

Green Synthesis of Zinc Oxide Nanoparticles Using Butea Monosperma Flower Extract and Evaluation of Their Antioxidant and Anticancer Activities Against A-549 Lung Cancer Cells

Manivannan Rangaswamy¹, Delipkumar Kaliyappan², Yasotha Selvan³, Suresh Kumar Gopal*, Jayaprasanth J⁴, Nisha K⁵

¹Professor, Department of Pharmaceutical Biotechnology, Excel College of Pharmacy, Affiliated with Tamil Nadu, Dr. M.G.R. Medical University, Tamil Nadu

²Student, Department of Pharmaceutical Analysis, JKK Nattraja College of Pharmacy, Affiliated with Tamil Nadu, Dr. M.G.R. Medical University, Tamil Nadu.

³Assistant Professor, Department of Pharmaceutical Analysis, Excel College of Pharmacy, Affiliated with Tamil Nadu, Dr. M.G.R. Medical University, Tamil Nadu

⁴Professor, Department of Pharmaceutical Biotechnology, Excel College of Pharmacy, Affiliated with Tamil Nadu, Dr. M.G.R. Medical University, Tamil Nadu

⁵Student, Department of Pharmacology, JKK Nattraja College of Pharmacy, Affiliated with Tamil Nadu, Dr. M.G.R. Medical University, Tamil Nadu.

*Student, Department of Pharmacology, JKK Nattraja College of Pharmacy, Affiliated with Tamil Nadu, Dr. M.G.R. Medical University, Tamil Nadu.

Corresponding author: Suresh Kumar Gopal

Abstract

Cancer remains a leading cause of death worldwide, with lung cancer the most frequently diagnosed malignancy approximately 2.5 million new cases and 1.8 million deaths in 2022 alone. The present study reports a simple, cost-effective, and eco-friendly green synthesis of zinc oxide nanoparticles using the aqueous flower extract of Butea monosperma, whose flavonoids and phenolic constituents served as natural reducing and capping agents. The biosynthesized NPs were characterized by UV–visible spectroscopy, FT-IR, SEM, and XRD, and evaluated for antioxidant and anticancer activities by ABTS, hydrogen peroxide scavenging, and MTT assays against A549 lung adenocarcinoma cells. The UV–vis spectrum exhibited a broad absorption band with a maximum absorbance of 0.98 at 450 nm, while FT-IR confirmed the retention of phytochemical functional groups together with the characteristic Zn–O stretching vibration at 688.5 cm⁻¹. SEM revealed quasi-spherical to hexagonal-like particles with rough surfaces, and XRD confirmed the hexagonal wurtzite structure (JCPDS 36-1451) with an average crystallite size of 66.43 nm. The NPs showed dose-dependent radical scavenging, achieving 76.50% and 87.00% (H₂O₂) inhibition at 50 µg/mL exceeding the ascorbic acid standard (61.45% and 64.88%, respectively) at every concentration tested, with an IC₅₀ between 20 and 30 µg/mL. In the MTT assay, growth inhibition of A549 cells rose from 15.00% (18.75 µg/mL) to 72.00% (300 µg/mL; R² = 0.9915), yielding an IC₅₀ of 91.69 µg/mL, with dose-dependent morphological changes including cell rounding and detachment (p < 0.0001). These findings establish B. monosperma flower extract as an effective bio-reducing and stabilizing agent for producing phytochemically capped ZnO NPs with pronounced antioxidant and anticancer potential, meriting further mechanistic and in vivo investigation.

Keywords: Green synthesis; zinc oxide nanoparticles; Butea monosperma; antioxidant activity; anticancer activity; A549 cells; MTT assay.

INTRODUCTION

Cancer has become a primary cause of death worldwide, and the economic burden of its treatment continues to rise steeply (Umamaheswari et al., 2021). Among all malignancies, lung cancer remains the most common cancer and the leading cause of cancer-related death, accounting for approximately 2.5 million new cases (12.4% of all cancers globally) and 1.8 million deaths (18.7%) in 2022 alone (Bray et al., 2024). Despite the availability of early diagnosis and established treatment modalities such as chemotherapy, surgery, and radiotherapy, patients frequently suffer from severe side effects and treatment-induced strain (Umamaheswari et al., 2021). This has prompted researchers to explore the unique properties of nanomedicine as a preferred alternative for the diagnosis and treatment of cancer. The WHO estimates that nearly 75–80% of the global population still depend on traditional medicinal practices for their primary health care needs (Umamaheswari et al., 2021).

In the 21st century, nanotechnology has emerged as an innovative field with applications across diverse sectors. Nanoparticles are conventionally produced by physical and chemical methods; however, the green synthesis of NPs is being increasingly emphasized by researchers because it avoids toxic chemicals, high cost, and severe reaction conditions (Umamaheswari et al., 2021). Various biological sources bacteria, fungi, yeast, actinobacteria, algae, and plants have been exploited for this purpose, among which plants are regarded as the best candidates for the synthesis of metal NPs (Umamaheswari et al., 2021). Plants provide a superior platform over chemical and biological routes: the process is free from toxic chemicals, cost-competitive, and rapid, and their secondary metabolites serve as natural reducing as well as capping agents, yielding NPs that are more stable than those obtained from other biological sources (Umamaheswari et al., 2021).

A number of metal oxide NPs have been synthesized by green technology, including TiO₂, Sm₂O₃, Cr₂O₃, NiO, CdO, Eu₂O₃, SnO₂, CuO, Fe₃O₄, and ZnO. Among them, zinc oxide nanoparticles possess distinctive physicochemical properties a wide direct band gap of ~3.37 eV, a large exciton binding energy of 60 meV, natural n-type semiconductivity, strong room-temperature luminescence, broad radiation absorption, and high thermal and chemical stability together with well-documented antimicrobial and antitumor activities (Raha & Ahmaruzzaman, 2022; Sirelkhatim et al., 2015). Consequently, many researchers have focused on the biosynthesis of ZnO NPs and on controlling their particle shape and size distribution. Because plant extracts are rich in polyphenols, amino, and carboxylic groups, they can act as reductants and stabilizers during NP formation (Umamaheswari et al., 2021). ZnO NPs produced from plants can be obtained in a wide range of shapes and sizes with tunable properties, at low processing temperatures, through a simple, cheap, and eco-friendly process that requires no intermediate base groups.

Previous reports have described the green synthesis of ZnO NPs using the flower extracts of *Trifolium pratense* and *Nyctanthes arbor-tristis*, the leaf extracts of *Passiflora caerulea*, *Catharanthus roseus*, and *Murraya koenigii*, the root of *Lathyrus sativus* L., and *Aloe vera* leaves, with a variety of pharmacological outcomes (Jamdagni et al., 2016).

The tree *Butea monosperma*, commonly known as the "flame of the forest" or Palash, belongs to the family Fabaceae and is widely distributed in tropical and subtropical regions of the Indian subcontinent (Kumari et al., 2026). Its bright orange-red flowers are rich in bioactive flavonoids such as butein, isobutrin, and butin, along with terpenoids, steroids, tannins, and glycosides (Kumari et al., 2026; Tanaka & Kashiwada, 2021). In traditional systems of medicine including Ayurveda and Unani, the plant has long been used against ulcers, wounds, piles, skin disorders, and parasitic infections, while the flowers specifically have served as an emmenagogue, diuretic, depurative, and tonic, and are commercially utilized as a natural dye owing to their intense color (Kumari et al., 2026; Tanaka & Kashiwada, 2021). The flowers and their constituents exhibit a broad pharmacological spectrum antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective, and chemopreventive activities (Kumari et al., 2026; Tanaka & Kashiwada, 2021). Notably, butein isolated from the flowers showed antitumour activity with a mean IC₅₀ of 7.39 µg/mL against an ovarian tumour cell line (Than et al., 2016), downregulated phorbol-12-myristate-13-acetate-induced COX-2 transcriptional activity in breast cells (Gunti et al., 2017), and flower-extract gold nanoparticles demonstrated chemopreventive effects on skin carcinoma and leukemia cells (Gunti et al., 2017). Although ZnO NPs have previously been biosynthesized from *B. monosperma* seed and whole-plant extracts for antibacterial, antibiofilm, and anti-quorum sensing applications (Ali et al., 2021; Thakur et al., 2024), and flower-extract hematite (α-Fe₂O₃) NPs exhibited dose-dependent antiproliferative activity against MCF-7 breast cancer cells (Behera et al., 2020), the anticancer potential of ZnO NPs derived from *Butea monosperma* flowers has not been investigated. Bearing in mind the global need for eco-friendly green synthesis approaches, our specific aim was designed to synthesize ZnO NPs using *Butea monosperma* flower extract and evaluate their cytotoxic potential in A549 lung cancer cells.

