

Formulation and Evaluation of Hydrogel Incorporated With Zinc Oxide Nanoparticles Mediated By Cassia Fistula L. Flower Extract For Anti-Aging Property

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Abstract

Green synthesis of metal oxide nanoparticles using plant-derived materials has attracted increasing interest as an alternative approach to conventional nanoparticle preparation. The present study investigated the plant-mediated synthesis and physicochemical characterization of zinc oxide nanoparticles (ZnO NPs) using Cassia fistula L. flower extract. The synthesized nanoparticles were characterized using UV–Visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and energy-dispersive X-ray (EDX) analysis. The synthesized material showed a reported UV–Visible absorption maximum at 370 nm. FTIR analysis exhibited characteristic absorption bands at approximately 3360, 1633, 1384, and 474 cm⁻¹, with the band at 474 cm⁻¹ attributed to Zn–O stretching. XRD analysis showed principal reflections at 2θ values of 31.7°, 34.4°, and 36.2°, corresponding to the (100), (002), and (101) planes of hexagonal wurtzite ZnO, respectively. The average crystallite size was reported as approximately 28 nm based on the Debye–Scherrer equation. SEM examination revealed predominantly spherical and hexagonal morphological features with relatively uniform distribution and some aggregation, while the thesis-reported TEM analysis indicated nanoparticle dimensions below 30 nm. EDX analysis demonstrated the presence of zinc and oxygen together with carbon and nitrogen, with Zn and O accounting for 29.16 wt% and 46.08 wt%, respectively. Overall, the characterization findings document the formation of a ZnO-containing nanoscale material obtained using C. fistula flower extract and provide physicochemical information relevant to its further investigation in topical formulation development.

Keywords: Cassia fistula L.; green synthesis; zinc oxide nanoparticles; plant-mediated synthesis; UV–Visible spectroscopy; FTIR; X-ray diffraction; SEM; TEM; EDX.

1. Introduction

Zinc oxide (ZnO) is an important metal oxide semiconductor that has received sustained attention in nanoscience because its physicochemical properties can vary with particle size, morphology, crystallinity and surface characteristics. At the nanoscale, ZnO exhibits properties that have supported investigation in biomedical, pharmaceutical, antimicrobial, catalytic and topical applications. Recent reviews have particularly highlighted the relevance of ZnO nanoparticles (ZnO NPs) to biomedical research, including their investigation in antimicrobial systems, wound-related applications, drug delivery and other nanomedicine platforms (El-Saadony et al., 2024). The physicochemical characteristics of ZnO NPs are closely related to their synthesis conditions and therefore require appropriate structural, optical, morphological and elemental characterization.

Several physical and chemical approaches have been developed for the preparation of ZnO NPs. However, conventional synthesis routes may involve harsh chemical reagents, multiple processing steps and relatively high energy requirements. These considerations have contributed to increasing interest in biological and plant-mediated approaches to nanoparticle synthesis. Phytochemical synthesis uses plant-derived materials as a source of biomolecules that can participate in nanoparticle formation and surface stabilization. Recent literature describes plant extracts as sources of diverse phytochemicals, including phenolic compounds, flavonoids, terpenoids and other constituents that can interact with metal ions and influence the formation, stabilization, morphology and physicochemical characteristics of the resulting nanoparticles (Al-darwesh et al., 2024a; Ravi & Babu, 2025). Importantly, the characteristics of plant-mediated ZnO NPs are not independent of the biological source or synthesis conditions; factors such as plant part, extraction solvent, extract concentration, precursor concentration, pH, temperature and reaction time can influence nanoparticle size, morphology and structural properties.

The use of plant extracts therefore provides a biologically based route for nanoparticle preparation while also introducing a source-dependent chemical complexity. Recent reviews have emphasized that the phytochemical composition of an extract can influence nanoparticle formation and that there is no single extraction or synthesis procedure that is universally applicable to all plant species and plant parts. Consequently, documenting the plant

material, extraction procedure and synthesis conditions is important for reproducibility and for understanding the physicochemical characteristics of the resulting nanomaterial(Jadoun et al., 2024; Khan et al., 2022).

Cassia fistula L. is a medicinal plant belonging to the family Fabaceae that has been investigated for its diverse phytochemical constituents and pharmacological properties. A recent comprehensive review reported the presence of several classes of bioactive constituents in C. fistula, including glycosides, tannins and flavonoids, and summarized reported antioxidant, antimicrobial, anti-inflammatory and other biological activities associated with the plant(Mwangi et al., 2021). Such phytochemical diversity provides a rationale for investigating C. fistula as a biological source for nanoparticle synthesis. However, the presence of phytochemicals in a plant extract alone does not establish their individual contribution to nanoparticle formation; their specific roles require experimental investigation.

Plant-mediated ZnO synthesis has consequently become an active area of research. Recent literature has described the use of different plant parts and extracts for ZnO nanoparticle preparation and has emphasized the relationship between synthesis conditions, nanoparticle physicochemical properties and potential application(Dey & Somaiah, 2022a) . In particular, recent reviews have identified the need for improved control of synthesis parameters, reproducibility, characterization and understanding of nanoparticle–biological interactions before broader biomedical translation can be established(Rahman et al., 2022) .

