

Research

Green synthesis and characterization of zinc oxide nanoparticles using *Monoon longifolium* leave extract for biological applications

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Abstract

The present study deals with the biosynthesis of zinc oxide nanoparticles (ZnO NPs) using the *Monoon longifolium* (*M. longifolium*) leaf extract. The prepared ZnO NPs were characterized by XRD, FTIR, UV-Vis, TGA/DTA, and SEM. The synthesis parameters, such as plant extract volume (10–50 mL), heating duration (15 min), zinc nitrate concentration (1 mM), reaction time (1 h), and temperature (60 °C), were optimized. The synthesized ZnO NPs exhibited significant antibacterial activity against *Staphylococcus aureus* (22 ± 0.57 mm) and *Escherichia coli* (19 ± 1 mm), as well as antifungal activity against *Candida albicans* (21 ± 0.16 mm), as determined by the agar-well-diffusion method. The minimum inhibitory concentration (MIC) of ZnO-NPs against *S. aureus* (6.25 µg/mL) and *E. coli* (12.5 µg/mL), respectively, while the minimum fungicidal concentration (MFC) was 25 µg/mL against *Candida albicans*. Additionally, the antioxidant activity of the ZnO NPs ranged from 0 to 78% (IC₅₀ = 12.5 µg/mL). These results demonstrate the potential of the synthesized ZnO NPs as effective antibacterial, antifungal, and antioxidant agents.

Keywords Nanotechnology · ZnO NPs · Green synthesis · *Monoon longifolium* leaves · Antimicrobiral activity

1 Introduction

Over the past three decades, there has been a noticeable increase in interest in the fascinating subjects of nanoscience and nanotechnology research. Nanoparticles (NPs), which are microscopic particles that range in size from 1 to 100 nm (nm), are fundamental components of nanotechnology [1]. These days, nanoscience and nanotechnology are widely applied in a variety of disciplines, such as electronics, materials science, biomedical devices, sensors, water purification, cosmetics, and catalysis. The catalytic, magnetic, electrical, and optical characteristics of nanomaterials can be significantly influenced by their size, crystallinity, and shape [2]. Common methods for synthesizing nanoparticles include physical, chemical, and biological processes.

Unfortunately, the conventional physical and chemical techniques for creating nanoparticles frequently entail the use of hazardous substances and high energy consumption, which can have a negative impact on the environment and public health [3, 4]. The synthesis of nanoparticles using environmentally friendly methods, such as green synthesis utilizing plant extracts, has gained popularity due to its cost-effectiveness, compatibility with the environment, and non-toxic nature [5]. Plant extracts contain enzymes (e.g., reductases and hydrogenases) and secondary metabolites (e.g., flavonoids, terpenoids, phenols) that can react with metal salts to rapidly form precursors of metal oxide nanoparticles

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[6]. As the result, the biosynthesis and evaluation of the biological activity of various nanoparticles, particularly inorganic materials like metals and metal oxides nanoparticles, have gained significant attention [5, 6].

ZnO NPs have become a subject of intense research due to their remarkable semiconductor properties, temperature-dependent behavior, and strong excitation binding energy [5]. ZnO's unique characteristics, including its crystal structure, surface defects, high surface area-to-volume ratio, antimicrobial, and antioxidant capabilities, as well as its non-toxicity and biocompatibility [7]. Recognizing these researchers have successfully employed eco-friendly, biological methods, such as the use of plant extracts, to synthesize ZnO nanoparticles, aligning with the growing emphasis on green chemistry and sustainable practices [8]. In this paper, the synthesis of ZnO NPs using *M. longifolium* leaf extract is reported.

M. longifolium, belongs to the family Annonaceae. Although it is native to southern India and Sri Lanka, tropical Asia and Africa have seen widespread introductions of it. *M. longifolium* is a soft evergreen tree that is widely planted for its ability to reduce noise pollution. Fever, gonorrhea, uterine diseases, mouth ulcers, and heart defects are treated with the leaves, and diabetes and hypertension are treated with the stem bark [9].

This work is aimed at the biosynthesis and characterization of ZnO NPs *M. longifolium* leaf extract for antibacterial, antifungal, and antioxidant activity. The current method has the advantages of less secondary pollution, superior biocompatibility, ease of work-up, and cost-effectiveness. To the best of our knowledge, this work is original and reported first time on the green synthesis and characterization of ZnO NPs for the antibacterial, antifungal, and antioxidant activities utilizing leaf extract from *M. longifolium*.

2 Material and methods

2.1 Chemicals

The following analytical-grade materials and chemicals like zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), hydrochloric acid (35.4%, HCl), and sodium hydroxide (98%, NaOH) were used in the study without any additional purification. Substances were employed without additional purification. 1 M of HCl and 1 M of NaOH were used to change the pH of the mixture.

2.2 Collection of plant

The fresh *M. longifolium* plant leaves (Fig. 1) were collected from Huruta in the Arsi Zone of Oromia region, Ethiopia. Huruta has a latitude and longitude of 8°09'N 39°21'E with an elevation of 1978 m. The area has a subtropical highland climate or temperate oceanic climate with dry winters. The area receives an average annual rainfall of 1200 mm, with the wettest months being March to May and September to November. The samples were collected with the permission of the the landowner following the ethical and standard procedure.

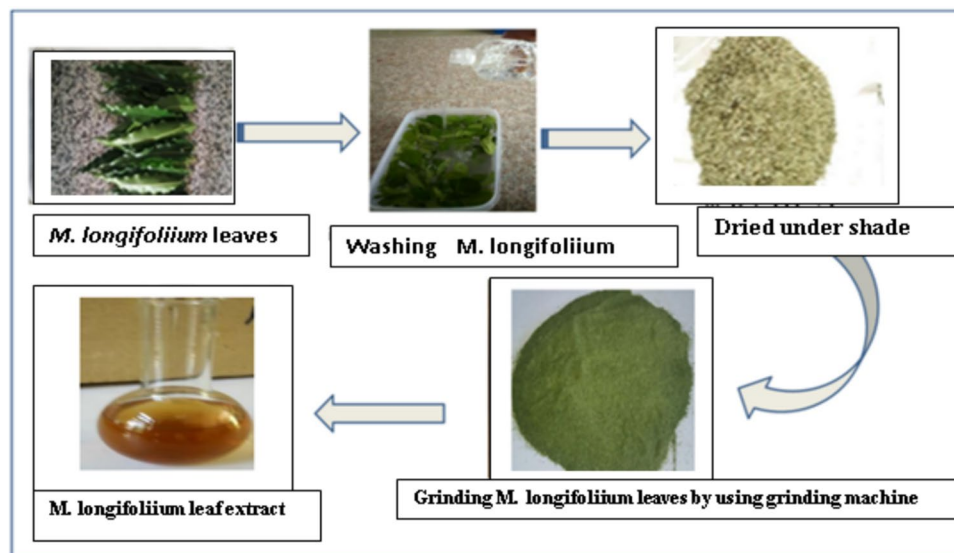
The collected *M. longifolium* leaves were transported to the Addis Ababa and identified by comparing them with already identified herbarium specimens at the national herbarium (ETH). The Botanist (Dr. Terefe Wondimu) was provided

Fig. 1 *M. longifolium* plant



Table 1 Botanical description of *M. longifolium*

Characteristic	Description
Family	Annonaceae
Subfamily	Malmeoideae
Species	<i>Monoon longifolium</i>
Common name	False ashoka
Kingdom	Plantae
Specimen ID	ETH-012
Collection date	2023-06-23
Collector name	Sisay Geda

Fig. 2 Preparation mechanism of *M. longifolium* leaf extract

detailed information about the herbarium specimens, as outlined in Table 1. After identification, the leaves were then brought to the chemistry lab of Adama Science and Technology University and after being placed in a plastic bag for further investigation.