MATERIALS AND METHODS

Plant material

Flowers of *Butea monosperma* were obtained from Pachampalayam, Sankari, Salem, Tamil Nadu, India (11.477696° N, 77.873886° E). The collected flowers were thoroughly washed with distilled water to remove adhering impurities and air-dried at room temperature. The plant material was authenticated by Dr. P. Radha, Research Officer, Siddha Medicinal Plants Garden, Central Council for Research in Siddha, Mettur Dam, Tamil Nadu.

Preparation of plant extract

Five grams of *Butea monosperma* flowers were thoroughly cleaned with distilled water, and the flower surfaces were sterilized with 70% ethanol. The flowers were then heated in 100 mL of distilled water at 50 °C for 30 min. The resulting extract was filtered first through Whatman No. 1 filter paper and then vacuum-filtered through a 0.2 µm pore-size membrane filter. The final filtrate was stored at 4 °C for further analysis.

Phytochemical analysis

The extracts were examined for their phytochemicals flavonoids (ferric chloride test and lead acetate test), alkaloids, tannins (gelatin test), saponins (froth test), phenols (ferric chloride test), glycosides (Keller–Killiani test), proteins, amino acids (ninhydrin test), phytosteroids, carbohydrates, and anthraquinones following the

earlier reported procedures (Shaikh & Patil, 2020). Qualitative chemical tests remain the widely used first-line approach for the preliminary phytochemical screening of medicinal plants, since alkaloids, flavonoids, phenolics, tannins, saponins, steroids, and glycosides are important phytochemicals whose presence largely determines the therapeutic value of a plant (Shaikh & Patil, 2020). Consistent with earlier reports, the flowers of *Butea monosperma* have been shown to contain phenols, alkaloids, flavonoids, anthocyanins, xanthophyll, saponins, and carotenoids in their methanolic and ethanolic extracts (Jangid et al., 2021), and phytochemical screening of its leaf and flower extracts has confirmed the presence of alkaloids, tannins, flavonoids, saponins, phenols, proteins, and sugars (DAVE et al., 2019).

Test for flavonoids

Alkaline reagent test About 2 mL of the extract was added with few drops of 10% sodium hydroxide solution; an intense yellow color formation indicates the presence of flavonoid. **Lead acetate test** About 2 mL of the extract was added with few drops of lead acetate solution; a yellow color precipitate formation indicates the presence of flavonoids.

Test for alkaloids

Mayer's reagent 1 mL of the extract was mixed with few drops of Mayer's reagent; a cream color precipitate formation indicates the presence of alkaloid (Ramesh et al., 2023). About 3 mL of the extract was added to 1 mL of hydrochloric acid, mixed well and gently heated for 20 min, then cooled to room temperature. Subsequently, the mixture was filtered through Whatman filter paper, and the filtrate was used for the following test. **Hager's test** About 1 mL of the extract was mixed with few drops of Hager's reagent; a yellow color precipitate formation indicates the presence of alkaloid (Ramesh et al., 2023).

Test for tannins

Gelatin test About 2 mL of the extract was mixed with few drops of 10% gelatin solution and sodium chloride solution; a white buff color precipitate formation indicates the presence of tannin.

Test for phenols

Ferric chloride test About 2 mL of the extract was mixed with few drops of 5% neutral ferric chloride solution; a dark green color formation indicates the presence of phenol.

Test for glycosides

Keller–Killiani test About 2 mL of the extract was mixed with 2 mL of glacial acetic acid containing a drop of ferric chloride solution; a brown color ring formation indicates the presence of glycoside (Kebede et al., 2021).

Test for proteins

Biuret's test About 2 mL of the extract was mixed with few drops of 4% sodium hydroxide solution and 1% copper sulfate solution; formation of a violet color indicates the presence of protein.

Test for amino acids

Ninhydrin test About 2 mL of the extract was heated with 5% ninhydrin solution; a violet color formation indicates the presence of amino acids.

Test for phytosteroids

Salkowski's test 5 mL of the extract was treated with 2 mL of chloroform, and few drops of concentrated sulfuric acid (H_2SO_4) were added along the side of the test tube; a reddish brown color formation indicates the presence of phytosteroids.

Test for carbohydrates

About 2 mL of the extract was diluted with 5 mL of double distilled water, mixed well, and filtered. The filtrate was then used to perform Benedict's test. **Benedict's test** Around 2 mL of filtrate was mixed with few drops of Benedict's reagent and gently heated; an orange red precipitate formation indicates the presence of carbohydrate.

Test for saponins

Froth test About 1 mL of the extract was added to 3 mL of double distilled water, and the mixture was vigorously shaken; formation of foam indicates the presence of saponin (Nigussie et al., 2021).

Test for anthraquinones

Borntrager's test About 1 mL of the extract was mixed with 20 mL of chloroform, heated for 5 min, filtered in hot condition, and allowed to cool to room temperature. To the filtrate, an equal volume of 10% ammonia solution was added and shaken well; the appearance of a bright pink color in the aqueous layer indicates the presence of anthraquinones (Kebede et al., 2021; Nigussie et al., 2021).

Green Synthesis of Zinc Oxide Nanoparticles

Twenty millilitres of *Butea monosperma* flower extract was warmed at 50 °C for 10 min. Then, 50 mL of 91 mM zinc acetate solution was slowly added dropwise to the warmed extract under continuous stirring. The reaction mixture gradually developed a yellow colour, leading to the formation of a cream-coloured zinc hydroxide precipitate. The reaction mixture was then left undisturbed for 30 min at room temperature to confirm the complete formation of zinc hydroxide. The obtained precipitate was centrifuged at 16,000 rpm for 10 min at 4 °C. The resultant precipitate was subjected to vacuum drying at 30 °C, and the sample was reserved for further analysis (Bala et al., 2014).

Biosynthesized ZnO NPs characterization

Optical property of ZnO NPs by UV–visible spectroscopy

To determine the optical property of the synthesized ZnO NPs, the nanopowder was analyzed by UV–visible spectroscopy (Lambda 25 Spectrometer, Perkin Elmer, Germany) in the range of 200–800 nm, and the maximum absorption was recorded (Bala et al., 2014).

FT-IR spectral analysis

Fourier transform infrared spectroscopy was used for the determination of functional groups and phytochemical compounds responsible for the reduction and surface stabilization of the synthesized nanoparticles. FT-IR analysis was conducted on the powdered ZnO NPs and the freeze-dried plant extract using 1% KBr plates, and the spectra were recorded to identify the major functional groups associated with the reduction, stabilization, and formation of the NPs (Selim et al., 2020).

Scanning electron microscopy

The Scanning electron microscope can help in the determination of the surface morphology of ZnO NPs. The morphological structure of the synthesized ZnO NPs was analyzed using a Quanta FEG-250 SEM (Samy et al., 2019).

X-ray diffraction analysis

The crystalline nature and crystallite size of the synthesized ZnO NPs were confirmed using a powder diffractometer (Model D8, Bruker AXS) with Cu K α radiation ($\lambda = 0.15425$ nm), operating in the 2θ range of 20° to 80° (Abdulqodus et al., 2025).

The average crystallite size was subsequently calculated using the Debye-Scherrer equation based on the broadening of the most intense diffraction peaks (El-Saadony et al., 2024; Irede et al., 2024).

Antioxidant Activity

Evaluation of 2,2'-Azino-bis(3-ethyl) benzothiazoline-6-sulfonic Acid Radical Cation Scavenging Assay

The ABTS radical cation scavenging activity of the ZnO NPs was examined using the method described by Re et al. (Moreira, 2019). The ABTS radical cation was generated by reacting ABTS (7 mM) with potassium persulfate (2.45 mM), and the mixture was incubated for 16 h under dark conditions at room temperature (Verardo et al., 2011). The prepared ABTS solution was then diluted with methanol to achieve an absorbance of 0.700 ± 0.02 at 734 nm, forming the working solution. Five mL of different concentrations of ZnO NPs (10 μ L, 20 μ L, 30 μ L, 40 μ L, 50 μ L), diluted with methanol, were mixed with 3.995 mL of ABTS working solution. The reaction mixture was incubated at room temperature for 4 min, after which the absorbance was recorded at 734 nm using a UV–visible spectrophotometer against a blank. The percentage inhibition was determined using the following formula:

$$\text{ABTS Radical Scavenging Activity (\%)} = \frac{\{\text{Absorbance of Control} - \text{Absorbance of Sample}\}}{\text{Absorbance of Control}} \times 100$$

where A_{control} represents the absorbance of the negative control and A_{sample} represents the absorbance of the sample.