Despite the growing body of research on plant-mediated ZnO nanoparticles, the physicochemical characteristics of nanoparticles produced from a particular plant source cannot be assumed solely from previously published reports because differences in plant material, extraction conditions and synthesis parameters may produce different nanoparticle characteristics(Asif et al., 2023) . Therefore, characterization using complementary analytical techniques is essential. UV–Visible spectroscopy can provide information on optical absorption; Fourier-transform infrared spectroscopy (FTIR) can identify functional groups associated with the nanoparticle material and surface-associated biomolecules; X-ray diffraction (XRD) can provide information regarding crystalline structure and crystallite size; scanning electron microscopy (SEM) and transmission electron microscopy (TEM) can provide morphological and dimensional information; and energy-dispersive X-ray (EDX) analysis can provide elemental composition(Shabbir Awan et al., 2023) .

Accordingly, the present study was undertaken to synthesize ZnO nanoparticles using Cassia fistula L. flower extract through a plant-mediated approach and to characterize the resulting material using UV–Visible spectroscopy, FTIR, XRD, SEM, TEM and EDX. The study focuses on documenting the optical, functional-group, crystalline, morphological and elemental characteristics of the synthesized material, thereby providing a physicochemical basis for its subsequent investigation in topical formulation development(Assad et al., 2024) .

2. Materials and Methods

2.1. Materials

Fresh flowers of Cassia fistula L. were used as the plant material for the plant-mediated synthesis of zinc oxide nanoparticles (ZnO NPs). Zinc nitrate hexahydrate [$\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$] was used as the zinc precursor and was obtained from Loba Chemie Pvt. Ltd., Mumbai, India. Distilled water was used during nanoparticle synthesis and washing. Ethanol was used during washing of the nanoparticle precipitate.

The principal instruments used for nanoparticle characterization were a Shimadzu UV–Visible spectrophotometer, Bruker Tensor 27 FTIR spectrometer, Bruker X-ray diffractometer, Thermo Fisher Scientific scanning electron microscope and transmission electron microscope, and Bruker energy-dispersive X-ray (EDX) analyzer. Additional equipment used during extract preparation and nanoparticle processing included a Remi grinder, Heidolph rotary evaporator, Remi centrifuge, Remi magnetic stirrer, Elma ultrasonicator and Kimaya hydraulic press.

2.2. Preparation of Cassia fistula L. Flower Extract

Fresh C. fistula flowers were thoroughly washed with distilled water to remove adhering particulate matter and visible contaminants. The flowers were shade-dried at room temperature for approximately 7–10 days and subsequently pulverized using a mechanical grinder. Approximately 50 g of the powdered flower material was subjected to maceration with 500 mL of extraction solvent for 48 h, with intermittent shaking during the extraction period. The extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator. The concentrated extract was stored at 4°C until further use.

2.3. Green Synthesis of Zinc Oxide Nanoparticles

A 0.1 M solution of zinc nitrate was prepared in distilled water. The prepared C. fistula flower extract was added dropwise to the zinc nitrate solution under continuous stirring. The reaction mixture was maintained within a

temperature range of 60–80°C for 2–3 h. A visible change in the reaction mixture was observed during the synthesis process and was considered an indication of nanoparticle formation.

Following completion of the reaction, the mixture was centrifuged at 10,000 rpm for 15 min to recover the nanoparticle precipitate. The precipitate was repeatedly washed with distilled water and ethanol to remove residual soluble components. The washed material was dried at 60°C and subsequently calcined at 400°C for 2 h. The resulting material was collected as the synthesized ZnO nanoparticle powder and subjected to further characterization.

2.4. Characterization of Zinc Oxide Nanoparticles

2.4.1. UV–Visible Spectroscopic Analysis

The optical characteristics of the synthesized ZnO NPs were examined using a Shimadzu UV–Visible spectrophotometer. The nanoparticles were dispersed in distilled water and ultrasonicated for approximately 10–15 min before analysis. The resulting dispersion was transferred to a quartz cuvette with a 1-cm optical path length. The absorption spectrum was recorded over the wavelength range of 200–800 nm using distilled water as the blank. Multiple scans were recorded, and the absorption maximum of the synthesized nanoparticles was identified from the resulting spectrum.

2.4.2. Fourier-Transform Infrared Spectroscopy

FTIR analysis was performed using a Bruker Tensor 27 spectrometer to investigate functional groups associated with the synthesized nanoparticles. The dried nanoparticle sample was finely powdered and mixed with spectroscopic-grade potassium bromide (KBr) at an approximate sample-to-KBr ratio of 1:100. The mixture was compressed into a pellet using a hydraulic press, and the infrared spectrum was recorded over the range of 4000–400 cm^{-1} . The resulting spectrum was examined for characteristic functional-group absorptions and bands attributable to metal–oxygen vibrations.

2.4.3. X-ray Diffraction Analysis

The crystalline characteristics of the synthesized ZnO NPs were evaluated using a Bruker X-ray diffractometer. The dried nanoparticle powder was subjected to X-ray diffraction using Cu K α radiation with a wavelength of 1.5406 Å. The diffraction pattern was recorded over a 2θ range of 20–80°. The obtained reflections were compared with standard reference data to identify the crystalline structure of the synthesized material.

The average crystallite size was estimated using the Debye–Scherrer equation:

$$D = K\lambda / \beta \cos \theta$$

where D is the mean crystallite size, K is the shape factor, λ is the wavelength of the incident X-ray radiation, β is the full width at half maximum of the selected diffraction peak, and θ is the corresponding Bragg angle.

The principal diffraction reflections reported for the synthesized material were at 31.7°, 34.4° and 36.2°, corresponding to the (100), (002) and (101) planes, respectively. An average crystallite size of approximately 28 nm was reported from the Debye–Scherrer calculation.

