from light blue to light brown and finally to dark brown, indicating the successful formation of nanoparticles. The obtained (IS-ZnONPs) were thoroughly characterized vai UV–Visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), and X-ray diffraction (XRD). The UV–Vis spectrum revealed a distinct absorption peak at approximately 373 nm for the *I. scabra* ZnO NPs. Additionally, the band gap energy was found to be 5.6 V. FTIR analysis revealed the functional phytochemical groups responsible for the reduction and capping of the nanoparticles. SEM analysis revealed quasispherical nanoparticles with a homogeneous morphology and a strong tendency to form aggregated clusters, whereas XRD confirmed their crystalline structure and high purity, with an average crystallite size of 14 nm.

Keywords: Green synthesis; *Iphiona scabra* leaves; ZnO NPs.

Introduction

Nanotechnology, a cornerstone of modern scientific advancement, is fundamentally based on nanoparticles (NP) materials which typically range from 1 to 100 nm and whose unique properties are governed by their nanoscale dimensions [1]. In recent years, the green synthesis of nanoparticles has gained increasing importance as an environmentally friendly and cost-effective alternative to traditional chemical and physical methods. Plant-mediated synthesis offers a dual role, where phytochemicals act both as reducing and stabilizing agents, enabling the formation of biocompatible nanoparticles with controlled morphology and size distribution [2,3]. Zinc oxide nanoparticles (ZnO NPs) are inorganic nanoparticles characterized by a hexagonal structure. They exhibit a unique combination of semiconductor properties, a high surface area-to-volume ratio, and biocompatibility. These properties make them highly valuable for diverse applications [4, 5, 6]. Despite this remarkable progress, certain medicinal plants remain underexplored. *Iphiona scabra*, a medicinal plant traditionally used for treating inflammatory and cardiovascular disorders [7].

Fig.1 Photograph of *Iphiona scabra*

Iphiona scabra, belongs to the Asteralaceae plant family, as shown in (Fig. 1). This plant is widely distributed in several countries, particularly Yemen, Sudan, Eritrea, Somalia, Ethiopia, Egypt (Sinai), and Saudi Arabia. In Yemen (Hadhramout), Iphiona scabra has a long history in traditional folk medicine and is widely used as an herbto treat various household remedy ailments. *Iphiona scabra* is a rough-textured, aromatic, yellow-flowered shrublet of arid landscapes, wadis (dry riverbeds) and desert plains, that is easily recognized by its scent strong, resinous, camphoraceous, and tubular florets. Despite this remarkable progress in this field, certain medicinal plants remain underexplored. These bioactive constituents make *Iphiona scabra* a promising candidate for green nanoparticle synthesis. However, to the best of our knowledge, no prior report has documented the use of *Iphiona scabra* leaf extract for the biosynthesis of zinc oxide nanoparticles (ZnO NPs). Therefore, this study aimed to synthesize and characterize ZnO NPs synthesized from *Iphiona scabra* leaf extract.

2. Materials and Methods

2.1. Reagents and chemicals

Zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$, 99.0%), sodium hydroxide (NaOH), dimethyl sulfoxide (DMSO, 99.9%) and deionized water were purchased from Alhosam for medical devices, Supplie and chemical agents Mukalla, Hadhramout, Yemen. Analytical-grade chemicals and reagents were all used exactly as needed, and no additional purification was needed.

2.2. Collection and Identification of *Iphiona scabra* DC. ex Decne

The plant *Iphiona scabra* DC. ex Decne is a common species in the region and is not endangered. Although a voucher specimen is not strictly required, one has been deposited in the Herbarium of the Department of Biology, Faculty of Education, Aden University, Yemen. Currently, it does not have an identification number and will be assigned one upon the herbarium's completion of international registration and obtaining an international code.

The species identity was further confirmed by comparison with a historical voucher specimen (Täckholm 28-01-1929 [CAI]) deposited at the Cairo University Herbarium (CAI), Egypt, an internationally recognized herbarium.

The taxonomical classification according to Plants of the World Online (POWO, 2026) is as follows:

Kingdom: Plantae

Phylum: Streptophyta

Class: Equisetopsida

Subclass: Magnoliidae

Order: Asterales

Family: Asteralaceae

Genus: *Iphiona*

Species: *Iphiona scabra* DC.ex Decne

On August30, 2023 *Iphiona scabra* was collected from Jabal Al-Des area, located at latitude 14° 46′ 10.05″ N and longitude 049° 15′ 59.91″ E, at an altitude of 117.9 m above sea level, approximately 180 km south of AL-Mukalla City, Hadhramaut Governorate, Yemen (Fig. 2). The plant was identified by Prof. Hana Al-Qahbi (Plant Ecologist), Department of Biology, Faculty of Education, Aden University, Yemen, based on comparison with relevant literature [8-11], as well as by consulting protologues and the Plants of the World Online database [8].