2.3 Preparation of extract from *M. longifolium* leaves

After a batch of the sample was brought to Adama Science and Technology University, double-distilled water was used to properly wash the freshly harvested *M. longifolium* leaves in order to get rid of any dust and undesirable visible particles. Then, the leaves were cut into little pieces and let ten days to dry in the shade. A mortar and pestle were used to pulverize the dry sample. Ultimately, 5 g of powdered *M. longifolia* was taken from the dried sample, weighed with an electronic balance, and then put into a 250 mL beaker with 200 mL of distilled water. On a hot plate, the mixture was boiled for one hour at 50 °C. Following filtering, the extract was allowed to cool to ambient temperature and then kept at 4 °C for later use (Fig. 2).

2.4 Test microorganisms

The gram-positive bacterium *S. aureus* and the gram-negative bacterium *E. coli* were received from the Microbial Biotechnology Laboratory at Adama Science and Technology University in Adama, Ethiopia, for use in this work. These microbes were chosen in order to evaluate the produced nanoparticles' antibacterial activity. These methods were applied by the Microbial Biotechnology Laboratory at Adama Science and Technology University in Adama, Ethiopia, to study the antifungal and antioxidant properties against *Candida albicans*.

2.5 Phytochemical screening test

The extracts from *M. longifolium* leaves were screened using established screening techniques to determine whether they contained any naturally occurring physiologically active substances, such as alkaloids, proteins, carbohydrates, tannins, phenols, saponins, flavonoids, terpenoids, steroids, and glycosides. The screening procedure adhered to the specified standard procedures. [9].

2.6 Biosynthesis of ZnO NPs using *M. longifolium* leave extract

After finding the optimum synthesis parameters, ZnO NPs were successfully synthesized using *M. longifolia* leaf extracts through the green process. Initially, 29.74 mg of zinc nitrate ($\text{Zn}(\text{NO}_3)_2$) was weighed using an electronic balance, then the measured zinc nitrate was added to a 500 mL Erlenmeyer flask containing deionized water and allowed to dissolve entirely by gently swirling the flask. After stirring for an hour, 25 mL of *M. longifolia* extract was added to the solution. Subsequently, NH_4OH was added to the starting solution slowly until the pH value reached 10. After centrifuging the resultant solution, the precipitate was dried for six hours at 40 °C in an oven. Then, the dried precipitate was grinded and calcined in a muffle furnace for three hours at 473 °C to produce the biosynthesized ZnO NPs (Fig. 3) [10].

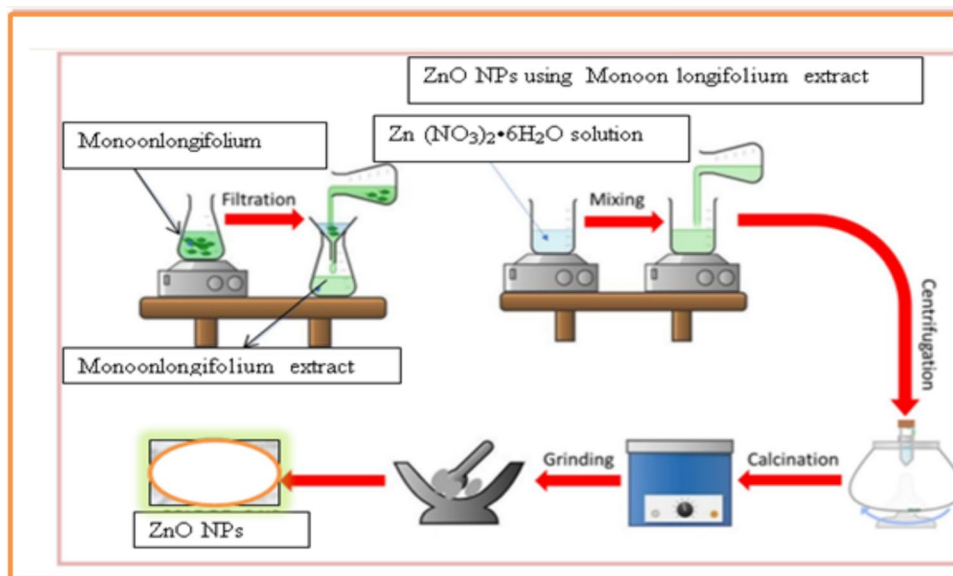
2.7 Effect of parameters on the biosynthesis of ZnO NPs

The effects were measured using spectrophotometry in the 300–400 nm wavelength range. The initial volume of *M. longifolia* aqueous extract, precursor concentration (0.25–1.50 mL), incubation times (0.5–2.50 h), temperature (0–100 °C), and heating time (10–300 min) were all examined. One by one, we changed these settings while maintaining the same values for the others.

2.8 Characterization

The synthesis of ZnO NPs was confirmed by various characterisation techniques. The absorbance of the samples was measured using UV–Vis spectroscopy throughout a 300–400 nm wavelength range, providing information about the optical properties of the generated NPs. [11]. Using a Shimadzu XRD-7000 device, an X-ray diffraction (XRD) analysis was performed to determine the average crystallite size, phase, and structure of the powdered NP samples [12]. The aqueous extract of *M. longifolia* and the produced NPs were subjected to functional group and chemical bonding investigation through the use of Shimadzu IR Affinity-1S instrument and Fourier-transform infrared spectroscopy (FTIR) [13]. Using a JSM-6390 device, scanning electron microscopy (SEM) allowed for a visual inspection of the morphological traits and

Fig. 3 The preparation process of ZnO NPs



dispersion of the produced NPs. These methods of characterisation collectively yielded valuable insights into the properties, structure, phase, and morphology of the synthesized ZnO NPs [10].

2.9 The antifungal and antibacterial properties of ZnO NPs

The agar well diffusion method was utilized to assess the antifungal and antibacterial characteristics of the green produced ZnO nanoparticles. Muller-Hinton agar plates were utilized for the antibacterial evaluation, and wells were filled with DMSO, an aqueous extract of *M. longifolium*, ZnO NPs at different concentrations (25, 50, and 75 µg/mL), and a common antibiotic (chloramphenicol). After 24 h of incubation at 37 °C, the diameter of the inhibitory zones was determined on the plates that had been infected with *Staphylococcus aureus* and *Escherichia coli* [12]. Similarly, on Muller-Hinton agar plates, where the wells were filled with the same range of NP concentrations, the antifungal activity of the ZnO NPs was assessed against *Candida albicans*. The inhibition zones were determined after 48 h of incubation [10].

2.10 Determination of MIC, MBC and MFC

Using the broth micro-dilution method, the minimum inhibitory concentration (MIC) of both conventional and nano-sized ZnO NPs against test pathogens was ascertained. After mixing the growth media with the ZnO NPs solution (100 µg/ml), the microbial strain (fungal or bacterial) prepared in 0.9% NaCl was added. Following serial dilutions, concentrations of 50, 25, 12.5, 6.25, 3.75, and 1.5 µg/ml were obtained. The bacteria were then cultured for 24 h at 37 °C, while the fungi were incubated for one week. The minimal concentration (MIC) that was found to be suppressing observable growth was noted. Then, 50 µl of each test tube that showed no signs of development was subcultured onto brand-new agar plates designated for that particular bacteria. The minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) was defined as the lowest concentration of ZnO NPs that prevented any microbial growth on the subculture plates [12].