2.4.4. Scanning Electron Microscopy

The morphology of the synthesized ZnO nanoparticles (ZnO NPs) was examined using a Thermo Fisher Scientific scanning electron microscope (SEM). The dried nanoparticle powder was mounted onto a conductive stub using double-sided carbon tape and subjected to conductive coating with gold or platinum prior to microscopic examination. SEM images were acquired at different magnifications to evaluate the particle morphology, distribution, and aggregation characteristics.

The synthesized ZnO NPs were observed to exhibit predominantly spherical and hexagonal morphologies. The particles showed a relatively uniform distribution, although some degree of aggregation was evident. These observations indicate the formation of nanoscale particles with distinct morphological characteristics.

2.4.5. Transmission Electron Microscopy

Transmission electron microscopy (TEM) analysis was performed using a Thermo Fisher Scientific transmission electron microscope to further examine the morphology and dimensions of the synthesized ZnO nanoparticles. The nanoparticles were dispersed in ethanol and ultrasonicated to facilitate uniform dispersion. A small portion of the resulting suspension was deposited onto a carbon-coated copper grid and allowed to dry under ambient conditions before microscopic examination.

TEM analysis revealed that the synthesized ZnO nanoparticles had dimensions below 30 nm. The TEM images were used to assess the particle morphology and nanoscale dimensions of the synthesized nanoparticles.

2.4.6. Energy-Dispersive X-ray Analysis

Elemental analysis of the synthesized ZnO nanoparticles was performed using a Bruker energy-dispersive X-ray (EDX) analyzer coupled with a scanning electron microscope (SEM). The analysis was carried out to examine the elemental composition of the synthesized material and to identify the characteristic elemental signals associated with zinc and oxygen.

The EDX spectrum exhibited prominent signals corresponding to zinc (Zn) and oxygen (O), confirming the presence of these elements in the synthesized nanoparticle sample.

3. Results

3.1 UV–Visible Spectroscopic Analysis

The UV–Visible absorption spectrum of the synthesized ZnO nanoparticles exhibited a distinct absorption maximum at 370 nm, as reported in the thesis. The observed absorption band was attributed to the characteristic optical absorption of ZnO nanoparticles. The corresponding UV–Visible spectrum is presented in Figure 1.

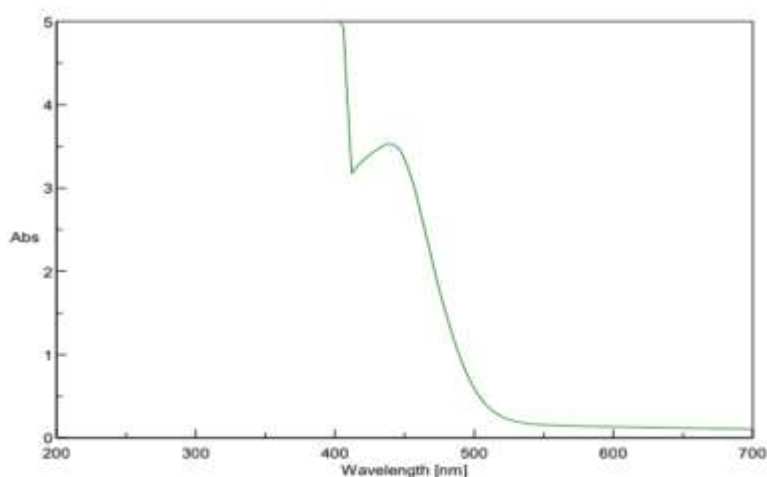


Figure 1 UV-Visible Spectroscopy graph

3.2 FTIR Spectroscopic Analysis

The FTIR spectrum of the synthesized ZnO nanoparticles showed several characteristic absorption bands. A broad absorption band was observed at approximately 3360 cm^{-1} , corresponding to O–H stretching vibrations. An absorption band at 1633 cm^{-1} was assigned to C=C stretching vibrations, while the band at 1384 cm^{-1} was associated with C–H bending vibrations. A distinct absorption band at 474 cm^{-1} was assigned to Zn–O stretching vibrations. The observed bands indicate the presence of organic functional groups associated with the plant extract together with the characteristic Zn–O vibration of the synthesized material. The FTIR spectrum is shown in Figure 2.

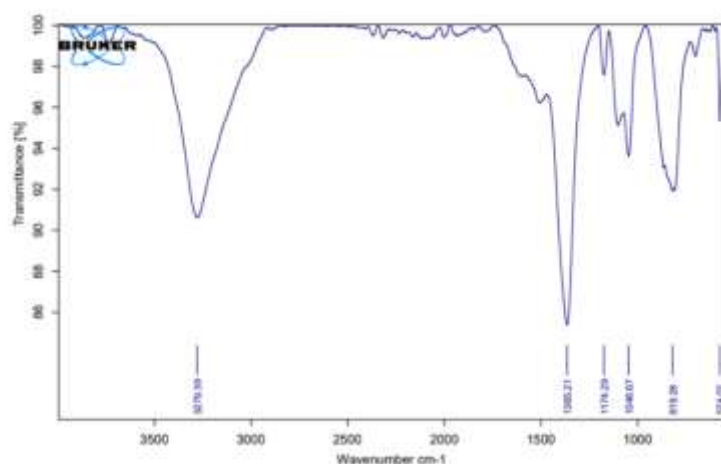


Figure 2. FTIR Spectroscopy graph

3.3 X-ray Diffraction Analysis

The XRD pattern of the synthesized nanoparticles exhibited multiple sharp and well-defined diffraction peaks. The principal diffraction peaks were observed at 2θ values of 31.7° , 34.4° , and 36.2° , corresponding to the (100), (002), and (101) crystallographic planes, respectively. These reflections were assigned to the hexagonal wurtzite structure of ZnO.