The plant's fresh leaves were collected and cleaned with deionized water (DIW), then chopped into small pieces and left to dry at room temperature. After drying, the leaves were ground into a fine powder, which was accurately weighed and stored in a jar in the refrigerator. This standard methodology ensures the preservation of the chemical and physical properties of the leaves, making them suitable for use in analysis.

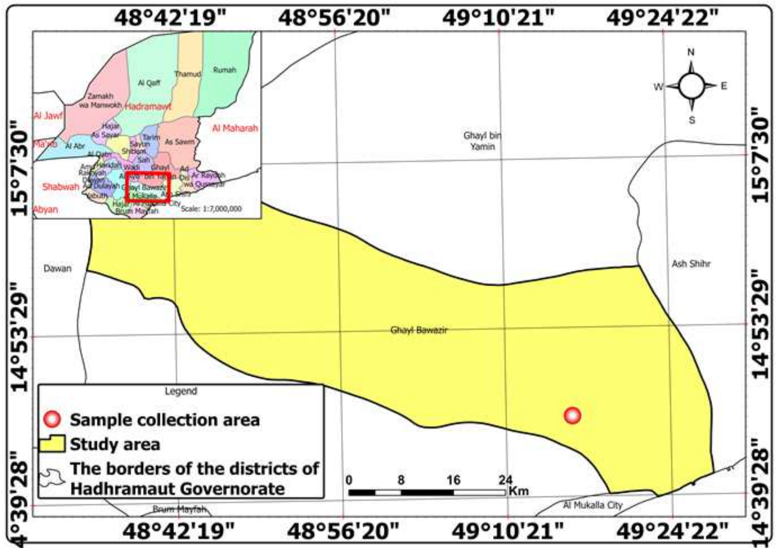


Fig. 2. A map showing the location of the plant collection site in Hadhramaut Governorate, Yemen. The red dot indicates the precise collection point at Jabal Al-Des area (latitude: 14° 46′ 10.05″ N, longitude: 049° 15′ 59.91″ E), approximately 180 km south of Al-Mukalla City. The map also shows the broader study area boundaries (marked by longitudes 48°42'19" to 49°24'22" E).

2.3. Preparation of *Iphiona scabra* leaf extract

After mixing 100 mL of (DIW) with approximately 20 g of powdered *Iphiona scabra (Is.L)*, the mixture was heated to 80°C for 60 minutes on a magnetic stirrer with continuous stirring according to the method of [12]. The solution turned pale brown. The resulting extract was filtered once it had cooled to room temperature (RT). To use the extract to create ZnO NPs, it was finally cooled and maintained at 4 °C. (Fig. 3) shows a pictorial representation of the *Iphiona scabra* extract production process.