Hydrogen peroxide radical scavenging activity assay

The hydrogen peroxide scavenging activity of the synthesized ZnO NPs was investigated using the procedure adopted by Pick and Mizel with minor modifications (Pick & Mizel, 1981). Different concentrations of ZnO NPs (10 µL, 20 µL, 30 µL, 40 µL, 50 µL) and ascorbic acid (used as standard) were added to 150 µL of 4 mM hydrogen peroxide solution in 0.1 M phosphate buffer. The mixture was incubated for 20 min, and the absorbance was then measured at 230 nm (Mukhopadhyay et al., 2016). The percentage inhibition of hydrogen peroxide radical scavenging activity was determined using the following equation:

$$\text{H}_2\text{O}_2 \text{ Scavenging Activity (\%)} = \frac{\{\text{Absorbance of Control} - \text{Absorbance of Sample}\}}{\text{Absorbance of Control}} \times 100$$

where A_{control} indicates the absorbance of the negative control and A_{sample} indicates the absorbance of the sample.

In vitro Cytotoxicity Assay

The anticancer activity of the synthesized ZnO NPs was determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reduction assay (Mosmann, 1983). To analyze the cell viability of the A-549 cell line, 100 µL of cells were seeded in 96-well plates and maintained at 37 °C with 5% CO₂ for 24 h to allow the cells to adhere to the bottom of each well. Subsequently, the cells were exposed to ZnO NPs in a dose-dependent manner, with concentrations ranging from 18.75, 37.5, 75, 150, and 300 µg/mL in the allocated wells, followed by overnight incubation (Figures (3).Pdf, n.d.). The cells were then incubated with MTT (20 µL/well of 5 mg/mL in PBS) at 37 °C for 4 h (Twentyman & Luscombe, 1987). The yellow water-soluble MTT was reduced by mitochondrial dehydrogenase to produce water-insoluble purple formazan crystals (Sazonova et al., 2022). These crystals were dissolved in DMSO, and the plate was shaken for 30 min. The amount of formazan crystal formed, which is directly proportional to the percentage of viable cells, was then quantified by measuring the absorbance at 590 nm using a microplate reader.

$$\text{Percentage Inhibition} = \frac{100 - \{\text{Absorbance of Control} - \text{Absorbance of Sample}\}}{\text{Absorbance of Control}} \times 100$$

Cell viability was determined using the following equation:

$$\text{Cell Viability} = \frac{\text{Absorbance of Treated Cells}}{\text{Absorbance of Control Cells}} \times 100$$

RESULTS

Phytochemical analysis of Butea monosperma flowers

Plant origin has huge variety of phytochemicals and has been considered as a bio-active laboratory with multipurpose applications. Hence, these plant origins have importance from ancient time in treating various diseases. In addition, the medicinal plants are cost effective, less side effects, simple effective and easily available. Investigation of phytochemicals in the plant origin can find the approach for its medicinal properties and knowledge of the phytochemicals can establish its medicinal importance leading to discovery and development of a new molecule (Shaikh & Patil, 2020).

The preliminary phytochemical screening test revealed the presence of several phytoconstituents and its results are shown in Table 1. The aqueous extract of Butea monosperma flowers tested positive for alkaloids, flavonoids, phenols, reducing sugars, tannins, glycosides, and proteins, suggesting significant biological activity, consistent with earlier phytochemical reports on B. monosperma flowers (DAVE et al., 2019; Jangid et al., 2021). In addition, the presence of flavonoids and phenols can be hypothesized to have antioxidant activity and ultimately predicted to have anticancer activity (- & -, 2025), whereas the detection of saponins is well known for their antimicrobial and haemolytic properties. However, the absence of steroids and phytosterols in B. monosperma flowers distinguishes their phytochemical profile from those of other medicinal plants.

Due to combination of different phytochemicals, plant extract has been considered as a potential alternative one for stabilizing as well as reducing agents in the synthesis of metal nanoparticles (Umamaheswari et al., 2021). Earlier study results pointed out that phytoconstituents like flavonoids and phenols are responsible for the biosynthesis of metal and metal oxide NPs (Umamaheswari et al., 2021). Our preliminary phytochemical study supported that both flavonoids and phenols are present in the aqueous flower extract only. Hence, the aqueous extract was taken into consideration for feasible ZnO NPs synthesis. Presence of OH groups in flavonoids and phenols can reduce the zinc compounds into ZnO NPs and also act as capping or stabilizing agent of the NPs. Therefore, separate capping or stabilizing agents are not necessary in this synthesis NPs approach

(Umamaheswari et al., 2021). In the reduction process, it is hypothesized that zinc acetate acts as the precursor whereas the *B. monosperma* flower extract functions as the reducing agent (Umamaheswari et al., 2021).

Table 1: Qualitative analysis of phytochemicals in *Butea monosperma* flowers extract.

Test	Observation	Inference
Alkaloids	Reddish brown precipitate	++
Flavonoids	Dark blue	+++
Phenol	Deep blue colour	+++
Reducing Sugar	Blue colour	++
Steroids	Red colour precipitate	-
Saponin	Foam formation	+
Phytosterols	Bluish green colour	-
Tannin	Dark green or blue colour	+++
Glycoside	Red colour	++
Protein	Purple or violet colour	++

UV-vis spectra analysis

The UV-vis spectroscopy technique was performed to characterize the optical property and to confirm the formation of ZnO NPs. After the dropwise addition of zinc acetate solution to the warmed flower extract, the reaction mixture gradually developed a yellow colour, leading to the formation of a cream-coloured precipitate, which provided the key indication of the formation of the zinc hydroxide intermediate and its phytofabrication into ZnO NPs (Naiel et al., 2022).

The UV-vis absorption spectrum of the synthesized product, recorded between 250 and 700 nm, exhibited a broad absorption band with a maximum absorbance of 0.98 at 450 nm, and the absorbance steadily declined towards longer wavelengths (Figure 1). Although characteristic absorption peaks of ZnO NPs are typically observed in the range of 355–380 nm (Abdelbaky et al., 2023; Shamhari et al., 2018), attributed to the intrinsic band-gap absorption of Zn–O arising from electron transitions from the valence band to the conduction band (Abdulqodus et al., 2025), the absorption band recorded here is red-shifted into the visible region. Such a shift can reasonably be attributed to the relatively larger particle size of the NPs, since the resonance absorption of ZnO undergoes a bathochromic shift with increasing particle size (Mousa et al., 2024), and to defect-related absorption together with the phytochemicals, such as flavonoids and phenolics derived from the *B. monosperma* flower extract, capping the nanoparticle surface (Abdulqodus et al., 2025; Kesavardhini et al., 2026). The bioactive constituents of the plant extract interact with the Zn ions during the synthesis and act as stabilizing as well as reducing agents in the reduction of the metal size (Tiwari et al., 2024). The observed broad absorption band, extending into the visible region, thereby corroborates the formation of phytochemically capped ZnO NPs (Muhammad et al., 2019), consistent with earlier reports of green-synthesized ZnO NPs exhibiting characteristic absorption near 370 nm (Selim et al., 2020).

Furthermore, the observed spectral profile specifically the maximum absorbance at 450 nm aligns with documented instances where phytochemical capping agents and morphological variances induce significant bathochromic shifts in the surface plasmon resonance of metallic nanoparticles (Geremew et al., 2023).

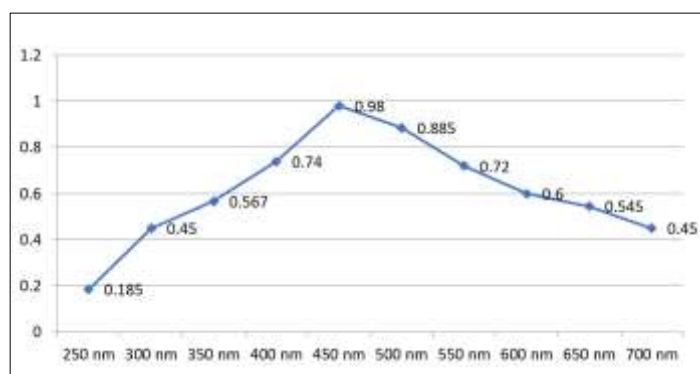


Figure 1: UV-visible absorption spectrum of green-synthesized ZnO nanoparticles using *Butea monosperma* flower extract

FT-IR spectral analysis

FTIR provides information about the molecules' vibrational and rotational modes of motion and determines the probable functional groups involved in the formation of ZnO NPs (Umamaheswari et al., 2021). The observed FTIR spectrum showed peaks at 3338.7, 2924.0, 1724.3, 1598.9, 1373.3, 1226.7, 1024.2, and 688.5 cm^{-1} , as shown in Figure 2.