2.11 Antioxidant activity

The antioxidant activity of ecofriendly synthesized ZnO NPs was assessed using the DPPH test. ZnO NPs were examined at doses of 6.45, 12.9, 25, 50, and 100 µg/mL for their scavenging activity. 750 µL of the samples and 750 µL of DPPH solution were combined and well blended for this experiment. For the negative control, 750 µL of DPPH was mixed with 750 µL of methanol, and for the positive control, 750 µL of DPPH was mixed with 750 µL of ascorbic acid. The samples and controls were then placed in a water bath at 37 °C for 30 min. Following incubation, the absorbance was recorded at 517 nm. The antioxidant activity was determined using a specific formula [13].

$$\text{Scavenging activity} = \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}} \times 100\% \quad (1)$$

3 Result and discussion

3.1 Phytochemical analysis

To identify the active components in the extract of *M. longifolium*, a qualitative analysis was performed. The results are shown in Table 2. Numerous phytochemically active substances, including saponins, alkaloids, phenols, tannins, steroids, flavonoids, proteins, and carbohydrates, were detected in the plant's floral aqueous extract. Terpenoids and glycosides, however, were absent from the extract. The flower extract in water showed a varied composition of proteins, carbohydrates, steroids, flavonoids, phenols, tannins, and saponins. In the process of creating ZnO NPs, these bioactive substances may function as reducing, capping, and stabilizing agents. [9].

The obtained results confirm the presence of various phytochemical constituents in the leaf extract of *Monoon longifolium*. These constituents include alkaloids, flavonoids, tannins, saponins, phenols, steroids, terpenoids, proteins, and carbohydrates, as indicated in Table 2. These compounds are responsible for the reducing properties of the leaf extract, which are crucial in the formation of ZnONPs, as well as for their capping and stabilizing effects [14]. This conclusion is

Table 2 Phytochemical screening of aqueous extract of *M. longifolium* extract

S. No.	Phytochemical	Name of the test	Result
1	Saponins	Foam test	+
2	Alkaloids	Wagner test	+
3	Phenols	FeCl ₃ test	+
4	Terpenoids	Chloroform test	–
5	Steroids	Chloroform test	+
6	Flavonoids	NaOH test	+
7	Glycosides	Salkowski's test	–
8	Tannins	KOH test	+
9	Proteins	Millon's test	+
10	Carbohydrate	Fehling's test	+

+ Present

– Absent

further supported by the analysis of the FTIR spectra. However, glycosides were not detected in the extract. These findings align well with previous studies that have reported the presence of alkaloids, saponins, tannins, flavonoids, steroids, phenols, and terpenoids in the aqueous extract of *Monoon longifolium* leaves [9].

3.2 Optimization of synthesis parameters

Using *M. longifolia* leaf extract, a number of experimental parameters, including temperature, reaction time, zinc nitrate concentration, heating duration, and amount of plant extract, were assessed for the green synthesis of ZnO NPs. To increase the yield of ZnO NPs, various reaction parameters were optimized. Using a UV–Vis spectrophotometer, the absorbance of the samples was determined between 300 and 450 nm [15].

3.2.1 Effect of heating time of extract

To study the effect of the heating time of extract on ZnO NPs production, different heating times of extract (10, 15, 20, 25, and 30 min) were used to synthesize ZnO NPs. It is observed that 15 min of heating time of extract results in maximum absorbance (Fig. 4a). The absorbance of ZnO NPs decreases when the extract is heated for longer than 15 min; this is because longer heating times break down the phytochemicals found in *M. longifolium* leaves. This work demonstrates that 15 min is the ideal heating time to prepare an aqueous extract of *M. longifolium* leaves, which is then used to synthesize ZnO nanoparticles. Similar results with our report [16].

3.2.2 Effect of zinc nitrate concentration

The findings of your research show that a concentration of 1 mM zinc nitrate hexahydrate was most effective in producing nanosized particles, as indicated by the absorbance peak at 330 nm in Fig. 4b. The higher absorbance at this concentration suggests a higher yield of nanoparticles (NPs). This result aligns with previous studies conducted by [17]. Furthermore, when zinc nitrate hexahydrate concentration was increased from 0.5 to 1 mM, the production of NPs increased as well. Nevertheless, absorbance decreased after 1 mM. This suggests that instead of the intended nanosized particles, bigger microparticles may emerge if zinc nitrate hexahydrate concentration is increased beyond 1 mM. At these increased precursor concentrations, metal oxide nanoparticles' characteristic absorbance was found to be low. These observations underscore the significance of metal ion concentration in nanoparticle formation. It is essential to optimize the concentration of the metal salt solution to achieve the desired nanosized particles with maximum yield and suitable absorbance properties.

3.2.3 Effect of reaction time (incubation time)

Using an aqueous extract of *M. longifolium* leaf extracts at room temperature, a change in absorbance over time was observed to comprehend the time-dependent kinetics of ZnO NPs formation. Figure 4c shows the analysis and

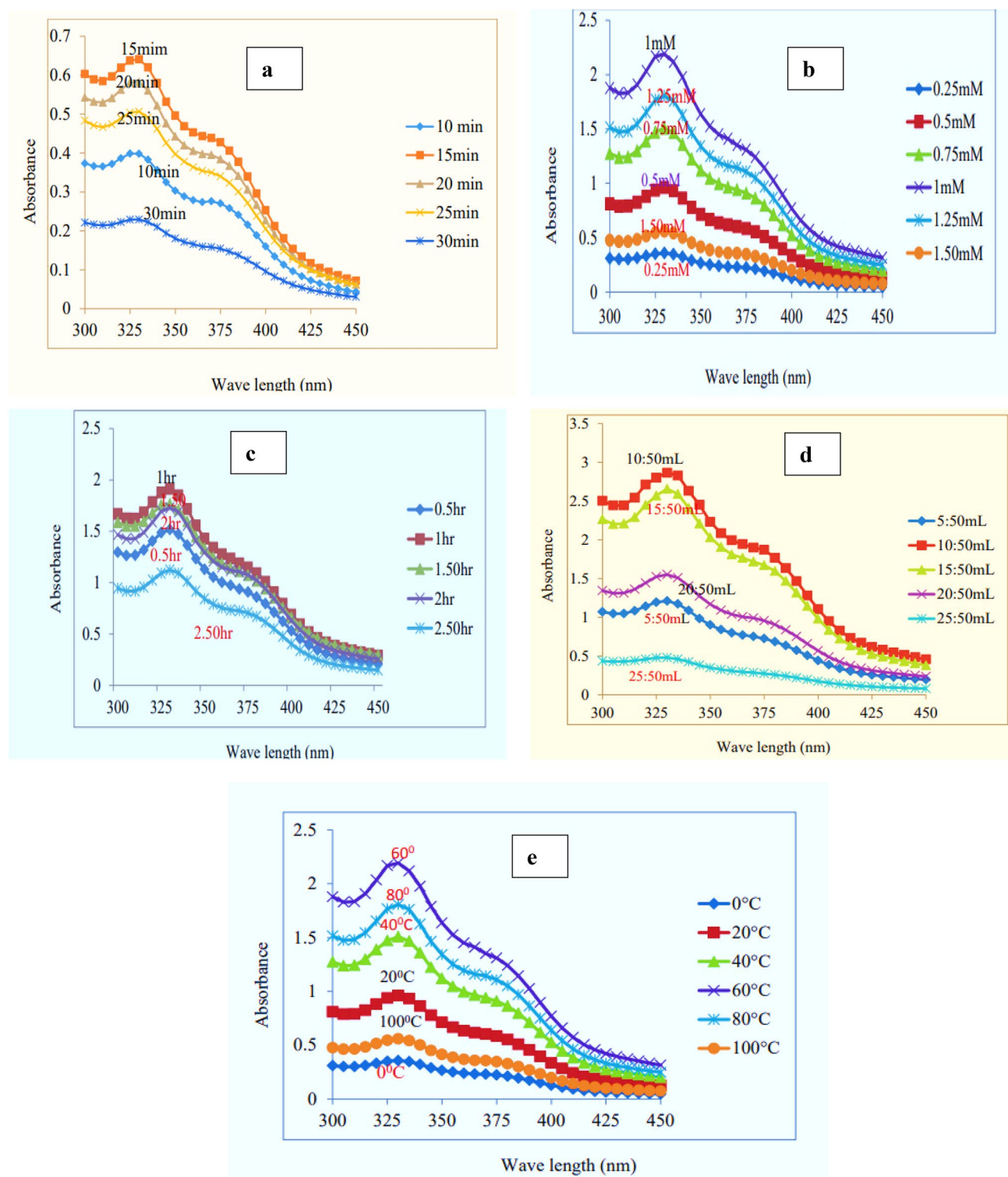


Fig. 4 Effective parameters on ZnO NPs synthesis. **a** Heating time of extract, **b** concentration of zinc nitrate, **c** Heating time of ZnO NPs, **d** Volume of extract and **e** Heating temperature of ZnO NPs