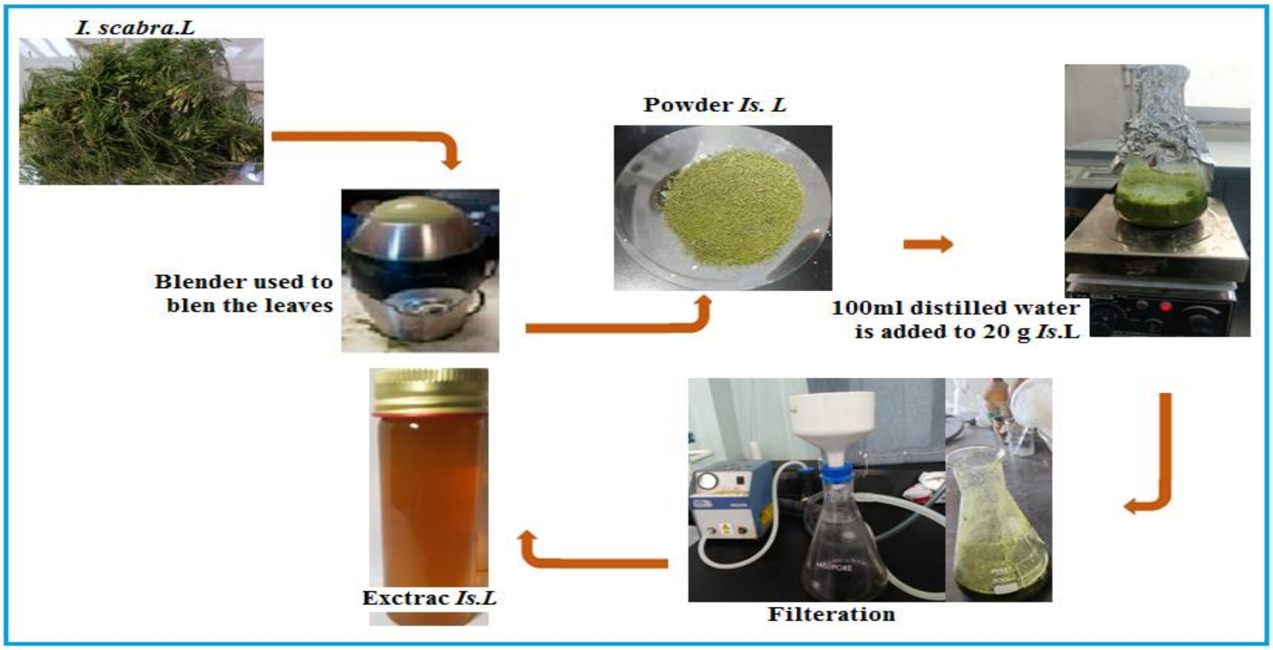


Fig. 3 Preparation mechanism of *I. scabra* leaf extract.

2.4. Green synthesis of ZnO NPs

A total of 20 ml of the produced aqueous leaf extract *(Iphiona scabra* .L) was combined with 80 ml of 0.4 M and colorless zinc nitrate hexahydrate (Zn $(NO_3)_2.6H_2O$) in a 250 mL beaker was slowly added dropwise into the solution. The mixture was agitated for 90 min vai a magnetic stirrer after 0.02 M NaOH solution was added to obtain a pH of 12. After the PH of the solution was adjusted, a white precipitate, formed, indicating the presence of zinc oxide nanoparticles [13,12]. After that, the mixture was centrifuged at 8000 rpm for 20 minutes. Finally, the dried product was baked for 120 minutes at 50 °C in a hot air oven. ZnO NPs were created by calcining the resulting white powder for four hours at 500 °C in a muffle furnace. The procedures used to prepare the ZnO NPs are shown in (Fig. 4).

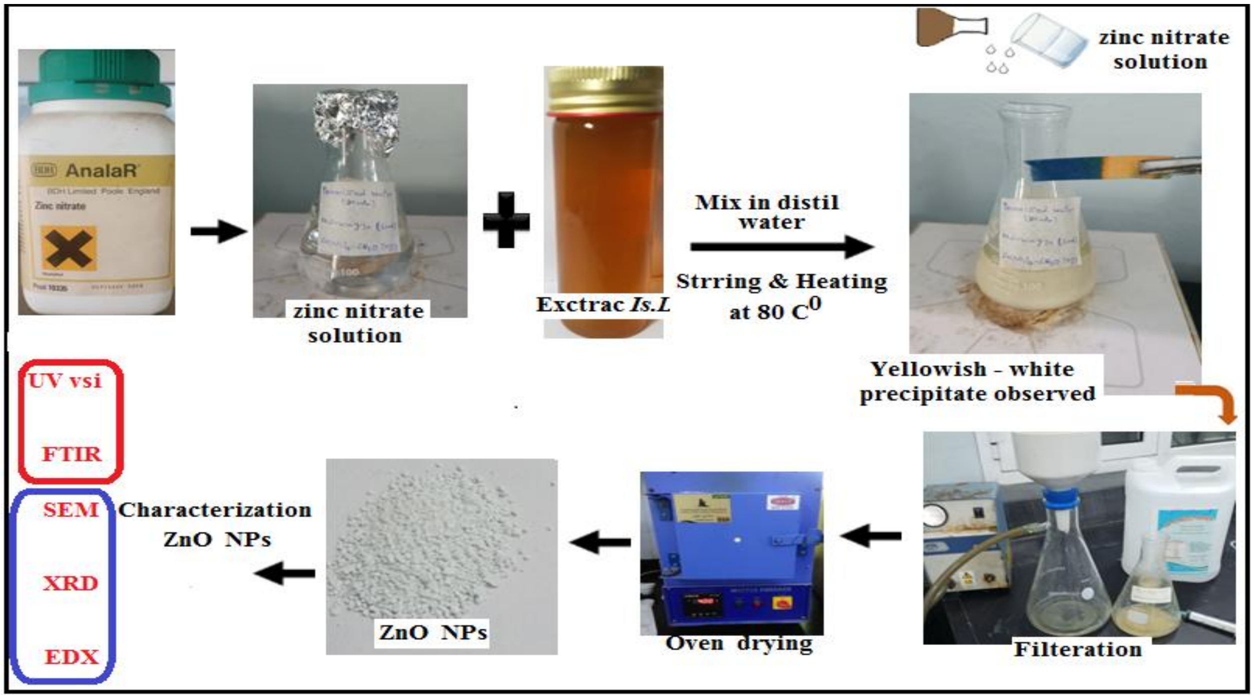


Fig. 4 Biosynthesis and characterization of ZnO NPs using *Iphiona scabra* leave extract