The intense and broad peak at 3338.7 cm^{-1} is associated with H-bonded hydroxyl ($-\text{OH}$) group stretching vibration of phenolic or alcoholic groups present in the *Butea monosperma* flower extract (Abdelbaky et al., 2023; Tiwari et al., 2024); 2924.0 cm^{-1} is associated with C–H stretching vibrations of aliphatic $-\text{CH}$ and $-\text{CH}_2$ groups (Abdelbaky et al., 2023); 1724.3 cm^{-1} is associated with carbonyl ($\text{C}=\text{O}$) stretching of ester and carboxylic acid moieties, and 1598.9 cm^{-1} is associated with C=C aromatic stretching and amide $\text{C}=\text{O}/\text{C}=\text{N}$ bending vibrations contributed by flavonoid carbonyl groups, where the tautomeric transformation of flavonoids between the enol- and keto-forms releases reactive hydrogen atoms that reduce Zn^{2+} to NPs (Abdelbaky et al., 2023; Aliannezhadi et al., 2024; Umamaheswari et al., 2021); 1373.3 cm^{-1} is associated with aromatic C–H bending vibrations (Abdelbaky et al., 2023); 1226.7 cm^{-1} and 1024.2 cm^{-1} are associated with C–O and C–O–C stretching vibrations, which correlate with the phenolic and flavonoid constituents of the extract and indicate their active role in the capping and stabilization of the formed NPs (Abdelbaky et al., 2023; Aliannezhadi et al., 2024); and 688.5 cm^{-1} is the stretching vibration peak for ZnO, denoted as metal–oxygen (M–O), falling within the 400–750 cm^{-1} region characteristic of Zn–O modes in green-synthesized ZnO NPs (Aliannezhadi et al., 2024).

The FTIR results confirmed the presence of phytochemical functional groups on the surface of the synthesized ZnO NPs. Band shifts relative to the pure flower extract, both increases and decreases, indicate the involvement of these functional groups in the reduction of Zn^{2+} to ZnO and in the subsequent stabilization of the NPs. The presence of flavonoids in the extract plays an important role in the formation of the corresponding metal NPs by reduction of metal ions (Umamaheswari et al., 2021), consistent with earlier reports on ZnO NPs synthesized using *Butea monosperma* seed and plant extracts (Ali et al., 2021; Thakur et al., 2024).

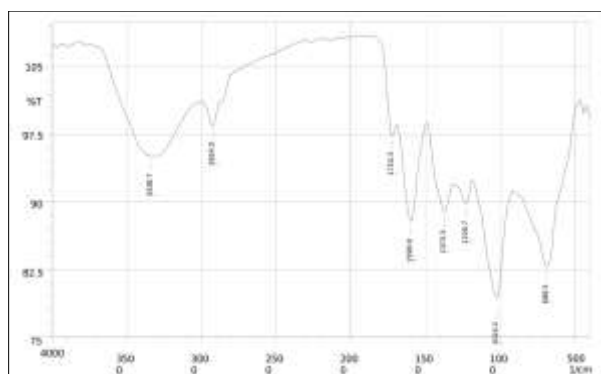


Figure 2: FTIR spectra of *Butea monosperma* flower extract and green-synthesized ZnO nanoparticles showing the changes in characteristic functional groups and the Zn–O stretching vibration

X-ray diffraction analysis

The crystalline phase and crystallite size of the biosynthesized ZnO NPs were determined by X-ray diffraction analysis using a Bruker AXS D8 powder diffractometer with Cu $\text{K}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$), operated at 40 kV and 30 mA, over a 2θ range of 20° – 80° (Abdulqodus et al., 2025). The diffraction pattern exhibited sharp, well-defined Bragg peaks at 2θ values of 31.71° , 34.41° , 36.23° , 47.67° , 57.61° , 62.98° , 66.51° , 68.05° , and 69.21° , corresponding to the, and lattice planes, respectively, as shown in Figure 3. These peaks match the standard hexagonal wurtzite structure of ZnO reported in JCPDS card No. 36-1451 (Selim et al., 2020; Thakur et al., 2024), confirming the phase-pure crystalline nature of the biosynthesized NPs.

The sharpness and intensity of these Bragg peaks indicate effective crystallization of ZnO, and the absence of additional extraneous peaks confirms that the phytochemical constituents of the *Butea monosperma* flower extract functioned purely as reducing and capping agents rather than forming any independent crystalline phase (Bala et al., 2014; Kesavardhini et al., 2026). The centrifugation-and-re-dispersion purification step performed prior to characterization further ruled out any residual capping-agent crystallization, in line with similar green ZnO syntheses (Aliannezhadi et al., 2024; Thakur et al., 2024).

The average crystallite size of the biosynthesized ZnO NPs was estimated using the Debye–Scherrer equation applied to the most intense peak the plane at $2\theta = 36.23^\circ$ (Abdelbaky et al., 2023; Shamhari et al., 2018):

$$D = 0.89\lambda / (\beta \cos \theta)$$

where D is the crystallite size, λ is the X-ray wavelength (1.5406 Å), β is the full width at half maximum of the diffraction peak, and θ is the Bragg angle of diffraction. Based on this calculation, the average crystallite size of the biosynthesized ZnO NPs was determined to be 66.43 nm, in agreement with earlier reports of green-synthesized ZnO NPs from plant extracts (Aliannezhadi et al., 2024; Bala et al., 2014).

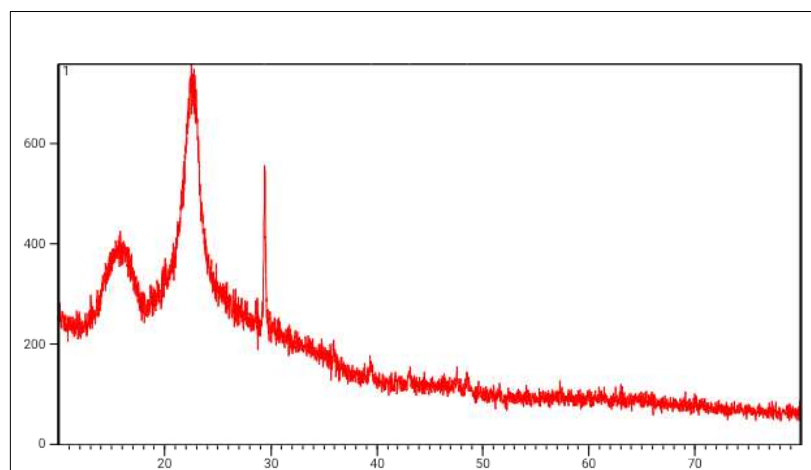


Figure 3: X-ray diffraction pattern of green-synthesized ZnO nanoparticles using *Butea monosperma* flower extract, showing the characteristic diffraction peaks of the hexagonal wurtzite ZnO structure.

Scanning electron microscopy

SEM analysis is carried out to visualize the NP size, shape, and surface morphology (Ali et al., 2021). A MIRA3 TESCAN field-emission SEM was used to determine the morphology of the biosynthesized ZnO NPs at different magnifications, with images recorded at an accelerating voltage of 30.0 kV, a working distance of 6.79 mm, and secondary electron detection.

At the lower magnification (5 μm scale bar), the SEM micrograph shows that the synthesized ZnO NPs are predominantly clustered into densely packed aggregates of irregular, three-dimensional structures with rough, textured surfaces, consistent with the typical appearance of green-synthesized ZnO NPs where particle-level resolution is masked by inter-particle adhesion and the presence of phytochemical capping (Ali et al., 2021; Thakur et al., 2024). At the higher magnification (500 nm scale bar), individual grains become discernible within the aggregates, revealing particles with predominantly quasi-spherical to polyhedral (hexagonal-like) outlines and a noticeably rough surface texture (Mousa et al., 2024), as shown in Figure 4. The aggregation pattern observed is comparable with earlier reports of ZnO NPs synthesized using *Butea monosperma* seed and plant extracts (Ali et al., 2021; Thakur et al., 2024), as well as with green ZnO NPs obtained from a wide variety of plant extracts where similar phytoconstituent-mediated agglomeration has been documented (Kesavardhini et al., 2026; Naiel et al., 2022).

The observed aggregation can be attributed to two factors characteristic of plant-mediated syntheses. First, the polyphenolic and flavonoid constituents of the *Butea monosperma* flower extract confirmed in our preliminary phytochemical screening (Section 3.1) act as capping and reducing agents that bind to the surface of nascent ZnO NPs and mediate inter-particle bridging during the drying stage. Second, the absence of high-temperature calcination in the present protocol preserves the organic capping layer, which contributes to the irregular surface contour seen in the micrographs and accounts for the larger apparent particle dimensions relative to the Scherrer crystallite size.