presentation of the ZnO NPs UV-Vis absorption spectra values that were generated in the reaction mixture at various reaction durations (0.2–2.5 h). It has been shown that the absorbance of ZnO NPs rises with incubation time. This is because the reaction medium produces additional ZnO NPs, which lengthens the incubation period by up to one hour. The absorbance does not significantly change after 1 h of reaction time, indicating that the ZnO NPs' stability has reached

its maximum. Hence, in the present study, 1 h of the reaction time was selected as the optimum condition to produce pure and maximum yield of ZnO NPs. This finding is consistent with previous studies conducted on *C. roseus*, where an absorption peak was recorded at 2 h [18].

3.2.4 Effect of volume of extract

The UV–Vis spectra in Fig. 4d illustrate how the volume *M. longifolia* leaf extracts affect the formation of ZnO NPs. This was accomplished by changing the volume of *M. longifolium* leaf extract from 5 to 25 mL while maintaining the same values for the other parameters. Figure illustrates how the absorbance of ZnO NPs rises from 5:50 to 10:50 in the volume of aqueous extract before declining. The increase in absorbance from 5:50 to 10:50 mL suggests that the *M. longifolia* leaf extract is continuously reducing the NPs. There is a decrease in ZnO NP absorbance when the volume of *M. longifolia* leaf aqueous extract used exceeds 10:50 mL. This is because more phytochemicals bind to the ZnO NP surface [19]. Additionally, the intensity of absorption diminishes and the solution becomes cloudy at greater extract concentrations, most likely as a result of an excess of biomolecules [21]. The ideal volume for the synthesis of ZnO NPs is 10:50 mL of *M. longifolia* leaf extract aqueous extract, which is why it was chosen for additional research.

3.2.5 Effect of temperature

According to UV–Vis studies, it was found that the ideal temperature for producing ZnO nanoparticles (NPs) is 60 °C, as shown in Fig. 4e. The figure demonstrates that the absorbance of the solution increased as the temperature rose from 0 to 60 °C, indicating a higher production of ZnO NPs. However, beyond 60 °C, the absorbance started to decrease, resulting in lower yields of ZnO NPs. The selection of 60 °C as the optimum temperature is consistent with previous research. Higher temperatures can cause the breakdown of active phytochemicals found in the plant extract used for synthesis. Conversely, lower temperatures may not be adequate for nanoparticle formation. Therefore, a temperature of 60 °C strikes a balance between promoting particle formation and preserving the integrity of the phytochemicals. Previous studies have also utilized similar temperature conditions for ZnO NP synthesis using plant extracts further [15], confirming the choice of 60 °C as the temperature for maximizing ZnO NP yield.

3.3 Characterization of ZnO nanoparticles

A noticeable color shift occurred when *M. longifolia* extract was added to the zinc nitrate solution, signifying a reduction reaction. UV–Vis, FTIR, XRD, and SEM techniques were used to characterize the produced ZnONPs in order to evaluate their optical qualities, thermal stability, chemical composition, crystal structure, and shape [19].

3.3.1 Visual observation

In the initial phase of the experiment, we dissolved the precursor in deionized water, which remains colorless (Fig. 5a). Then, the leaf extract (Fig. 5b) was added while the mixture was being constantly stirred. The mixture was then heated to 60 °C for 15 min, and as the reaction progressed, the color shifted from brown to pale yellow, as observed in Fig. 5c.

Fig. 5 Color of **a** precursors solution, **b** Aqueous extract of *M. longifolium* leaves, **c** precursor solutions with aqueous extract of *M. longifolium* leaf

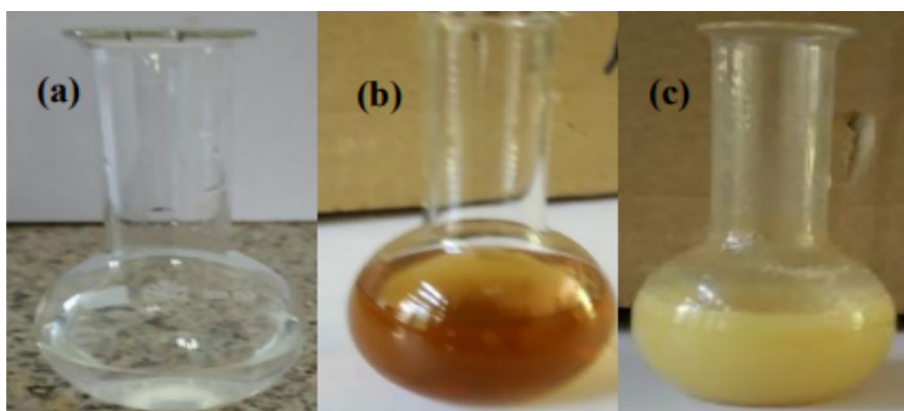


Fig. 6 . UV–Vis spectra of ZnO nanoparticles

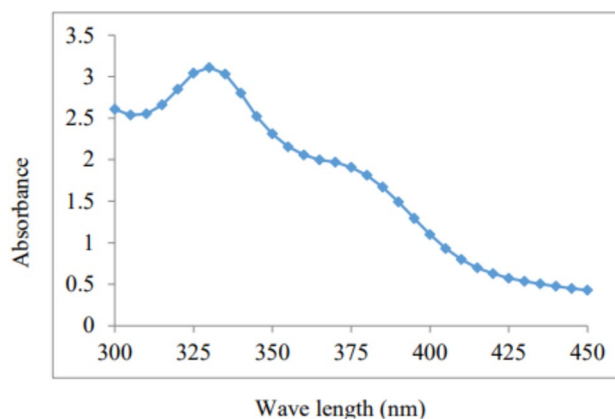
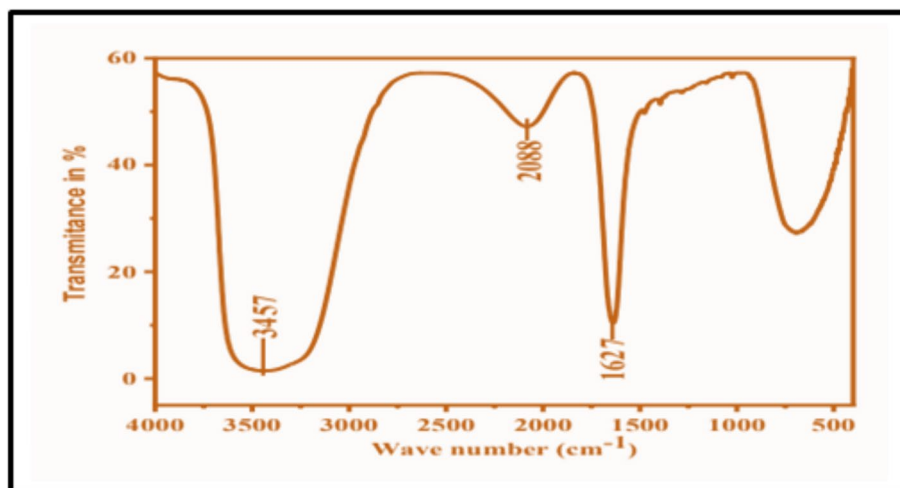


Fig. 7 FTIR spectrum of aqueous extract of *M. longifolium* leaves



Surface plasma resonance (SPR) is the result of synchronized oscillation of electron gas at NP surfaces, which is the cause of this modification [16]. The reduction of metal salts is shown by the color shift, which shows that the synthesis of NPs was effective [20].