The thesis reported the material as comprising approximately 49.4% crystalline phase and 50.6% amorphous phase. The average crystallite size was reported as approximately 28 nm, calculated using the Debye–Scherrer equation. The XRD pattern is presented in Figure 3.

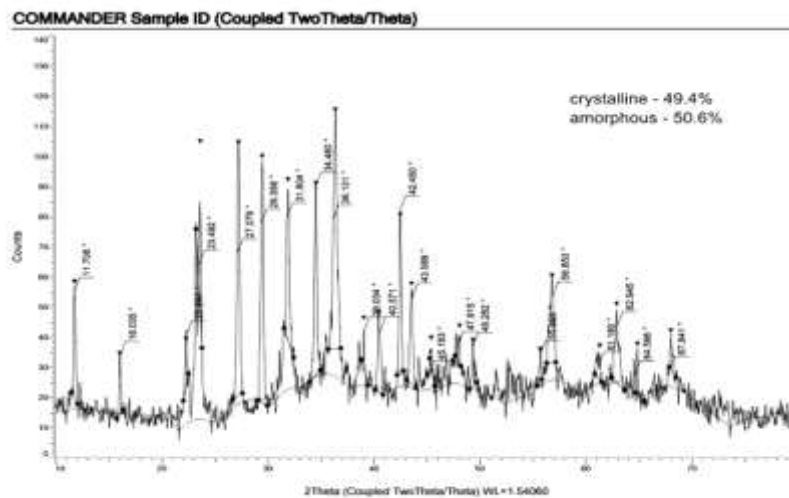


Figure 3. XRD Pattern

3.4 Scanning Electron Microscopy Analysis

SEM examination of the synthesized ZnO nanoparticles revealed predominantly spherical and hexagonal morphological features with a relatively uniform distribution. Some degree of particle aggregation was also observed in the micrographs. The SEM images are presented in Figures 4A and 4B.

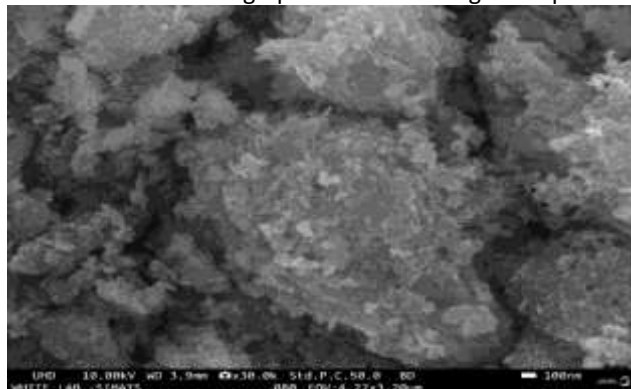


Figure 4A. SEM Micrograph of ZnO

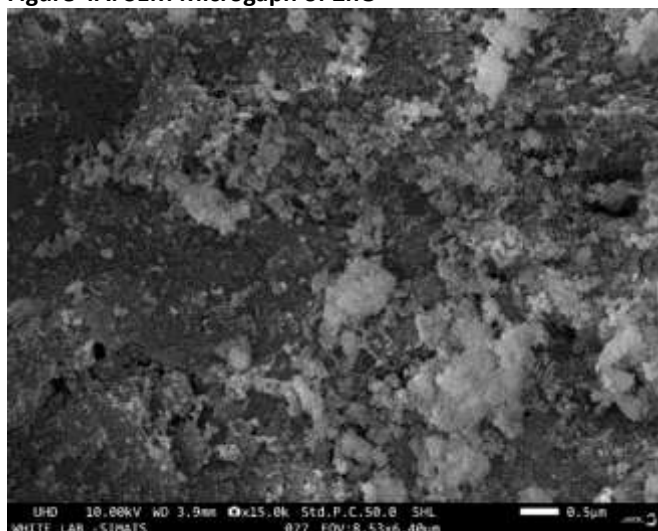


Figure 4B. SEM Micrograph of ZnO

3.5 Transmission Electron Microscopy Analysis

The thesis reported that TEM analysis showed nanoparticle sizes in the range of <30 nm. This observation was reported as being consistent with the nanoscale crystallite size obtained from XRD analysis. Representative microscopy images are presented in Figures 5 and 6.