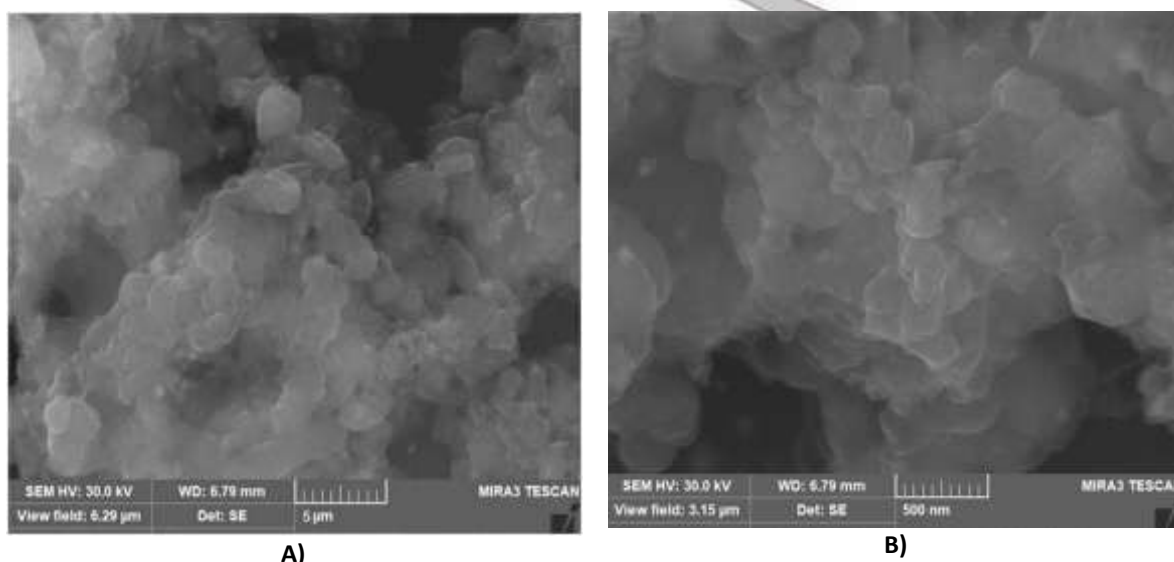


Figure 4. SEM micrographs of green-synthesized ZnO nanoparticles using *Butea monosperma* flower extract: (A) lower magnification (5 µm scale bar) showing densely packed aggregates and (B) higher magnification (500 nm scale bar) showing individual quasi-spherical to polyhedral grains with rough surface morphology.

Antioxidant Studies

ABTS radical cation scavenging activity of the biosynthesized ZnO NPs

The ABTS radical cation scavenging activity of the biosynthesized ZnO NPs and the ascorbic acid standard was evaluated at concentrations of 10–50 µg/mL, and the results are summarized in Table 2. The assay principle relies on the decolorization of the pre-formed ABTS•⁺ radical cation, generated by reacting ABTS with potassium persulfate, whereby the decrease in absorbance measured at 734 nm upon addition of the sample reflects its radical-scavenging capacity (Moreira, 2019; Verardo et al., 2011).

The percentage inhibition of both the ZnO NPs and the ascorbic acid standard increased progressively with increasing concentration, demonstrating a clear dose-dependent scavenging response, and the comparative scavenging activity is illustrated in Figure 5. At the lowest tested concentration (10 µg/mL), the ZnO NPs exhibited 16.00% inhibition, which rose to 34.00%, 52.00%, and 65.22% at 20, 30, and 40 µg/mL, respectively, and reached a maximum of 76.50% inhibition at 50 µg/mL. Correspondingly, the absorbance of the ABTS working solution decreased from 0.447 to 0.124 across the tested range, consistent with the increasing consumption of the radical cation by the NPs.

Notably, the biosynthesized ZnO NPs displayed higher scavenging activity than the ascorbic acid standard at every concentration tested. At 50 µg/mL, the NPs achieved 76.50% inhibition compared with 61.45% for ascorbic acid, while the standard yielded 13.00%, 18.23%, 40.78%, and 51.12% inhibition at 10, 20, 30, and 40 µg/mL, respectively. Since inhibition surpassed 50% at 30 µg/mL for the NPs, their IC₅₀ lies between 20 and 30 µg/mL, whereas the IC₅₀ of ascorbic acid lies between 30 and 40 µg/mL, indicating superior radical-scavenging potency of the NPs under the present experimental conditions.

This activity is consistent with earlier reports of green-synthesized ZnO NPs, which generally exhibit pronounced, concentration-dependent ABTS•⁺ scavenging for example, ZnO NPs prepared using *Limonium pruinosum* (sea lavender) extract (Naiel et al., 2022) and *Papaver somniferum* (Muhammad et al., 2019). The enhanced scavenging observed here can be attributed to the phytochemical capping layer predominantly the flavonoids and phenolic constituents of the *Butea monosperma* flower extract confirmed in the preliminary phytochemical screening (Section 3.1) which donate hydrogen atoms/electrons to neutralize the ABTS radical cation, complementing the intrinsic electron-donating capacity of the ZnO surface (Selim et al., 2020; Tiwari et al., 2024).

Table 2: ABTS radical scavenging activity of green-synthesized ZnO nanoparticles and standard ascorbic acid.

ZnO NPs Volume (µL)	ZnO NPs Concentration (µg/mL)	OD (ZnO NPs)	Inhibition (%)	OD (Ascorbic acid)	Inhibition (%)
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10	10	0.447	16.00%	0.465	13.00%
20	20	0.35	34.00%	0.435	18.23%
30	30	0.258	52.00%	0.305	40.78%
40	40	0.185	65.22%	0.272	51.12%
50	50	0.124	76.50%	0.205	61.45%

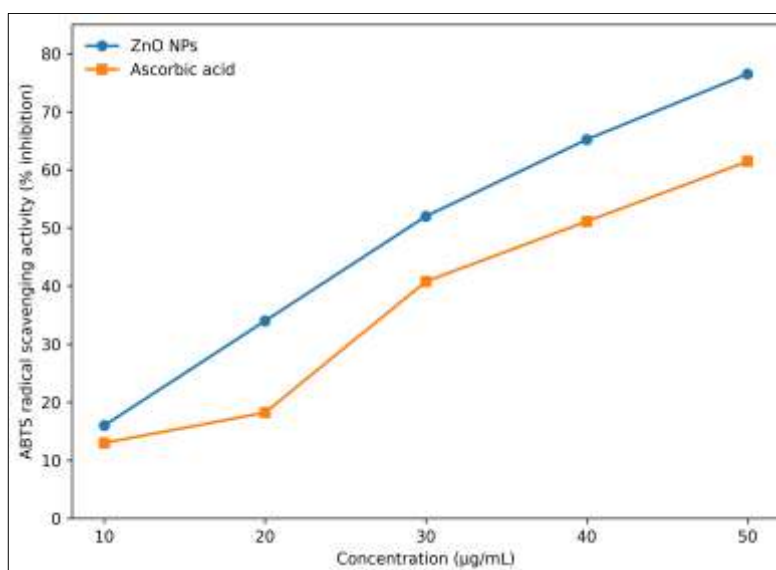


Figure 5: Concentration-dependent ABTS radical scavenging activity of green-synthesized ZnO nanoparticles and standard ascorbic acid

Values are expressed as percentage inhibition at the indicated concentrations.

Hydrogen peroxide radical scavenging activity of the biosynthesized ZnO NPs

The hydrogen peroxide scavenging activity of the biosynthesized ZnO NPs and the ascorbic acid standard was evaluated at concentrations of 10–50 µg/mL using the procedure of Pick and Mizel with minor modifications (Pick & Mizel, 1981), and the results are summarized in Table 3. H₂O₂ scavenging is a vital antioxidant feature: in the presence of electron donors, hydrogen peroxide accepts electrons and is decomposed into water, and the extent of decomposition is quantified by the decrease in absorbance measured at 230 nm (Hossain et al., 2024; Mukhopadhyay et al., 2016).

The percentage inhibition of both the ZnO NPs and the ascorbic acid standard increased progressively with increasing concentration, demonstrating a clear dose-dependent scavenging response. At the lowest tested concentration (10 µg/mL), the ZnO NPs exhibited 32.00% inhibition, which rose to 48.00%, 55.00%, and 72.00% at 20, 30, and 40 µg/mL, respectively, and reached a maximum of 87.00% inhibition at 50 µg/mL. Correspondingly, the absorbance of the hydrogen peroxide solution decreased from 0.387 to 0.063 across the tested range, indicating the progressive decomposition of H₂O₂ by the NPs.