3.3.2 UV–Vis spectral analysis

Figure 6 shows the UV–Vis absorption spectrum of the ZnO NPs solution. It was observed that the absorption peak was centered at a wavelength of 325 nm, which is in the range of 310–360 nm and is specific for ZnO NPs [21]. Similar absorption peaks have been reported for ZnO NPs synthesized using *Cayratia pedata* leaf extract [22]. This indicates the comparability of the bio-assisted ZnO NPs produced in this study. Notably, the absence of any other peaks in the spectrum suggests the high purity of the ZnO NPs synthesized through this rapid and environmentally friendly method.

3.3.3 FTIR spectral analysis

The utilization of FT-IR spectroscopic analysis revealed the detection of phytochemicals functioning as capping agents during the synthesis and stabilization process of ZnO NPs. These phytochemicals include phenols, amines, ethers, and carboxylic acids. Moreover, the analysis was conducted to assess the functional groups present in ZnO NPs, as well as in the leaf extract of *M. longifolium* [23]. Figure 7 displays the spectrum of the aforementioned leaf extract. Within the spectrum, notable peaks were observed at 3457, 2088, and 1627 cm^{-1} . The prominent and wide band at 3457 cm^{-1} signifies the presence of OH^- functional groups, which can be attributed to alcohol and phenolic substances found in the plant extract. Moreover, the stretching of the C–N bond in the cyanide group is indicated by the absorption peak at 2088 cm^{-1} . The absorption range between 1600 and 1760 cm^{-1} , displaying complex absorptions, implies the existence of aldehydes, ketones, and esters and corresponds to the C=O stretching characteristic of these functional groups.

Fig. 8 FTIR spectrum of bio-synthesized ZnO NPs

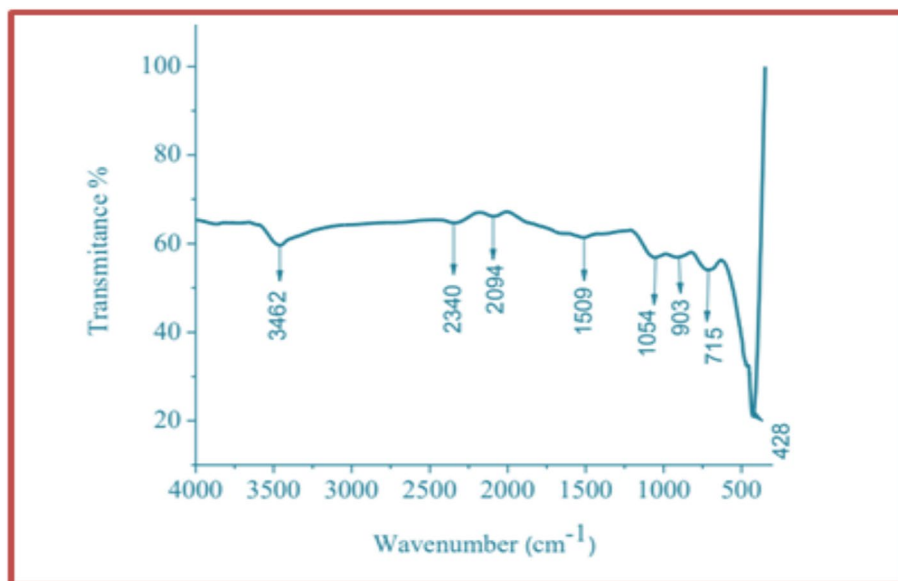
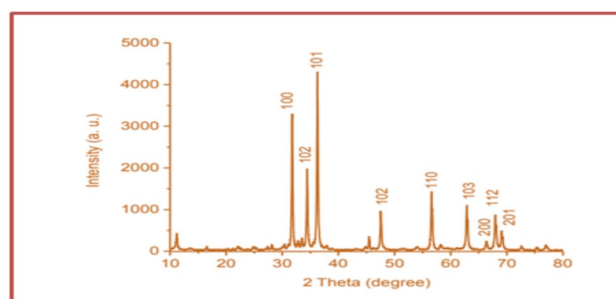


Fig. 9 XRD pattern of biosynthesized ZnO NPs



ZnO NPs that were biosynthesized showed peaks at particular wave numbers in their FTIR spectra 3462, 2340, 2094, 1509, 1054, 903, and 470 cm^{-1} are some of these peaks Fig. 8. The signal at 3462 cm^{-1} shows that phenols and alcohols contain O–H groups. The peak at 1509 cm^{-1} is indicative of amides' N–H bending and C=O stretching. The C–O stretching of alcohols, carboxylic acids, esters, and ethers, or the C–N stretching of aliphatic amines, is represented by the peak at 1054 cm^{-1} . The Zn–O stretching vibration is attributed to the peak in the range of 428 to 715 cm^{-1} [24], indicating that *M. longifolium* leaf extract is used as a capping and reducing agent during the synthesis of ZnO NPs. Various ZnO band locations emerge between 428 and 715 cm^{-1} in the FTIR spectra of different ZnO NPs samples generated utilizing plant extracts, as evidenced by these samples [25]. were used to manufacture ZnO NPs, and band locations between 428 and 715 cm^{-1} were discovered. These outcomes confirm what we discovered from the synthetic ZnO NPs' FTIR spectra.

3.3.4 XRD pattern

A versatile, non-destructive analytical method for recognizing and quantifying the various crystalline forms found in both powder and solid materials is called X-ray diffraction (XRD) [33]. Figure 9 displays the XRD pattern of sensitized ZnO NPs powder, revealing a series of diffraction peaks across the entire spectrum of 2θ values ranging from 26 to 49°. The characteristic diffraction peaks, with 2θ values of 31.72°, 34.48°, 36.72°, 47.55°, 56.84°, 62.88°, 67.96°, 68.24°, and 69.09°, respectively, correspond to the crystallographic planes (100), (002), (101), (102), (110), (103), (200), (112), and (201). This confirms the crystalline structure of the HAp NPs produced, consistent with similar findings reported by [10]. The XRD pattern of the synthesized ZnO NPs is compared to the standard values of JCPDS file no. 89–1397. All detectable peaks correspond to the pure hexagonal phase of ZnO NPs, confirming the successful formation of ZnO NPs. Additionally, the green-synthesized ZnO NPs exhibit an average crystallite size of 49.25 nm, and the nano-sized structure of the composite particle formed was determined using Debye–Scherrer's equation (Eq. (1)) [12].

$$D = \frac{k\lambda}{\beta \cos \theta} \quad (2)$$

Applying the Scherrer equation, which makes use of the subsequent variables: The ZnO nanoparticles that were calcined at 500 °C for three hours had a crystal size (D) of 49.25 nm. The X-ray source wavelength (λ) is the CuK α radiation (1.5406 Å). The full width at half-maximum (FWHM) of the diffractogram peak is expressed as β in radians. The Bragg's diffraction angle is represented by θ .