2.5. Characterization of the IS-ZnO NPs

UV-Vis, FT-IR, XRD, SEM and EDX analyses confirmed the successful synthesis and characterization of the ZnONPs.

2.5.1. UV–Visible spectroscopy analysis

UV-visible spectroscopic analyses of the IS-ZnONPs (dissolved in DMSO) and aqueous leaf extract of *Iphiona scabra* Yemeni (ISY) were conducted vai (Jasco V-730 with serial number A063361798) spectroscopy across a 200–800 nm wavelength range. The stability of the synthesized IONPs was assessed through periodic UV-Vis measurements. All stability studies were conducted under controlled temperature (25 ± 2°C) dark storage.

2.5.2. Fourier transform infrared spectroscopy

Fourier transform infrared (FT-IR) spectroscopy was used to characterize the aqueous leaf extracts of *Iphiona scabra* Yemeni (ISY) and the IS-ZnONPs. The analysis was accomplished with a Perkin Elmer spectrum IR Veirsion 10.6.2, and the IR spectra were characterized at wavenumbers of 4000 - 400 cm^{-1}.

2.5.3. X-ray diffraction

The XRD pattern was recorded vai a Bruker D8 Discover (Germany) diffractometer at ambient temperature. Radiation (λ = 1.54060 Å) was generated and a copper source at 40 kV, and 40 mA was used. Diffraction patterns were recorded over a 2θ range of 5° to 100°.

2.5.4. Scanning electron microscopy (SEM)

The morphology of the IS-ZnONPs was examined vai TESCAN Ultra-High Resolution and SEM from Germany. X-ray energy dispersive spectroscopy (EDS) was also performed with a scanning electron microscope (20 kV). The nanoparticles were dispersed in DMSO, and sonicated for 20 minutes, after which one drop was placed on a copper grid coated with carbon to create the samples. Prior to examination, the samples were vacuum-dried for 24 hours at a 15 kV accelerating voltage. The method allowed for extensive structural analysis of the nanoparticles by providing high-resolution imaging capabilities of 1 nm at accelerating voltage of 15 kV.

2.5.5. Energy-dispersive X-ray (EDX) microanalysis

The elemental analysis and distribution of the IS-ZnONPs were confirmed via energy-dispersive X-ray spectroscopy.

3. Results and Discussion

3.1. Characterization of the IS-ZnO NPs

3.1.1 Visual observation

In the initial phase of the experiment, we dissolved the precursor zinc nitrate hexahydrate in DW, which remained colourless (Fig. 5a). A noticeable color shift occurred when *Iphiona scabra* Yemeni (ISY) extract was added to the zinc nitrate hexahydrate solution, indicating a reduction reaction (Fig. 5b) , while the mixture was constantly stirred. The mixture was then heated to 80 °C for 20 min, and as the reaction progressed, the color shifted from pale yellow to white, as observed in (Fig. 5c).

Fig. 5 Color of (a) the zinc nitrate hexahydrate solution, (b) when the (*IS*Y) extract was added, and (c) the color shifted at pH (12).