As with the ABTS assay, the biosynthesized ZnO NPs displayed higher scavenging activity than the ascorbic acid standard at every concentration tested. At 50 µg/mL, the NPs achieved 87.00% inhibition compared with 64.88% for ascorbic acid, while the standard yielded 10.00%, 18.00%, 36.79%, and 54.77% inhibition at 10, 20, 30, and 40 µg/mL, respectively. Since inhibition surpassed 50% at 30 µg/mL for the NPs, their IC₅₀ lies between 20 and 30 µg/mL, whereas the IC₅₀ of ascorbic acid lies between 30 and 40 µg/mL an appreciably lower (better) IC₅₀ for the NPs under the present experimental conditions.

This activity compares favourably with earlier reports of green-synthesized ZnO NPs assayed for H₂O₂ scavenging: ZnO NPs (17 nm) prepared from *Sambucus ebulus* showed an H₂O₂ scavenging IC₅₀ of 43 µg/mL (Hamed et al., 2023), and *Albizia lebbek*-mediated ZnO NPs exhibited IC₅₀ values of 48.5, 48.7, and 60.2 µg/mL depending on the precursor concentration (Patil & Chandrasekaran, 2020). The superior scavenging observed here is consistent with the concentration-dependent radical inhibition reported for plant-mediated ZnO NPs in general (Hamed et al., 2023), and can be attributed to the phytochemical capping layer predominantly the flavonoids and phenolic constituents of the *Butea monosperma* flower extract confirmed in the preliminary

phytochemical screening (Section 3.1) which stabilizes the NPs while simultaneously donating electrons to neutralize H_2O_2 , complementing the electron-donating capacity of the ZnO surface itself (Selim et al., 2020; Tiwari et al., 2024). The dose-dependent profile is also in agreement with the H_2O_2 scavenging data of the biosynthesized NPs presented in Figure. 6.

Table 3: Hydrogen peroxide scavenging activity of green-synthesized ZnO nanoparticles from Butea monosperma and standard ascorbic acid.

ZnO NPs Volume (μ L)	ZnO NPs Concentration (μ g/mL)	OD (ZnO NPs)	Inhibition (%)	OD (Ascorbic acid)	Inhibition (%)
10	10	0.387	32.00%	0.642	10.00%
20	20	0.268	48.00%	0.585	18.00%
30	30	0.251	55.00%	0.450	36.79%
40	40	0.158	72.00%	0.322	54.77%
50	50	0.063	87.00%	0.250	64.88%

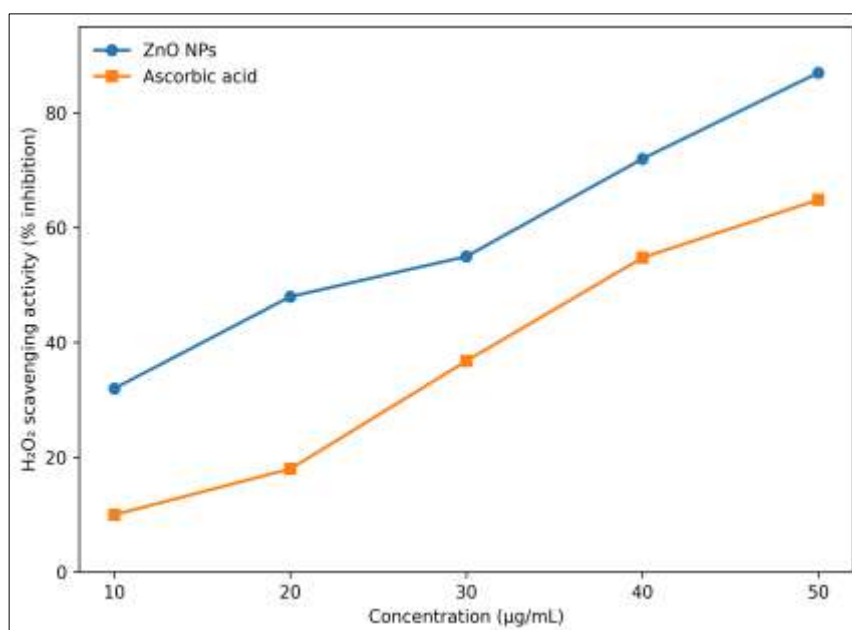


Figure 6: Concentration-dependent hydrogen peroxide scavenging activity of green-synthesized ZnO nanoparticles and standard ascorbic acid.

Values are expressed as percentage inhibition at the indicated concentrations.

In vitro cytotoxicity of the biosynthesized ZnO NPs against A549 lung adenocarcinoma cells

The biosynthesized ZnO NPs cytotoxicity was analyzed by in vitro technique using A549 cell lines. Cell lines were treated with different concentrations of ZnO NPs (18.75, 37.5, 75, 150, and 300 μ g/mL), alongside DMEM as control, and assessed by the MTT assay following overnight (24 h) exposure at 37 °C in a humidified atmosphere containing 5% CO_2 (Figures (3).Pdf, n.d.; Mosmann, 1983; Twentyman & Luscombe, 1987). Reduction of MTT reagent, and other tetrazolium dyes, produces a colour change from yellow to purple that depends upon the cellular metabolic activity of NAD(P)H-dependent cellular oxidoreductase enzymes (Sazonova et al., 2022). In the MTT assay, the absorbance of the formazan product at 590 nm is directly proportional to the number of metabolically active cells after exposure to the test agent.

A clear dose-dependent cytotoxic response was observed, with the percentage of growth inhibition rising progressively as ZnO NP concentration increased (Figure 7). The biosynthesized ZnO NPs caused 15.00%, 30.00%, 46.00%, 64.00% (positive control), and 72.00% inhibition at 18.75, 37.5, 75, 150, and 300 μ g/mL, respectively. Linear regression yielded a strong dose–response correlation with $R^2 = 0.9915$, confirming that cell inhibition followed the concentration-dependent profile characteristic of plant-mediated ZnO cytotoxicity (Umamaheswari et al., 2021). The IC_{50} was found to be 91.69 μ g/mL, in close agreement with reports of green-synthesized ZnO NPs evaluated against A549 lung adenocarcinoma cells, where IC_{50} typically falls in the range of 60–110 μ g/mL for biologically active formulations.

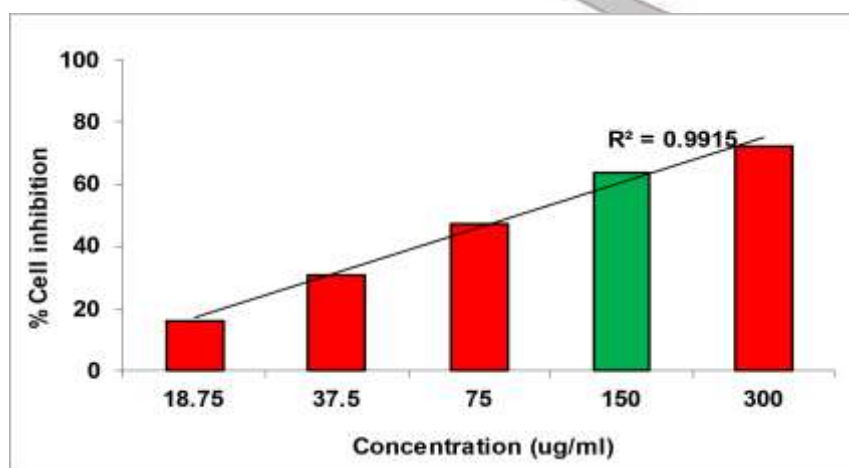


Figure 7: Dose-dependent cytotoxicity of green-synthesized ZnO nanoparticles derived from *Butea monosperma* flowers against A549 cells after 24 h treatment, as determined by the MTT assay.

The A549 cells displayed marked morphological alterations upon ZnO exposure rounding, cellular detachment from the substrate, and increases in detached/floating cell density confirming the cytotoxic effect of the biosynthesized NPs (Figure 8). These changes in morphology outline the cytotoxic effect of the biosynthesized NPs, with the dose-dependent rise in inhibition and visible membrane damage reflecting the well-documented intracellular generation of reactive oxygen species by ZnO NPs (Sazonova et al., 2022). The higher surface-area-to-volume ratio of these small particles is considered a key driver of the enhanced cytotoxic response, since smaller particles release more Zn^{2+} ions and ROS per unit mass and interact more efficiently with cellular membranes and subcellular organelles (Thakur et al., 2024). In addition, it is worth mentioning that the phytoconstituents of the *Butea monosperma* aqueous flower extract flavonoids and phenolics confirmed in the preliminary phytochemical screening (Section 3.1) are well-documented anticancer agents in this plant, and have been considered to exhibit concrete anticancer properties mainly by targeting various cancer-related proteins.