3.3.5 Scanning electron microscopy

Surface morphology of the prepared ZnO NPs were examined through SEM analysis at various magnification levels (Fig. 10). The SEM images revealed well-shaped particles, with a majority exhibiting a hexagonal shape. Agglomerations of individual zinc oxide particles were observed in the images, indicating the tendency of the nanoparticles to form clusters. Further examination of these agglomerated lumps revealed the presence of nanoparticle aggregates within them. The SEM analysis provided valuable insights into the surface morphology and structure of the ZnO NPs, highlighting their well-defined shapes and the presence of agglomerations and aggregates [25].

3.3.6 Thermogravimetric analysis (TGA)

Thermogravimetry/Differential Thermal Analysis (TG/DTA) was performed to confirm the formation and determine the thermal stability of a synthesized ZnO NPs phase. Figure 11 displays the TG/DTA curve of ZnO NPs, as recorded at a heating rate of 10 °C/min between 50 and 800 °C under a nitrogen (N₂) atmosphere. The thermal decomposition of the ZnO NPs occurs in several steps, and a 6.41% residue remains after thermal analysis up to 800 °C.

The first weight loss of 18.17% from room temperature to 168.6 °C is due to the thermal dehydration of ZnO NPs. During this thermal dehydration, water molecules are lost. Upon further heating to 262.4 °C, the sample exhibited a second weight loss of 14.19%, which is attributed to the decomposition of some of the organic moieties in the phytochemicals serving as capping agents and reductants [5]. A final weight loss of 61.23% was observed around 473 °C. after that there

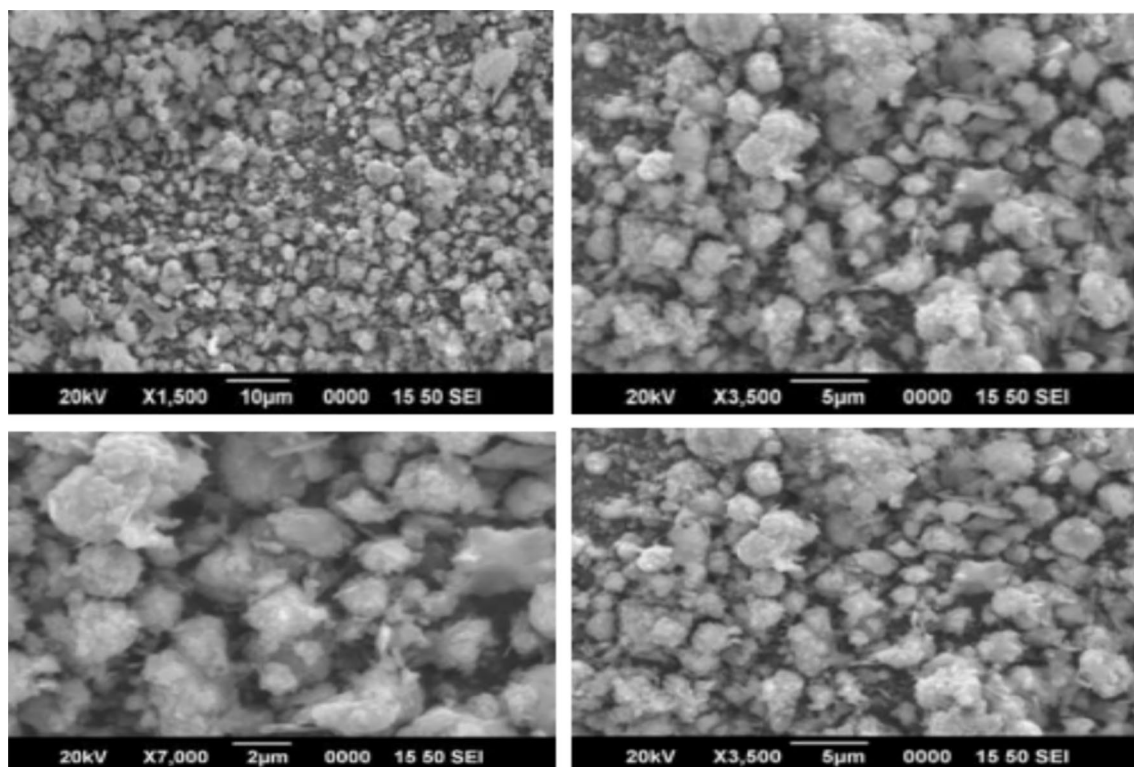
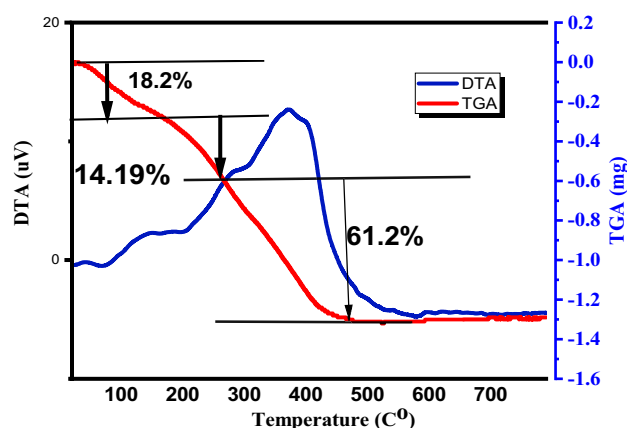


Fig. 10 SEM images of biosynthesized ZnO NPs

Fig. 11 TGA/DTA graph of biosynthesized ZnO NPs



is no any further weight loss was observed upon further heating to 800 °C [26]. The blue curve represents DTG, indicating weight change rates with peaks at 300 °C and 450 °C, aligning with significant weight loss events in the TGA curve. The DTG peaks highlight critical temperature points of rapid weight loss changes.

3.3.7 Antibacterial activity of ZnO NPs

The ZnO NPs showed exceptional antibacterial efficiency against gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacterial strains, according to the antibacterial data. At a dosage of 75 µg/mL, the ZnO NPs had a robust inhibitory impact against *S. aureus*, with a zone of inhibition measuring roughly 22 ± 0.57 mm. The 23 mm zone of inhibition found for the antibiotic chloramphenicol was similar to this. Comparably, the zone of inhibition for chloramphenicol was 21 mm, and the zone for *E. coli* measured 19 ± 0.52 mm.

ZnO NPs' antibacterial activity rose with concentrations, as Table 3 and Fig. 12a, b demonstrate. Because of its large surface area and small particle size, which raise surface reactivity and strengthen the antibacterial qualities, ZnO NPs have been found to have improved antibacterial activity. The leaf extract of *M. longifolium* shown antibacterial activity against both kinds of bacteria. The leaf extract demonstrated a zone of inhibition of 15 ± 0.54 mm against *S. aureus* and 10 ± 0.45 mm against *E. coli* at a dose of 75 µg/mL (Fig. 12c, d and Table 3). Even though these values were less than those found for the ZnO NPs, the leaf extract's antibacterial capability was nevertheless evident. Plant extracts possess antibacterial properties derived from their secondary metabolites, which have the ability to destroy genetic material, disrupt bacterial cells, and inhibit enzymes, ultimately resulting in growth suppression or death.