3.1.2. UV–Vis analysis

Green synthesis of IS-ZnONPs using *Iphiona scabra* Yemeni leaf extract was indicated by UV spectroscopy in the 200–900 nm wavelength range. The UV-visible spectrum of the pure *Iphiona scabra* Yemeni leaf extract (Fig. 6 (a)), exhibited two distinct absorption bands at 227 nm, and 286 nm, indicating that the extract compounds acted as reducing and capping agents to synthesize the IS-ZnONPs [14]. (Fig. 6 (b)) shows the UV-Vis absorption spectra of the synthesized ZnO NPs in zinc nitrate hexahydrate ($Zn\ (NO_3)_2.6H_2O$) in the *Iphiona scabra* leaf extract. The UV-Vis spectrum of the IS-ZnONPs showed a peak at 373 nm, which was in accordance with the previously reported literature [15], whereas the peak at 374 nm was obtained for the ZnO NPs obtained from an aqueous extract of *C. spinosa* (Fig. 6 (c)) shows the band gap calculation of the green synthesized IS-ZnONPs by using the Tauc relation. The calculated band gap of the synthesized (IS-ZnONPs) is 2.38 eV. A similar result was reported by [16], where a strong peak at 400 nm was obtained. In a study conducted by [17], where ZnONPs were synthesized from *M. longifolium* leaf extract, a corresponding absorption peak at 325 nm was obtained. A study of the coffee ground mediated synthesis of ZnONPs was carried out by [18], and yielded an absorption peak at 320 nm, whereas a study of the *L. pruinosum* extract carried out by [19], yielded an absorption peak at 375 nm.

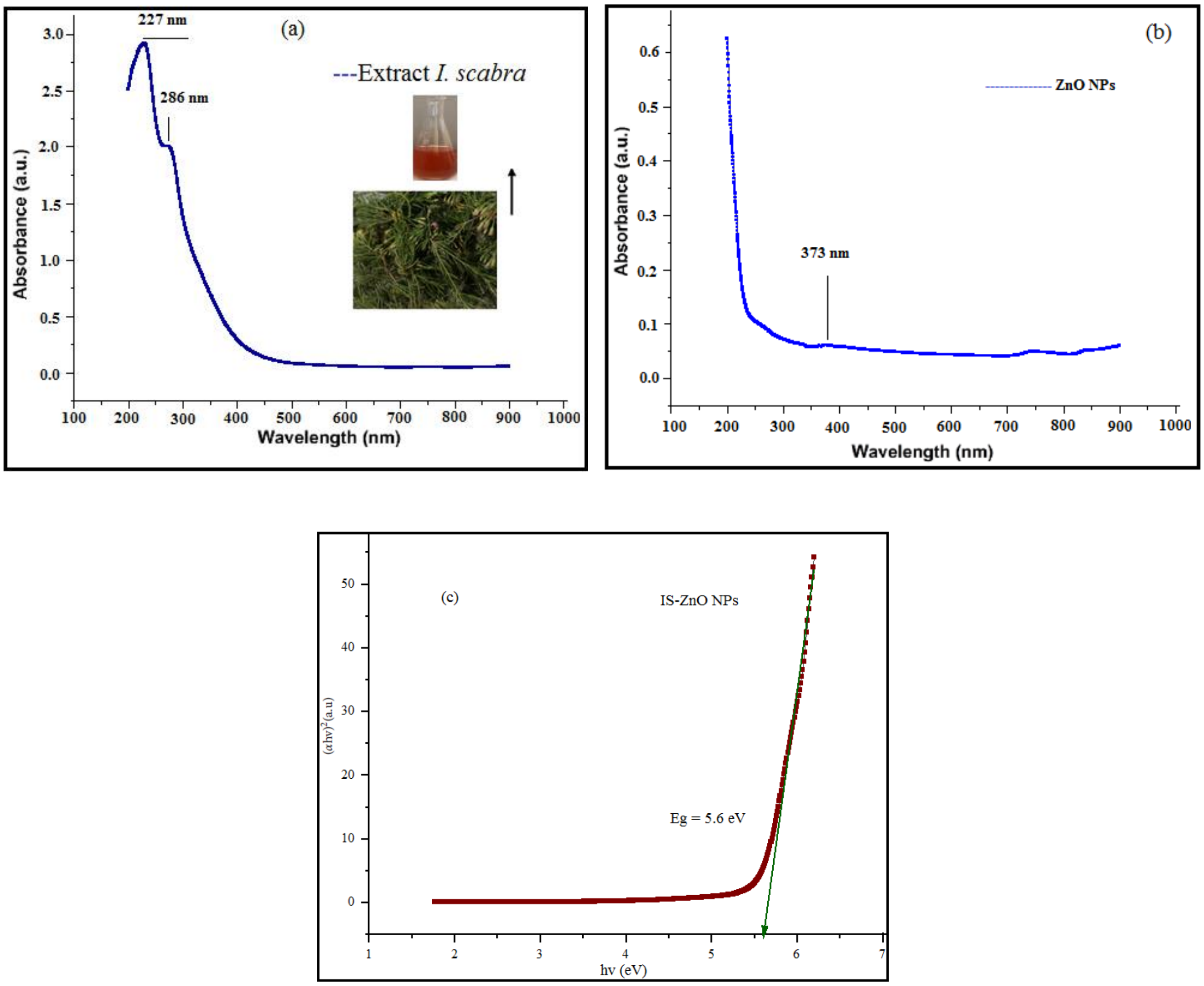
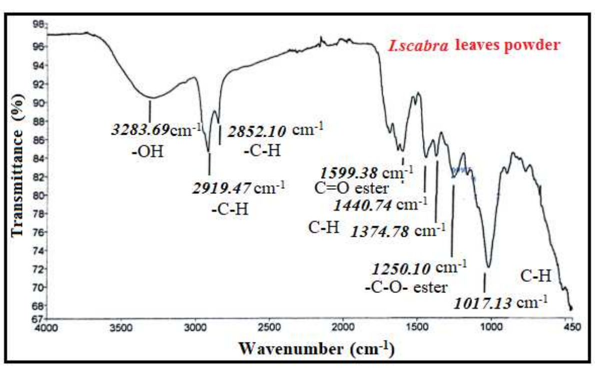