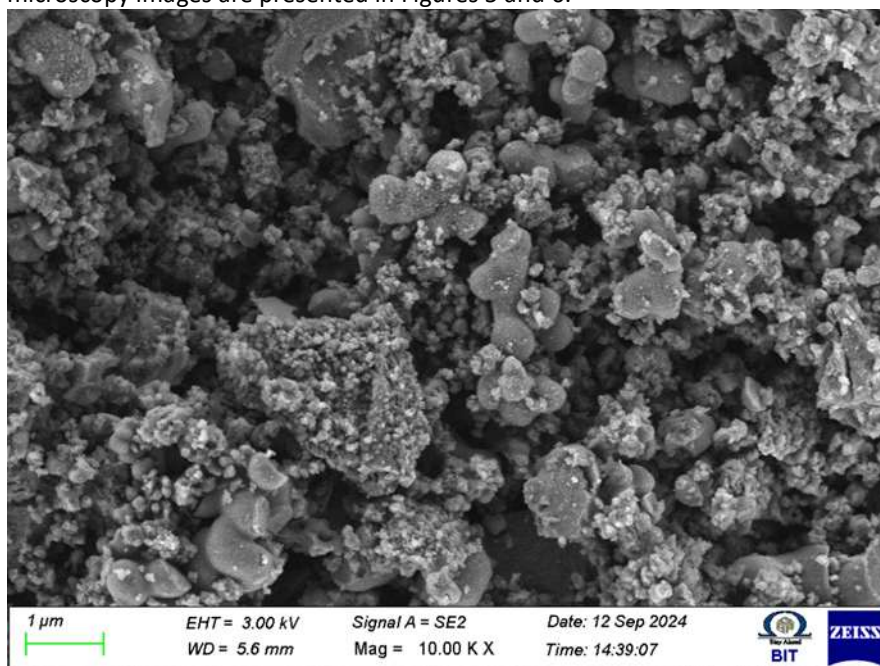


Figure 5. TEM Micrograph of ZnO

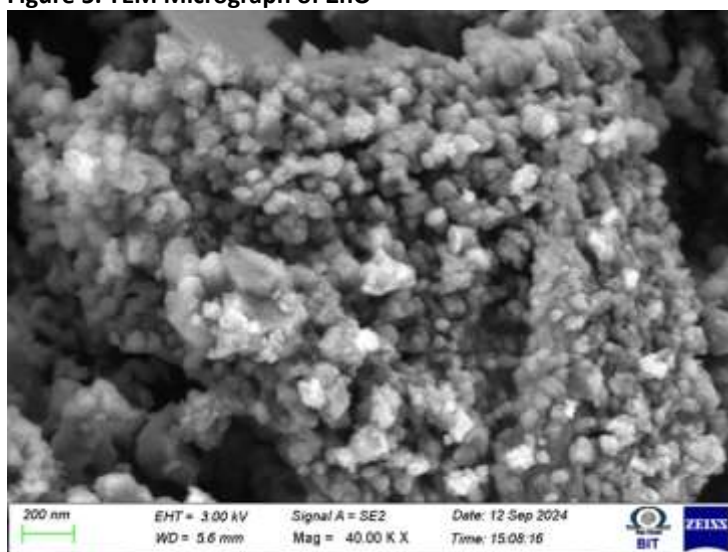


Figure 6. TEM Micrograph of ZnO

3.6 Energy-Dispersive X-ray Analysis

EDX analysis demonstrated prominent signals corresponding to oxygen (O) and zinc (Zn) in the synthesized material. Additional signals corresponding to carbon (C) and nitrogen (N) were also detected.

The quantitative EDX analysis reported the following elemental composition:

Element	Weight (%)	Atomic (%)
C K	5.99	9.66
N K	18.76	25.93
O K	46.08	55.77
Zn L	29.16	8.64

Table 1A. Elemental composition of green-synthesized ZnO nanoparticles determined by EDX

The EDX spectrum and quantitative elemental composition are presented in Figure 8 and Table 1, respectively.

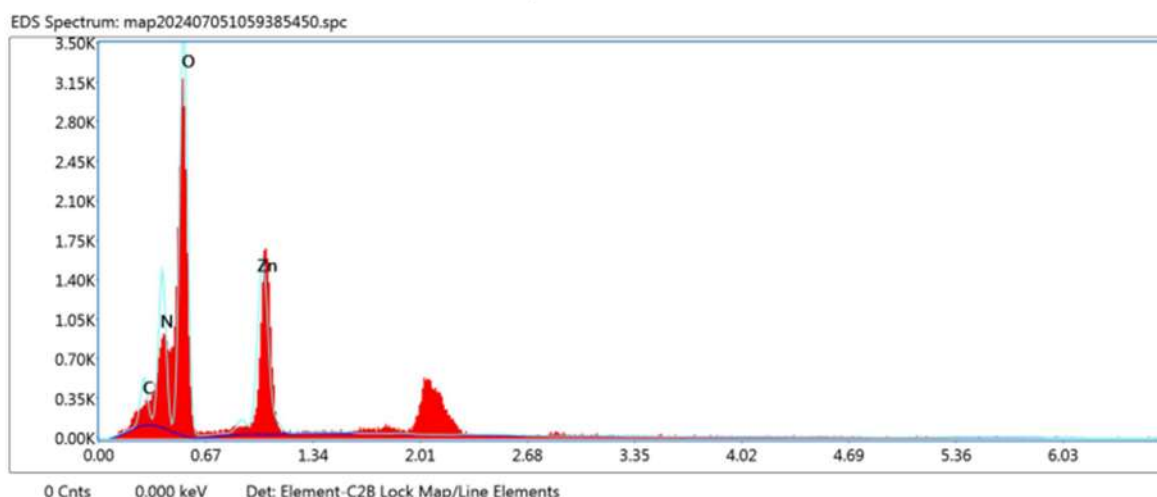


Figure 7.EDX Analysis

Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	5.99	9.66	26.92	11.04	0.0295	1.1399	0.4322	1.0000
N K	18.76	25.93	97.72	7.73	0.1144	1.1075	0.5507	1.0000
O K	46.08	55.77	279.49	7.43	0.2568	1.0794	0.5163	1.0000
ZnL	29.16	8.64	88.70	4.49	0.1958	0.7742	0.8674	0.9997