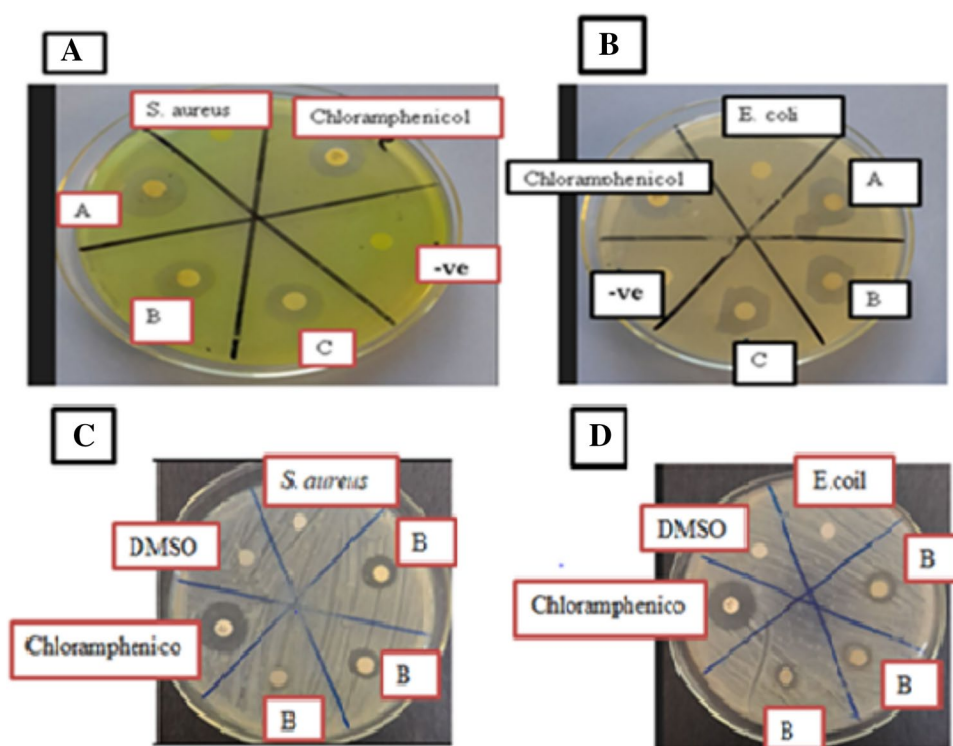
At a concentration of 75 µg/mL, the leaf extract showed a zone of inhibition of 15 ± 0.54 mm against *S. aureus*, and 10 ± 0.45 mm against *E. coli* Fig. 12c, d and Table 3. These values were lower than those observed for the ZnO NPs, but still demonstrated the antibacterial potential of the leaf extract. The antibacterial activity of a plant extract is risen from their secondary metabolites, which can disrupt bacterial cells, inhibit enzymes, and damage genetic material, leading to growth inhibition or death.

When the current results were compared to earlier research, the produced ZnO NPs showed strong antibacterial activity. For example, the inhibition values reported by [29] for 22 mg/mL of ZnO NPs against *S. aureus* and *E. coli* were 17.7 mm and 24 mm, respectively [30]. Similar results were reported by [33] for ZnO NPs against *S. aureus* and *E. coli*, with values of 21 mm and 18 mm, respectively. In comparison, our results show significant activity, with biosynthesized ZnO NPs demonstrating a zone of inhibition of 22 ± 0.57 mm against *S. aureus* at 75 µg/mL and 19 ± 0.52 mm against *E. coli* at 75 µg/mL. In comparison, chloramphenicol (the control) displayed the highest antibacterial activity of 23 ± 0.56 mm

Table 3 Zone of inhibition (mm) of ZnO NPs with extract of *M. longifolium* leaves

		Diameter of zone of inhibition (mm)			
		Bacteria strains	25 µg/mL	50 µg/mL	75 µg/mL
ZnO NPs	<i>S. aureus</i>		7 ± 0.58	11 ± 0.57	22 ± 0.57
	<i>E. coli</i>		5 ± 0.57	8 ± 0.58	19 ± 1
<i>M. longifolium</i> leaf extract	<i>S. aureus</i>		5 ± 0.53	8 ± 0.52	15 ± 0.54
	<i>E. coli</i>		4 ± 0.57	5 ± 0.54	10 ± 0.45

Fig. 12 Zone of inhibition of ZnO NPs, against *S.aureus* (A) and against *E. coli* (B) and Zone of inhibition of *M.longifolium* leaf extract *S.aureus* against (C) and against *E. coli* (D)



against *S. aureus* at 75 $\mu\text{g/mL}$ and the lowest activity of 21 ± 0.57 mm against *E. coli* at 75 $\mu\text{g/mL}$. The antibacterial activities were concentration-dependent, with ZnO NPs exhibiting stronger activity against *S. aureus* than against *E. coli*.

ZnO NPs' intricate antibacterial action mechanism is impacted by various elements, including the makeup of the bacterial cell wall. Previous investigations have indicated that ZnO NPs had stronger antibacterial activity than zinc nitrate. ZnO NPs have an antibacterial impact because of their high surface area to volume ratio, which encourages microbe interaction. As a result, Zn^{2+} ions dissolve and become more efficient as a biocide. [27]. The exact mechanism of ZnO NPs' antibacterial activity is still under debate, with proposed mechanisms including direct contact with bacterial cell walls, disruption of cell integrity, the release of antimicrobial ions such as Zn^{2+} , and generation of reactive oxygen species (ROS) [28]. However, the toxicity mechanism may vary in different environments due to changes in dissolved Zn species and the physicochemical properties of ZnO NPs. Further research is necessary to gain a comprehensive understanding of the antibacterial mechanisms of ZnO NPs in different contexts.

3.3.8 Antifungal activity of ZnO NPs

Using the disc diffusion experiment, the antifungal efficacy of ZnONPs and a plant extract against *Candida albicans* was assessed. The observation of distinct zones of inhibition encircling the discs with different concentrations of ZnONPs indicates that the nanoparticles considerably impeded the growth of fungi (Fig. 13). This suggests that ZnONPs have fungicidal qualities, meaning they can kill or stop the growth of fungus. The biologically produced ZnONPs were found to have dose-dependent antifungal activity, with bigger inhibitory zones appearing at higher doses (Table 4). The ZnONPs demonstrated antifungal efficacy against *Candida albicans* at test concentrations of 25, 50, and 75 $\mu\text{g/mL}$. Their inhibition zones varied from 15 ± 0.21 mm to 21 ± 0.16 mm, which was marginally bigger than the positive control's 23 ± 0.16 mm inhibition zone. As opposed to the positive control, which had an inhibition zone of 21 ± 0.16 mm, the plant extract had a lesser antifungal impact, with inhibition zones ranging from 6 ± 0.21 mm at 25 $\mu\text{g/mL}$ to 15 ± 0.16 mm at 75 $\mu\text{g/mL}$.

The distinct and prominent inhibition zones caused by the ZnONPs can be attributed to the interaction between the nanoparticles and the fungal cell wall. Biochemicals such as flavonoids and tannins on the surface of the ZnONPs can directly contact fungal cell wall proteins, causing the cell wall to rupture and leading to the observed antifungal effects [29]. However, these surface biochemicals may have been weakened during the manufacturing and processing of the ZnONPs, and the antifungal activity is primarily due to the impact of the nanoparticles.