Fig. 6 UV-Vis absorption spectra of the *Iphiona scabra* leaf extract (a), ZnO NPs (b) and bend gap (c).

3.1.3. FT-IR spectroscopy analysis

Fourier transform infrared (FTIR) spectroscopy was employed to identify the functional groups present in the *I. scabra* powder, the aqueous extract of *Iphiona scabra* leaves, and the biosynthesized Zinc oxide nanoparticles (IS-ZnO NPs). The resulting spectra, presented in Figures (7, 8, and 9), revealed characteristic absorption bands across different spectral regions. A prominent broad band was observed in the single bond stretching region, which shifted from 3283.69 cm^{-1} in the *Iphiona scabra* powder (Fig. 7) and the aqueous extract (Fig. 8) to 3307.55 cm^{-1}, suggesting the presence of hydroxyl (OH) groups associated with alcohols and phenolic compounds. This band may also be attributed to the presence of water molecules adsorbed on the samples. In the *Iphiona scabra* powder spectrum (Fig. 7), weak absorption peaks at 2919.47 cm^{-1} and 2848.86 cm^{-1} confirmed the stretching vibrations of aliphatic (–CH) functional groups which are found in organic components of the *Iphiona scabra* powder [13]. A minor peak observed at 2102.51 cm^{-1} in the aqueous extract spectrum (Fig. 7) could be attributed to the stretching vibration of O=C=O. This may arise from trace impurities introduced during the synthesis process or from specific measurement conditions [20]. The absorption band at 1635.21 cm^{-1} in the aqueous extract spectrum (Fig. 7) is likely due to the stretching vibration of the (C=C) group, which is characteristic of phenolic compounds present in the plant extract, these results match those of previous studies [15,13]. The peak at 1599.38 cm^{-1} in the *Iphiona scabra* powder spectrum (Fig. 7) may be attributed to the asymmetrical and symmetrical stretching modes of carboxylate ions (O=C=O) [21]. The bending vibrations of C–H bonds (-CH$_2$ and -CH$_3$) are indicated by the peaks observed at 1440.74 cm^{-1} and 1374.84 cm^{-1} in the spectra of the *Iphiona scabra* powder (Fig. 7). The band at 1250.10 cm^{-1} could be assigned to the C-O stretching vibration, potentially of ester or ether linkages. The prominent peak at 1017.13 cm^{-1} in the aqueous extract spectrum (Fig. 8) is attributed to the C-OH bending vibration, which is indicative of the presence of carbohydrates within the *Iphiona scabra* extract [22,20]. The spectrum of the synthesized (IS-ZnONPs) (Fig. 9), exhibited a small absorption peak at 2981.45 cm^{-1}, indicating that the corresponding –OH stretching band, at1433.48 cm^{-1} represented alkanes, the, peak at 952.26 cm^{-1} represented the shift of the C–O stretching band of carboxylic acid, and another peak was observed at 448.12 cm^{-1}, which was consistent with the Zn-O stretching mode, confirming the formation of the IS-ZnO NPs. This observation aligns with previously reported literature on the FTIR characterization of ZnO nanoparticles synthesized via biological methods [13,14].



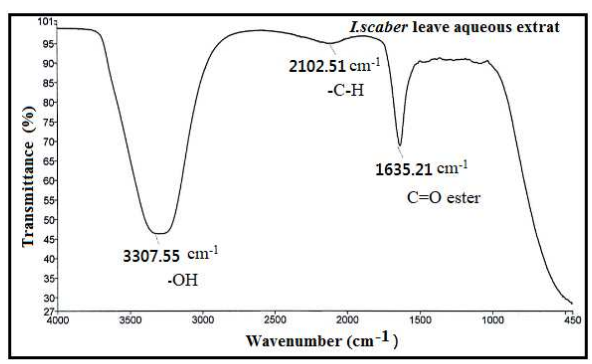


Fig. 7 FT-IR spectrum *I. scabra* leaves powder

Fig. 8 FT-IR spectrum of the aqueous extract from *I. scabra*.