Table 1B. Elemental composition of the synthesized ZnO from EDX Analysis

3.7 Overall Characterization Findings

Collectively, the characterization results documented the formation of a ZnO-containing nanoscale material obtained using Cassia fistula flower extract. UV–Visible spectroscopy showed the reported absorption maximum at 370 nm, FTIR analysis demonstrated characteristic Zn–O and organic functional-group bands, and XRD analysis showed the reported diffraction pattern associated with hexagonal wurtzite ZnO with an average crystallite size of approximately 28 nm. SEM revealed spherical and hexagonal morphological features with some aggregation, while the thesis-reported TEM analysis indicated a particle size of <30 nm. EDX analysis detected Zn and O as well as C and N, with the quantitative elemental composition given in Table 1A and 1B. A consolidated summary of the major characterization findings and their corresponding interpretations is presented in Table 2, providing an integrated overview of the optical, functional-group, crystalline, morphological, nanoscale, and elemental characteristics of the synthesized material.

Technique	Main observation	Interpretation
UV–Vis	Reported absorption maximum at 370 nm	Optical characteristic consistent with ZnO
FTIR	Zn–O-associated band at 474 cm ⁻¹	Supports Zn–O structural component
XRD	31.7°, 34.4°, 36.2°	Hexagonal wurtzite ZnO
SEM	Spherical/hexagonal features; aggregation	Morphological characterization
TEM	<30 nm, thesis-reported	Nanoscale particle observation; requires verification
EDX	Zn, O, C and N detected	Elemental composition

Table 2. Summary of physicochemical characterization findings of Cassia fistula L. flower-mediated zinc oxide nanoparticles. The table summarizes the principal observations obtained from UV–Visible spectroscopy, FTIR, XRD, SEM, TEM, and EDX analyses, together with their corresponding interpretations. The TEM observation is identified as thesis-reported and requires verification against the original microscopy record

4. Discussion

The present study investigated the plant-mediated synthesis of zinc oxide nanoparticles using *Cassia fistula* L. flower extract and characterized the resulting material using complementary optical, spectroscopic, structural, microscopic and elemental techniques. The combined characterization findings provide evidence for the formation of a ZnO-containing nanoscale material and demonstrate the importance of evaluating the synthesized product using multiple analytical approaches rather than relying on a single characterization technique (Al-darwesh et al., 2024b).

4.1. Optical Characteristics

The synthesized material exhibited a reported UV–Visible absorption maximum at 370 nm. Absorption in this region is consistent with the optical response commonly reported for nanoscale ZnO prepared through plant-mediated approaches. For example, green-synthesized ZnO nanoparticles obtained using *Limonium prinosum* extract showed an absorption maximum around 370 nm (Naiel et al., 2022), while ZnO nanoparticles synthesized using *Thryallis glauca* leaf extract exhibited an absorption maximum at approximately 359 nm (Dey & Somaiah, 2022b). Similarly, plant-mediated ZnO synthesis using *Alnus nepalensis* has been reported to produce an absorption feature at 361 nm. These reports demonstrate that absorption features in the near-UV region are frequently observed for phylogenically synthesized ZnO materials (Jaishi et al., 2024).

The 370-nm absorption reported in the present study therefore provides optical evidence consistent with the formation of nanoscale ZnO. However, the absorption maximum should not be interpreted alone as definitive proof of ZnO formation because UV–Visible absorption can also be influenced by particle size, morphology, surface states, aggregation and synthesis conditions. The optical result is consequently more appropriately interpreted together with the FTIR and XRD findings.

4.2. FTIR Analysis and Surface-Associated Functional Groups

FTIR analysis of the synthesized material showed bands at approximately 3360, 1633, 1384 and 474 cm^{-1} . The band at approximately 474 cm^{-1} was assigned to Zn–O stretching, supporting the presence of the Zn–O structural component. The bands in the higher-wavenumber region indicate the presence of organic functional groups associated with the plant-derived material.

Similar observations have been reported for plant-mediated ZnO synthesis, where FTIR analysis has been used to identify both Zn–O vibrations and functional groups associated with biomolecules originating from the plant extract. In green synthesis, such plant-derived constituents may remain associated with the nanoparticle surface and can potentially participate in nucleation, surface interaction or stabilization. Recent studies have reported the involvement of functional groups derived from phenolic, aromatic, hydroxyl-containing and other plant constituents in plant-mediated ZnO synthesis (Jayachandran et al., 2021).

In the present study, the observed FTIR profile is therefore compatible with the coexistence of Zn–O-associated vibrations and organic functional groups derived from the plant-mediated synthesis process. However, FTIR alone cannot establish which individual phytochemicals were responsible for nanoparticle formation or surface stabilization. Identification of specific compounds and their contribution would require additional techniques such as chromatographic or mass-spectrometric analysis and appropriate mechanistic experiments (Correa Pinto et al., 2025).

4.3. Crystalline Structure and Crystallite Size

The XRD pattern showed principal reflections at 2θ values of 31.7°, 34.4° and 36.2°, assigned to the (100), (002) and (101) planes, respectively. These reflections are characteristic of the hexagonal wurtzite structure of ZnO and are consistent with patterns reported for plant-mediated ZnO nanoparticles in recent studies (Bouasla et al., 2024). For example, plant-mediated ZnO prepared using *Thryallis glauca* was reported to exhibit a hexagonal wurtzite structure, while ZnO synthesized using *Cayratia pedata* and *Senna auriculata* extracts has likewise been characterized using XRD with nanoscale crystalline dimensions (Dey & Somaiah, 2022c) (Jayachandran et al., 2021) (Chandrasekaran et al., 2023).