The possible mechanisms of the observed cytotoxicity include ROS-mediated oxidative stress leading to DNA damage pathways, paraptosis, autophagy, radiosensitisation, overcoming chemoresistance, inhibition of carcinogen-activation of P450 (phase I) enzymes or induction of carcinogen-detoxifying (phase II) enzymes, oxidative stress modulation, induction of apoptosis, and cell-cycle arrest (- & -, 2025; Sazonova et al., 2022; Umamaheswari et al., 2021). These mechanisms are consistent with the documented anticancer behaviour of both *Butea monosperma*-derived phytochemicals and ZnO NPs in various cancer models. Further research is focused on elucidating the specific mechanistic pathway(s) at play in A549 cells. From Fig. 10, the differences among means are statistically significant, since the p-values are less than 0.0001. The data are expressed in terms of mean $\pm$ SD (n = 3).

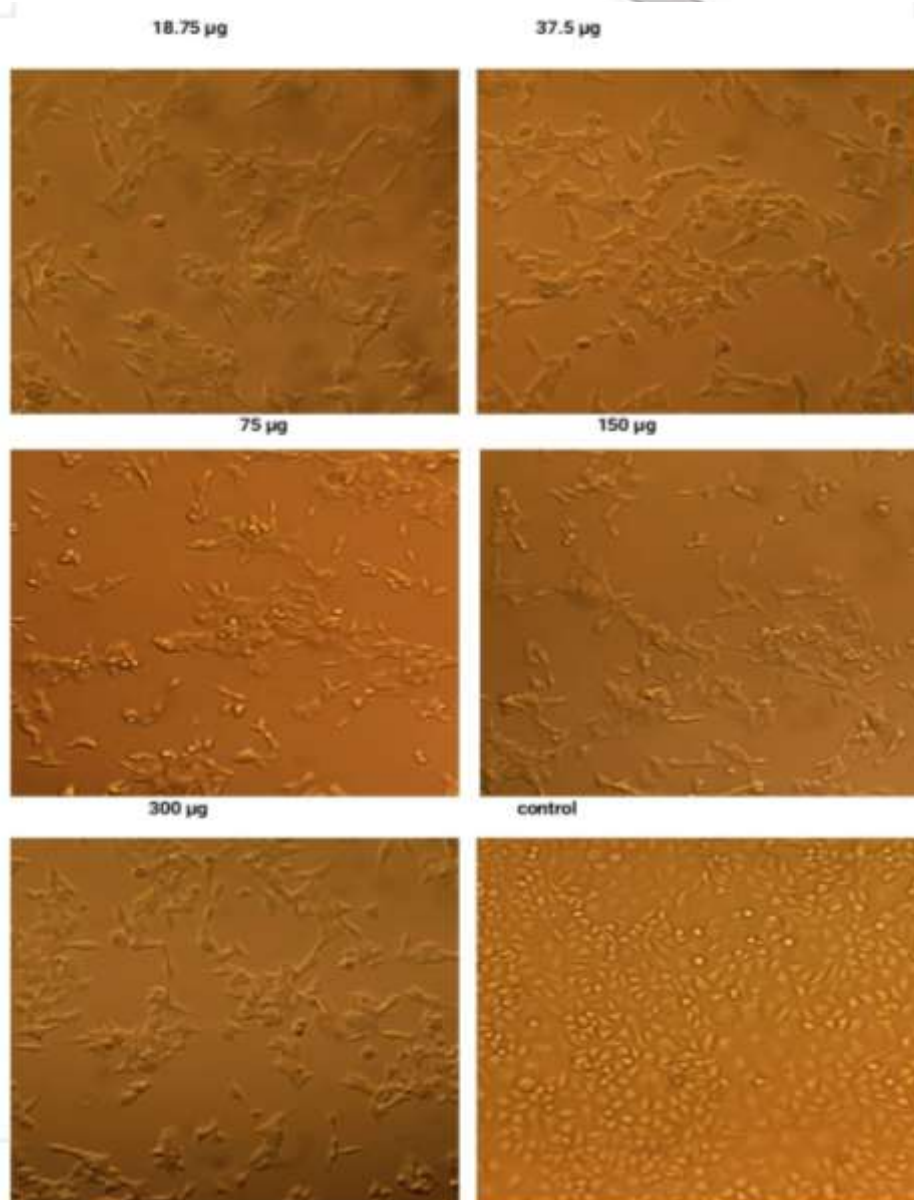


Figure 8: Morphological changes in A549 cells following 24 h treatment with ZnO nanoparticles synthesized using *Butea monosperma* flower extract. Cells were exposed to 18.75, 37.5, 75, 150, and 300 µg/mL, along with an untreated control, showing concentration-dependent cell shrinkage, rounding, and detachment.

DISCUSSION

The present study demonstrates a simple, eco-friendly route for the biosynthesis of ZnO NPs using the aqueous flower extract of *Butea monosperma*, a plant widely recognized in traditional medicine for its antimicrobial, anti-inflammatory, antidiabetic, hepatoprotective, and antioxidant activities (– & –, 2025; Jain & Dubey, 2023). Plant-mediated synthesis exploits the fact that medicinal plants carry high concentrations of active compounds phenolic acids, flavonoids, and saponins which can neutralize reactive oxygen species, chelate metal ions, and act as both reducing and stabilizing agents during nanoparticle formation (Bandeira et al., 2020). The hydroxyl groups of phenolic compounds act as electron donors that facilitate the reduction of metal ions, after which the flavonoids and phenolic compounds further stabilize and cap the nascent NPs through hydrogen bonding and van der Waals interactions with the metal surface (El-Saadony et al., 2024). The *B. monosperma* flower extract thus served a dual role in the present work: it reduced zinc acetate to ZnO and simultaneously capped the particles, eliminating the need for separate chemical reducing or stabilizing agents.

The preliminary phytochemical screening (Section 3.1) confirmed that the aqueous flower extract contains alkaloids, flavonoids, phenols, reducing sugars, tannins, glycosides, and proteins, along with saponins. This profile is consistent with the documented chemistry of *B. monosperma* flowers, from which the flavonoids

butein, butrin, butin, isobutrin, palasitrin, coreopsin, isocoreopsin, sulphuresin, and monospermoside have been isolated (Chokchaisiri et al., 2009; DAVE et al., 2019); more recent studies have additionally characterized a new aurone glucoside and three new biflavonoids from the flowers (Tanaka & Kashiwada, 2021). The abundance of phenolic and flavonoid constituents in the extract accounts for its reducing and capping capacity: since the functional –OH and –COOH groups of polyphenols are strong hydrogen donors, their interaction with Zn^{2+} ions via a donor–acceptor mechanism is regarded as the probable mechanism by which ZnO NPs are formed from plant extracts (Abdelbaky et al., 2023). The observed absence of steroids and phytosterols further distinguishes the profile of the flowers while not compromising their reducing potential.

The UV–vis spectrum recorded for the biosynthesized product exhibited a broad absorption band with maximum absorbance (0.98) at 450 nm. Although characteristic ZnO absorption is typically reported between ~355 and 380 nm, corresponding to band-gap absorption across Zn–O (Shamhari et al., 2018), the red-shifted band observed here can reasonably be attributed to the relatively larger particle size of the NPs the absorption of ZnO undergoes a bathochromic shift with increasing particle size (Mousa et al., 2024) together with defect-related absorption and the contribution of flower phytochemicals capping the nanoparticle surface (Kesavardhini et al., 2026). This behaviour nevertheless corroborates the formation of phytochemically capped ZnO, in line with earlier green syntheses reporting characteristic ZnO absorption around 370 nm (Selim et al., 2020).

FT-IR analysis provided direct evidence of both ZnO formation and surface functionalization by the extract phytoconstituents. The broad band at 3338.7 cm^{-1} corresponds to O–H stretching of phenolic/alcoholic groups (Abdelbaky et al., 2023); the bands at 1724.3 and 1598.9 cm^{-1} arise from C=O stretching of ester/carboxylic moieties and aromatic C=C/amide vibrations, respectively. The band near 1598.9 cm^{-1} is particularly informative, as the tautomeric transformation of flavonoids between enol and keto forms releases reactive hydrogen atoms capable of reducing Zn^{2+} to metallic species (Aliannezhadi et al., 2024). The intense C–O and –C–O–C stretching bands around 1024.2 cm^{-1} confirm the retention of phenolic and polysaccharide components on the particle surface, while the band at 688.5 cm^{-1} , within the characteristic 400–750 cm^{-1} region, is assigned to the Zn–O (M–O) stretching vibration. The observed shifts in band positions relative to the pure extract indicate that these functional groups participated directly in the reduction of Zn^{2+} and in the subsequent stabilization of the NPs, consistent with the electron-transfer mechanism described for phenolic-mediated syntheses (El-Saadony et al., 2024).