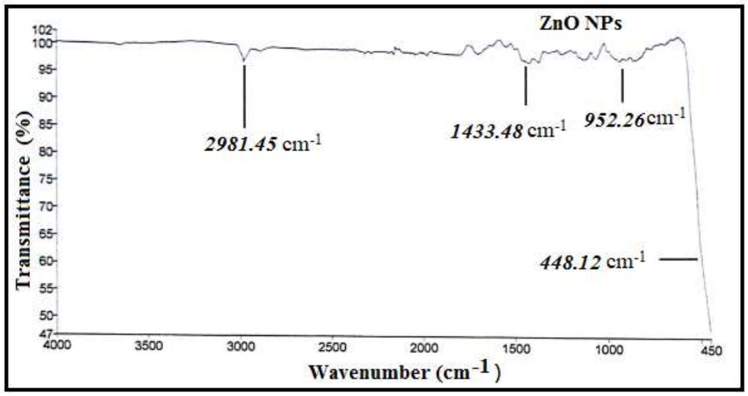


Fig. 9 FT-IR spectra of the synthesized ZnO NPs.

3.1.4. XRD analysis

XRD confirmed the crystallinity of the green-prepared ZnO NP based leaf extract of *Iphiona scabra* (Fig. 10). The XRD diffractogram at 2θ values revealed that the main integrated peaks at 31.63, 34.23, 36.12, 47.37, 56.67, 62.82, 66.28, 67.81 and 68.93° corresponded to the (100), (002), (101), 102, (110), (103), (200), (112), (201), (004) and (202) diffraction planes, respectively. The results of the XRD analysis of ZnO are in line with those of previous reports, which revealed the crystal structure of ZnO NPs created from plant extracts [17, 23, 2]. Our results matched those of [20], who biosynthesized crystal ZnO NPs via *Laurus nobilis* L. By using Deby-Scherrer's equation (Eq. (1)) , which was calculated from the full width at half maximum (FWHM) of the most intense peak connected to the (101) plane, which is located at 36. 12°, the average crystalline size of the ZnO NPs synthesized with the leaves of *Iphiona scabra*, was determined to be 14 nm. Additionally, the characteristic ZnO peaks were the only peaks detected, indicating the purity of the synthesized ZnO NPs by Yemeni *Iphiona scabra* leaf extract, The (IS-ZnO NPs) were calcined at 500 °C for two h.

$$\Phi = K\lambda / \beta \cos\theta \quad \text{……………………………………} (1)$$

where, Φ is the crystalline size, λ is the wavelength of X–ray used; K is the shape factor, β is the full line width at the half–maximum elevation of the main intensity peak, and θ is the Bragg angle.

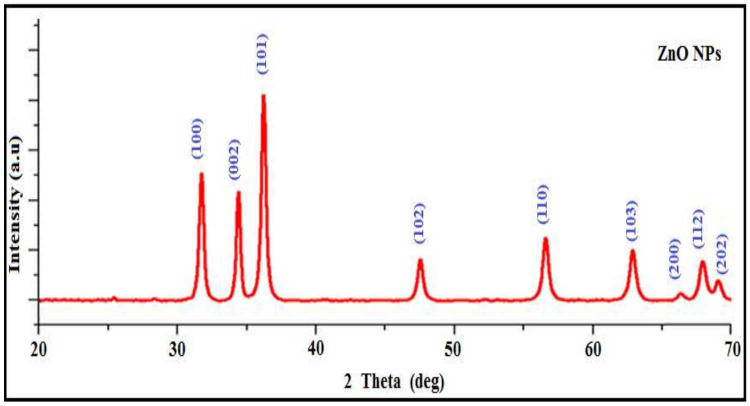
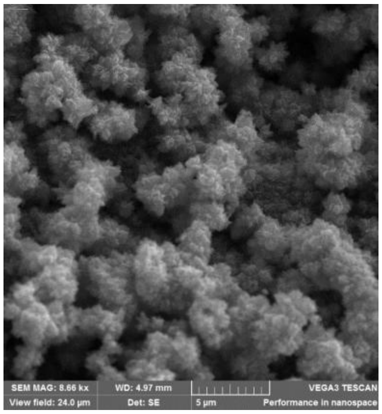


Fig. 10 XRD pattern of the ZnO-NPs.