The average crystallite size in the present study was reported as approximately 28 nm based on the Debye–Scherrer equation. This value is within the nanoscale range reported for many phylogenically synthesized ZnO materials, although direct comparison between studies should be made cautiously because crystallite size is affected by precursor concentration, plant-extract composition, reaction conditions, calcination and the specific diffraction peak used for calculation. Furthermore, XRD-derived crystallite size represents the coherently diffracting crystalline domain and should not automatically be interpreted as the physical particle size observed by electron microscopy.

The source material also reports approximately 49.4% crystalline and 50.6% amorphous fractions. Because the calculation procedure for these percentages is not documented in the available experimental information, these values should be treated as reported observations rather than independently interpreted quantitatively.

Additional quantitative phase analysis would be required to establish the crystalline-to-amorphous proportion using a defined analytical method.

4.4. Morphological Characteristics

SEM examination revealed predominantly spherical and hexagonal morphological features, with relatively uniform distribution and some degree of aggregation. Morphological variation and aggregation are commonly observed in plant-mediated ZnO nanoparticles because particle growth can be influenced by precursor concentration, reaction temperature, extract composition, surface-associated biomolecules and post-synthesis thermal treatment(Al-darwesh et al., 2024b) .

The observed aggregation does not necessarily indicate failure of nanoparticle formation. Nanoparticles possess high surface energy, and interactions between particles can promote aggregation during drying and sample preparation. Similar aggregation has been reported in other plant-mediated ZnO systems. For example, ZnO nanoparticles synthesized using *Cayratia pedata* extract were reported to occur in an agglomerated form. Therefore, the aggregation observed in the present SEM images can reasonably be described as a morphological characteristic of the synthesized powder rather than as evidence of unsuccessful synthesis.

The reported coexistence of spherical and hexagonal features also indicates that the morphology was not completely uniform. Because the available study does not provide a quantitative particle-size distribution from SEM images, the morphological findings should remain descriptive rather than being expressed as a numerical size distribution(Kaningini et al., 2021) .

4.5. TEM Findings and Relationship with XRD

The thesis-reported TEM analysis indicated nanoparticle dimensions below 30 nm. This observation is broadly comparable with the reported XRD crystallite size of approximately 28 nm. Plant-mediated ZnO nanoparticles with nanoscale dimensions have also been reported in recent literature, although the values vary substantially according to the plant source and synthesis conditions.

The apparent agreement between the reported TEM dimension and XRD crystallite size provides supportive evidence that the synthesized material contains nanoscale domains. Nevertheless, these two measurements describe different physical characteristics. XRD estimates the size of crystalline domains, whereas TEM provides direct morphological information about individual particles or particle features visible in the analyzed micrographs. Therefore, similar numerical values should not be interpreted as demonstrating that the crystallite and particle are necessarily identical.

Importantly, the TEM images and original microscopy record should be verified before publication because the manuscript currently relies on the thesis-reported <30 nm value. A quantitative TEM size distribution would strengthen the characterization if the original image files and measurement data are available(Peta & Singh, 2025) .

4.6. Elemental Composition

EDX analysis detected zinc and oxygen as major elements together with carbon and nitrogen. The quantitative analysis reported 29.16 wt% Zn and 46.08 wt% O, while carbon and nitrogen accounted for 5.99 wt% and 18.76 wt%, respectively. The detection of Zn and O is consistent with the expected elemental composition of a ZnO-containing material and complements the structural information obtained by XRD.

The additional detection of carbon and nitrogen is noteworthy in the context of plant-mediated synthesis. Plant extracts contain a complex mixture of organic constituents, and residual or surface-associated organic material may contribute to carbon- and nitrogen-containing signals. Similar plant-mediated ZnO studies have used EDX to demonstrate the presence of Zn and O together with additional elements associated with biological synthesis or sample composition. However, EDX elemental detection alone cannot establish the chemical identity of the organic compounds responsible for these signals.

The quantitative EDX composition should therefore be interpreted descriptively. In particular, the presence of carbon and nitrogen means that the material should not be described simply as an analytically pure ZnO phase on the basis of EDX alone. Rather, the results support the presence of Zn and O within the synthesized material together with detectable C- and N-containing components(Zaater et al., 2024) .

4.7. Integrated Interpretation

Taken together, the characterization findings provide mutually supportive evidence for the formation of a nanoscale ZnO-containing material through the use of *C. fistula* flower extract. The reported UV-Visible absorption maximum at 370 nm provides optical evidence consistent with nanoscale ZnO; FTIR demonstrates Zn-O-associated vibration together with organic functional groups; XRD identifies reflections consistent with hexagonal wurtzite ZnO and reports an average crystallite size of approximately 28 nm; SEM demonstrates spherical and hexagonal morphological features with some aggregation; and the thesis-reported TEM observation indicates dimensions below 30 nm. EDX further demonstrates the presence of Zn and O together with C and N.

These findings are broadly consistent with recent reports demonstrating that plant-mediated synthesis can produce ZnO nanoparticles with characteristic optical absorption, wurtzite crystallinity and nanoscale morphology. However, the comparison also illustrates that nanoparticle characteristics vary between plant species and synthesis protocols. Consequently, the present findings should be regarded as specific to the *C. fistula* flower extract and experimental conditions used in this study rather than generalized to all plant-mediated ZnO systems.