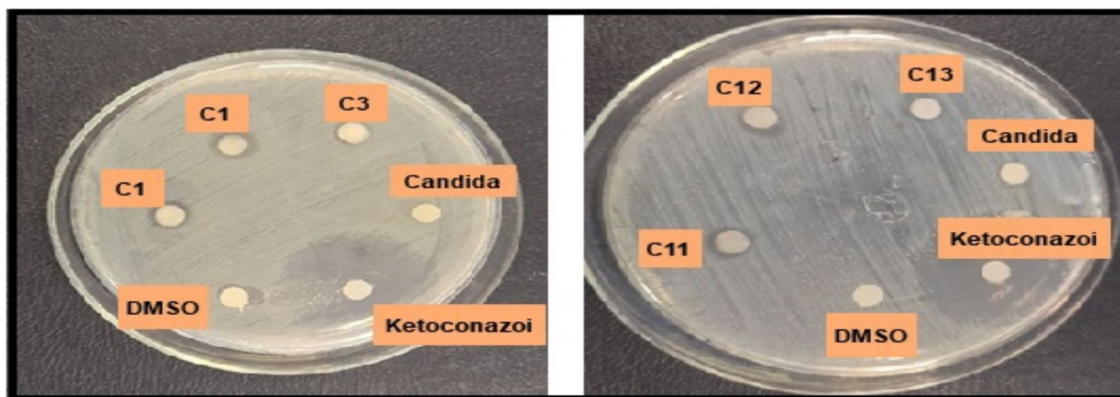


Fig. 13 Antifungal testing with zone formation; ZnO NPs, against *Candida albicans*

Table 4 Zone of inhibition (mm) of ZnO NPs with extract of *M. longifolium* leaves

Tested fungus		Diameter of zone of inhibition (in mm)			
		25 µg/mL	50 µg/mL	75 µg/mL	– Tv control
<i>Candida albicans</i>	ZnONPs	15 ± 0.21	17 ± 0.56	21 ± 0.16	0 ± 0
	Plant extract	6 ± 0.21	7 ± 0.56	15 ± 0.16	0 ± 0

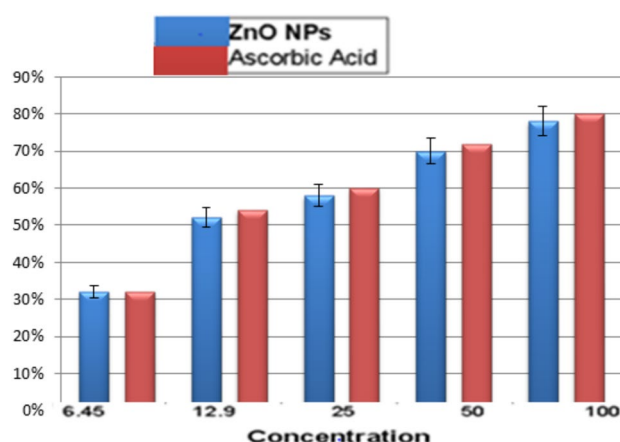
Table 5 MIC, MBC and MFC of ZnO nanoparticles

Bacterial strain	ZnO NPs	
	Microbicidal concentration (µg/ml)	
	MIC	MBC
<i>E. coli</i>	12.5	25
<i>S. aureus</i>	6.25	12.5
Fungi strain	MIC	MFC
<i>Candida albicans</i>	12.5	25

The antifungal properties of biologically produced ZnONPs make them promising candidates for the development of bioactive food packaging materials. Previous research has also demonstrated their potential in this application [30]. The inhibitory zones observed were 21 ± 0.16 mm at 75 µg/mL, 17 ± 0.56 mm at 50 µg/mL, and 15 ± 0.21 mm at 25 µg/mL (represented by C11, C12, and C13). Additionally, the plant extract alone exhibited potential in treating systemic fungal infections, with inhibitory zones of 15 ± 0.16 mm at 75 µg/mL, 7 ± 0.56 mm at 50 µg/mL, and 6 ± 0.21 mm at 25 µg/mL respectively. The zone of inhibition values of leaf extract were lower than those observed for the ZnO NPs, but still demonstrated the antifungal potential of the leaf extract.

3.3.9 MIC, MBC and MFC of ZnO nanoparticles

By measuring ZnO NPs' minimum inhibitory concentration (MIC) and minimum bactericidal/ fungicidal concentration (MBC/MFC) against bacterial and fungal pathogens, the antibacterial efficiency of the material was proven. With minimum inhibitory concentrations (MIC) of 12.5 µg/ml against *Escherichia coli* and 6.25 µg/ml against *Staphylococcus aureus*, the ZnO NPs were found to have strong antibacterial properties (Table 5). Moreover, it was discovered that the MBC values were two times greater than the matching MIC values, suggesting that somewhat greater ZnO NP concentrations were needed to attain full bactericidal action. Comparable patterns were noted in the ZnO NPs' antifungal capabilities, as they demonstrated a 12.5 µg/ml MIC and a 25 µg/ml MFC against *Candida albicans*. These ZnO NPs' remarkable antibacterial ability is highlighted by their low MIC and MBC/MFC values, which also point to their prospective uses in the creation of cutting-edge antimicrobial coatings and treatments.

Fig. 14 Antioxidant activity of ZnO NPs**Table 6** IC₅₀ values of ZnO NPs by DPPH, compared to ascorbic acid

Compounds	Absorbance of samples	IC ₅₀ (%)
Control of DPPH	0.505	0
Ascorbic-acid	0.564	54
ZnO NPs	0.52	52

3.3.10 Antioxidant activity of ZnO NPs

The antioxidant activity of the synthesized ZnO NPs was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, as shown in Fig. 14. DPPH is a stable free radical that exhibits a dark violet color when dissolved in organic solvents. The synthesized ZnO NP samples showed a decrease in DPPH absorbance at 517 nm, indicating their potential as free radical scavengers [13].

The IC₅₀ values, presented in Table 6, provide further insight into the antioxidant potency of the ZnO NPs. The control DPPH sample showed 0% inhibition, while the ascorbic acid (vitamin C) positive control achieved a 54% inhibition. Remarkably, the synthesized ZnO NPs demonstrated a 52% inhibition of the DPPH radicals, highlighting their significant antioxidant activity. The ZnO NPs also exhibited potent DPPH radical scavenging, with the scavenging percentages increasing from 32 to 78% as the concentrations were raised from 6.45 to 100 µg/mL. Though slightly lower than the ascorbic acid reference, the ZnO NPs' antioxidant performance was still impressive, likely attributed to their surface functionalization with bioactive components. This property makes them suitable for various biomedical applications due to their high biocompatibility and antioxidant activity. Further surface modifications may unlock even greater potential for the ZnO NPs.

4 Conclusion

Using an extract from the leaves of the medicinal plant *M. longifolium*, the current study successfully demonstrated the green production of ZnO NPs. Investigating the shape, structure, particle size, crystallinity, and functional groups of the produced ZnO NPs was done by extensive characterisation techniques. These techniques included SEM, TGA/DTA, XRD, UV-Vis, and FT-IR. The ZnO nanoparticles that were produced showed remarkable antibacterial properties. They showed significant antifungal efficacy against *Candida albicans* as well as notable antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Additionally, the nanoparticles showed impressive antioxidant properties. Based on their multifunctional characteristics, the green-synthesised ZnO NPs seem to have significant promise as antibacterial, antifungal, and antioxidant agents. This synthesis process is environmentally friendly, economical, and sustainable, making it a viable tactic for the creation of valuable nanomaterials with a range of biological uses.

Author contributions Sisay Geda Bekele (1) contributed to the writing of the main manuscript text. Dawit Darcha Ganta (2) prepared the figures for the manuscript. Muluneh Endashaw (3) reviewed and provided feedback on the manuscript. All authors have read and approved the final version of the manuscript.

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Data availability The datasets that were collected and utilized for analysis will be made available upon request from the corresponding author, who has full access to all study data and is in charge of ensuring that the data is accurate and reliable in the analysis.

Declarations

Ethics and consent to participate Voucher specimens were deposited in the *Monoon longifolium*, with deposition numbers ETH-001. The specimens were identified by Dr. Terefe Wondimu. This article does not contain any studies involving human, animal, or patient participants performed by any of the authors.

Research involving plants We confirmed that all methods (field studies/collection) involving *M. longifolium* leaves used for the green synthesis of NPs in the current study were in accordance with relevant guidelines/regulations/legislation.

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

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