3.1.5. SEM and EDX analysis

SEM analysis revealed that the synthesized (IS-ZnONPs) are predominantly spherical to quasi-spherical in shape and fall within the nanometer size range (Fig. 11). The nanoparticles tend to form agglomerates, a characteristic feature attributed to the capping and stabilizing effects of phytochemicals present in the plant extract used for synthesis. The observed uniformity in particle size and morphology indicates that the synthesis method provides effective control over the nucleation and growth phases. These findings are in strong agreement with previous reports on ZnO nanoparticles synthesized via similar green chemistry routes, thereby validating the efficacy of the employed method. Compositional analysis was performed via EDS, as shown in the corresponding spectrum (Fig. 12 a, b). The spectrum confirms that the material is composed primarily of zinc (Zn) and oxygen (O), with no detectable impurities. The signature peaks for zinc were identified at approximately 1.0 keV (Lα), 8.6 keV (Kα), and 9.5 keV (Kβ), whereas the oxygen (O) peak was observed at approximately 0.5–0.6 keV. Quantitative analysis revealed that the elemental composition was approximately 80.7% zinc and 19.3% oxygen by weight, which closely matches the theoretical stoichiometry of zinc oxide. These results confirm the successful synthesis of high-purity (IS-ZnONPs), rendering them suitable for advanced applications in optoelectronics, photocatalysis, and biomedicine [13,14].



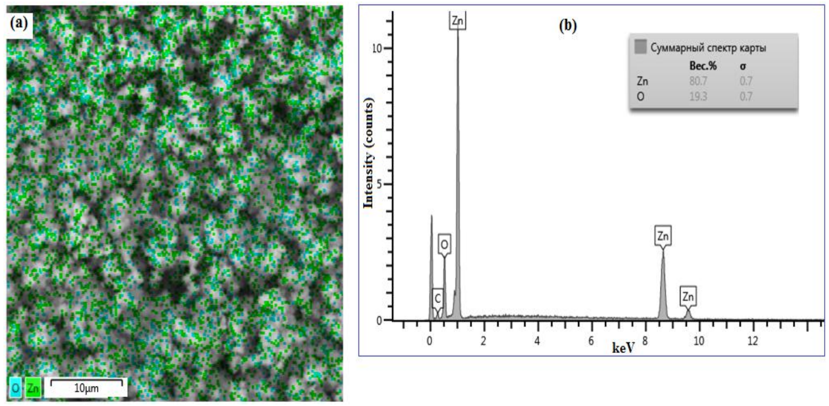


Fig. 11 SEM micrograph of the IS-ZnO NPs. **Fig. 12** (**a** and **b**) EDX micrograph of the IS-ZnO NPs.

4. Conclusions

This study demonstrated the successful preparation of zinc oxide nanoparticles (IS-ZnONPs) using the aqueous leaf extract of *Iphiona scabra* from Yemen as a natural reducing and stabilizing agent. The proposed approach is simple, eco-friendly, and sustainable, highlighting its potential as an alternative strategy to mitigate the global challenge of antibiotic resistance. Unlike conventional chemical and physical synthesis routes, green synthesis offers a nontoxic pathway that minimizes environmental hazards. The phytochemicals present in different parts of *Iphiona scabra* including leaves, fruits, seeds, and roots play crucial roles in mediating nanoparticle formation. The synthesis of the (IS-ZnONPs) was confirmed by UV–Vis spectroscopy, which revealed a characteristic absorption peak at approximately 373 nm. Morphological features such as size, shape, and stability were determined through scanning electron microscopy (SEM). Fourier transform infrared (FTIR) spectroscopy confirmed the capping of the nanoparticles with bioactive compounds derived from the plant extract, whereas X-ray diffraction (XRD) analysis verified their crystalline structure.

Author contributions

Mahmood Mohammed Ali Saleh (1) (Data curation: Supporting; Formal analysis: Lead; Investigation: Lead; Writing – original draft: visualization, methodology, formal analysis, Writing – review & editing: Lead).

Hana Abdulrahman Saleh AL-Quhbi (2) Formal analysis: Methodology; Validation: Supporting; Visualization:

Writing – original draft Supporting:; Writing – review & editing: Supporting).

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Ethics and Consent to Participate declarations: Not applicable.

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Competing interests the author declares that there are no competing interests.

Data availability statement: All the data generated or analyzed during this study are included in this published article.

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