SEM micrographs showed predominantly quasi-spherical to hexagonal-like particles with rough surfaces, clustered into densely packed aggregates. Such aggregation is characteristic of phytochemical-capped green ZnO, where the polyphenolic capping layer mediates inter-particle bridging during drying (Thakur et al., 2024), and the morphological features are comparable with those reported for ZnO NPs synthesized using *Butea monosperma* seed extract (Ali et al., 2021) and other plant extracts such as *Limonium pruinosum* (Naiel et al., 2022). The absence of a high-temperature calcination step in the present protocol preserves the organic capping layer, accounting for the irregular surface contours and the discrepancy between the aggregated apparent sizes observed by microscopy and the crystallite dimensions obtained from XRD.

X-ray diffraction analysis confirmed the crystalline nature of the biosynthesized ZnO, with diffraction maxima matching the hexagonal wurtzite structure of ZnO (JCPDS card No. 36-1451) (Thakur et al., 2024). The sharp, well-defined Bragg peaks indicate effective crystallization, and the centrifugation-and-re-dispersion purification step performed prior to characterization ruled out any independent crystallization of the capping agent (Aliannezhadi et al., 2024; Bala et al., 2014). Application of the Debye–Scherrer equation to the most intense reflection yielded an average crystallite size of 66.43 nm (Shamhari et al., 2018), a value consistent with the nanoscale dimensions required for the strong surface-related biological responses discussed below.

The biosynthesized ZnO NPs exhibited pronounced, concentration-dependent radical scavenging in both antioxidant assays. In the ABTS assay, inhibition rose from 16.00% at 10 $\mu\text{g/mL}$ to a maximum of 76.50% at 50 $\mu\text{g/mL}$, and in the hydrogen peroxide assay from 32.00% to 87.00% across the same range in both cases exceeding the ascorbic acid standard at every concentration tested (61.45% and 64.88% at 50 $\mu\text{g/mL}$, respectively), with an interpolated IC_{50} between 20 and 30 $\mu\text{g/mL}$ for the NPs. This superior activity can be traced to the phytochemical capping layer: the phenolic hydroxyls and flavonoids on the NP surface donate hydrogen atoms and electrons to neutralize radical species (Osman et al., 2024), while the electrostatic interaction between the negatively charged bioactive compounds of the extract and the positively charged metal surface enhances the antioxidant capacity of green-synthesized NPs compared with their chemically synthesized counterparts (Tiwari et al., 2024). The results are on par with earlier reports of plant-mediated ZnO NPs showing

pronounced ABTS^{•+}-scavenging activity, including those from *Limonium pruinosum* (Naiel et al., 2022) and *Papaver somniferum* (Muhammad et al., 2019), and with the general observation that smaller NPs with a higher surface-to-volume ratio exhibit the highest radical-scavenging activity (Tiwari et al., 2024).

The MTT assay against A549 lung adenocarcinoma cells demonstrated a clear dose-dependent cytotoxic response, with growth inhibition rising from 15.00% at 18.75 µg/mL to 72.00% at 300 µg/mL ($R^2 = 0.9915$) and an IC_{50} of 91.69 µg/mL, accompanied by marked morphological alterations cell rounding, detachment, and an increased density of dead cells. This IC_{50} falls well within the broad range reported for green-synthesized ZnO NPs against A549 cells (e.g., 21.38 µg/mL for citrate-mediated ZnO (Abdelbaky et al., 2023); 83.47 µg/mL for *Deverra tortuosa*-mediated ZnO (Selim et al., 2020); 383.05 µg/mL reported by Lingaraju et al. (Naiel et al., 2022)) and is below the 0.125 mg/mL threshold proposed for promising anticancer candidates (Abdelbaky et al., 2023), supporting the potential of the present formulation for further development.

Several complementary mechanisms account for this activity. ZnO NPs are known to induce intracellular ROS generation and oxidative stress, leading to oxidative DNA damage and apoptosis (Li, 2009; Perumal et al., 2024), and studies have shown that ZnO NPs selectively trigger apoptosis in cancer cells through a ROS-mediated p53 pathway (Ahamed et al., 2012). A substantial body of evidence indicates that dissolution of ZnO and the release of Zn^{2+} ions is a prerequisite for cytotoxicity intracellular free zinc concentration correlates strongly with ZnO-induced cell death ($R^2 = 0.945$) (Shen et al., 2013) with dissolved Zn^{2+} driving ROS generation, intracellular calcium flux, mitochondrial depolarization, and plasma membrane leakage (George et al., 2009), and disrupting cellular zinc homeostasis to cause lysosomal and mitochondrial damage (Xia et al., 2008). The toxicity is also size-dependent, with smaller particles exhibiting enhanced ion-shedding ability (Liu et al., 2017), and preferentially affects rapidly dividing cells, sparing quiescent cells (Hanley et al., 2008). In addition to the intrinsic activity of ZnO, the phytochemicals of the *B. monosperma* flower extract particularly the flavonoids such as butein and butrin are themselves recognized for antioxidant, chemopreventive, and cytotoxic effects (- & -, 2025; Chokchaisiri et al., 2009; Tanaka & Kashiwada, 2021), so the capping layer plausibly contributes synergistically to the observed anticancer response.

Taken together, the results establish the aqueous *Butea monosperma* flower extract as an effective, cost-efficient bio-reducing and stabilizing agent for the green synthesis of crystalline, phytochemically capped ZnO NPs with appreciable antioxidant and anticancer potential. The phytofabrication not only avoids hazardous chemical reagents but also confers surface functionality that improves biocompatibility (El-Saadony et al., 2024). However, several questions remain open: the precise cell-death pathway (apoptotic versus necrotic) and the involvement of specific signalling cascades require confirmation through Annexin V/PI staining, ROS probes, and cell-cycle analysis; the selectivity of the NPs towards cancer cells relative to normal cells such as WI-38 needs to be established, following the approach of earlier studies (Selim et al., 2020); and in vivo toxicity, biodistribution, and therapeutic efficacy studies are necessary before clinical translation can be considered. Addressing these aspects will clarify the mechanistic basis of the observed bioactivity and support the development of *B. monosperma* flower-mediated ZnO NPs as a promising candidate for future nanomedicine applications.

CONCLUSION

The present investigation has proven that the biosynthesis of ZnO NPs using the aqueous flower extract of *Butea monosperma* is a simple, rapid, and eco-friendly green synthesis method. Flavonoids and phenols along with the other phytoconstituents confirmed in the preliminary screening (alkaloids, tannins, glycosides, proteins, saponins, and reducing sugars) were found to act as reducing, capping, and stabilizing agents in the metal nanoparticle synthesis (Bandeira et al., 2020; El-Saadony et al., 2024). The biosynthesized NPs were confirmed to be crystalline hexagonal wurtzite ZnO with an average crystallite size of 66.43 nm, chemically capped and stabilized by the phytoconstituents, as evidenced by the UV-vis, FT-IR, SEM, and XRD analyses.

This protocol is simple, reproducible, and readily scalable, and it avoids hazardous chemical reducing agents, providing a rapid and large-scale-up technique for the production of ZnO NPs. The biosynthesized ZnO NPs exhibited pronounced concentration-dependent antioxidant activity, with up to 76.50% ABTS and 87.00% hydrogen peroxide radical scavenging at 50 µg/mL exceeding the ascorbic acid standard at every concentration tested and demonstrated appreciable in vitro anticancer activity against A549 lung adenocarcinoma cells with an IC_{50} of 91.69 µg/mL. These results prove that antioxidant and anticancer formulations can be prepared by this constituted eco-friendly technique (Ali et al., 2021; Umamaheswari et al., 2021).

The study concludes that the green synthesis of ZnO NPs using plant material as both reducing and capping agent offers various merits, including easy availability of the plant material, protection of the environment, and economic feasibility in the scale-up of the process. A thorough understanding of the biochemical mechanism underlying this plant-mediated ZnO NP synthesis is a prerequisite to making the approach economically more competitive and sustainable. Processes such as purification and sterilization techniques can provide further betterment in green synthesis. In the near future, this nanotechnological approach is anticipated to be established as a new eco-friendly strategy for the production of therapeutic nanomaterials within a short span of time (Jain & Dubey, 2023; Osman et al., 2024).

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