The physicochemical characterization provides a basis for subsequent investigation of the synthesized material in topical formulations. Nevertheless, the present study establishes characterization findings rather than demonstrating a specific therapeutic or cosmetic effect. Further investigations involving quantitative particle-size analysis, surface chemistry, phase quantification, reproducibility studies and appropriately designed biological or formulation evaluations would be required before specific application-related claims can be established (Król-Górniak et al., 2023).

5. Limitations

Several limitations should be considered when interpreting the present findings.

First, some synthesis parameters were not fully documented in the available experimental record. In particular, the exact volume of plant extract, volume of zinc nitrate solution, extract-to-precursor ratio, reaction pH, stirring speed, number of washing cycles, drying duration and exact reaction temperature and duration were not available. These parameters may influence nanoparticle formation and physicochemical characteristics and should therefore be documented in future studies.

Second, the extraction procedure does not provide sufficient information to establish the exact extraction solvent and several extraction-related parameters, including extraction yield, final extract concentration and extraction temperature. Consequently, the influence of extraction conditions on nanoparticle formation cannot be quantitatively assessed from the present dataset (Kambale et al., 2023).

Third, the reported crystallite size of approximately 28 nm was calculated using the Debye–Scherrer approach. XRD-derived crystallite size represents a crystalline domain dimension and should not be considered equivalent to the physical particle size measured by electron microscopy. A quantitative particle-size distribution based on appropriately documented microscopy images would provide additional information.

Fourth, the TEM result is reported as a particle dimension below 30 nm, but the available experimental information does not document the number of particles measured, measurement procedure or statistical distribution. Verification of the original microscopy record and quantitative image analysis would strengthen this finding.

Fifth, the EDX analysis detected carbon and nitrogen in addition to zinc and oxygen. Although these additional signals may be associated with plant-derived or other sample-associated components, EDX alone cannot establish their molecular identity or demonstrate which individual phytochemicals participated in nanoparticle formation or stabilization.

Finally, the study does not experimentally establish the individual phytochemical constituents responsible for nanoparticle formation, stabilization or surface association. Additional phytochemical profiling and mechanistic studies would therefore be required to determine the specific contribution of *C. fistula* flower constituents to nanoparticle synthesis.

Despite these limitations, the complementary characterization results provide a useful physicochemical description of the synthesized material and establish a basis for subsequent investigation (Vera et al., 2023).

6. Conclusion

The present study investigated the plant-mediated synthesis of zinc oxide nanoparticles using *Cassia fistula* L. flower extract and characterized the resulting material using complementary analytical techniques. The synthesized material exhibited the reported UV–Visible absorption maximum at 370 nm and characteristic FTIR bands, including a Zn–O-associated band at approximately 474 cm^{-1} . XRD analysis showed principal reflections corresponding to the (100), (002) and (101) planes of hexagonal wurtzite ZnO, with an average crystallite size of approximately 28 nm as reported from the Debye–Scherrer calculation. SEM examination revealed predominantly spherical and hexagonal morphological features with some aggregation, while the thesis-reported TEM analysis indicated dimensions below 30 nm. EDX analysis demonstrated the presence of zinc and oxygen together with detectable carbon and nitrogen.

Collectively, these findings support the formation of a ZnO-containing nanoscale material obtained through a *C. fistula* flower-mediated synthesis approach. The characterization data provide a physicochemical basis for further investigation of this material in topical formulation development. However, the present study is limited to the

characterization findings and does not by itself establish a specific therapeutic, cosmetic or biological effect. Further studies with fully documented synthesis parameters, quantitative particle-size analysis, surface-chemical characterization, reproducibility assessment and application-specific evaluation would strengthen the evidence for potential topical applications (Salamatullah et al., 2021) (Gaikwad et al., 2023).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

FUNDING

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AUTHOR CONTRIBUTIONS

AUTHOR 1- Conceptualization, Methodology, Writing-original draft, AUTHOR 2- Formal Analysis, Supervision, Project administration, AUTHOR 3 -Writing – Review & editing, Supervision, Data curation, AUTHOR 4-Writing – Review & editing.

Declaration Of Generative AI And AI-Assisted Technologies In The Writing Process

During the preparation of this work, the author(s) used Grammarly, Inc. to check the grammar and Turnitin to check the plagiarism. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

DATA AVAILABILITY

The data supporting the findings of this study are contained within the article. Original characterization records and underlying experimental data are available from the corresponding author upon reasonable request, subject to their availability and institutional requirements.

ETHICS STATEMENT

This study did not involve human participants or experimental animals and therefore did not require human or animal ethical approval.

ABBREVIATIONS

ZnO — Zinc oxide

NPs — Nanoparticles

UV-Vis — Ultraviolet-Visible spectroscopy

FTIR — Fourier-transform infrared spectroscopy

XRD — X-ray diffraction

SEM — Scanning electron microscopy

TEM — Transmission electron microscopy

EDX — Energy-dispersive X-ray spectroscopy

KBr — Potassium bromide

Cu K α — Copper K-alpha radiation

FWHM — Full width at half maximum

rpm — Revolutions per minute

wt% — Weight percentage

at% — Atomic percentage

°C — Degree Celsius

nm — Nanometre

μm — Micrometre

cm^{-1} — Reciprocal centimetre

2θ — Diffraction angle

D — Crystallite size

K — Scherrer shape factor

λ — X-ray wavelength

β — Full width at half maximum of the diffraction peak

θ — Bragg angle

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