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Anti-Proliferative Activity of Biosynthesized Zinc Oxide Nanoparticles Against Breast Cancer Mcf-7 Cells

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ABSTRACT

In this study, we employed *Tecoma stans* extract to create green synthetic ZnO nanoparticles (NPs), which we then analyzed using FTIR, XRD, SEM with EDX, TEM, and UV-Vis methods. By measuring the antioxidant activity, ZnO NPs were assessed for viability in MCF-7 cells. ZnO NPs have a rather consistent size distribution, as seen by the histogram's mean size of about 25 nm. ZnO NPs' inhibitory concentration (IC-50) was determined to be 36.58μg/ml, while that of ascorbic acid (standard) was 34.89. Comparison with Paclitaxel, the Standard Drug: At 12.5 μg/ml, paclitaxel inhibition was 8.97%, while at 400 μg/ml, it was 93.61%. The concentration needed to block 50% of cells is known as the IC50 value. Extract from *Tecoma stans* Leaves 186.61 μg/ml Standard paclitaxel dosage: 147.42 μg/ml. This study highlights the promise of nanotechnology, namely the application of green-synthesized ZnO NPs, in the treatment of cancer. Optimizing their safety and effectiveness for clinical applications should be the main goal of future research.

***Key words**: Zinc oxide nanoparticles were characterized using XRD, FTIR, UV-Vis, SEM, EDX, and TEM analyses.*

Introduction

Nanomaterial's are particles having nanoscale dimension, and nanoparticles are very small sized particles with enhanced catalytic reactivity, thermal conductivity, non-linear optical performance and chemical steadiness owing to its large surface area to volume ratio (Agarwal *et al.,* 2017). The growth and development of mankind have always been closely associated with the progress of materials technology. In the last decades, the field of nanotechnology and associated researches were continuously in rapid growth due to the experience and capitalization of accumulated knowledge (Hassan *et al.,* 2018). The growing need of environmental friendly nanoparticles, researchers are using green methods for the of various metal nanoparticles for pharmaceutical applications (Vidya *et al.,* 2013). In literature, ZnO nanoparticles are synthesized from conventional methods like chemical reduction (Raut, Thorat, and Thakre, 2013). *Tecoma stans* contains a number of bioactive phytochemicals and is mainly known for its phytochemical reserpine, which was widely used as an antihypertensive drug. But very least research has been published on the leaves of plant *Tecoma stans* (Nandhini and Vijistella Bai, 2014). The alkaloids isolated from the plant material show various pharmacological activities such as antipsychotic, antimicrobial, anti-inflammatory, anticancer, antihypertensive, antidiarrheal, and antioxidant (Singh *et al.,* 2019). Nanoparticles are generally effective in a wide variety of sectors that if their production is based on green chemistry, they have great applications in the fields of food, medicine, cosmetics and health (Ren *et al.*, 2022). Several bioactive compounds like quinic acid, quercetin, rutin, scopoletin, palmitoleic acid, and naringenin have been found in Prunus species (Biresaw and Taneja, 2020). Such as silver, gold, palladium, copper oxide, zinc oxides (Padmaja *et al.*, 2021). Many pharmacological properties were observed in the plant, including hypolipidemic, respiratory, immunological, car- diovascular, anti-inflammatory, antipyretic, anticancer, antioxidant, antimicrobial and many others (Karthick *et al.*, 2023) *in vitro* antioxidant activity (Sobiyana, Manikandan, and Anburaj 2019). The effect of the samples on the proliferation of MCF-7 was expressed as the % cell viability and % Cell growth inhibition using the following formula (Anburaj *et al*., 2016). The zinc oxide nanoparticles (ZnO-NPs) are easier to generate, nontoxic, eco-friendly, bio-compatible, and bio-safe that makes theme as potential biological application (Seshadri, 2021).

Materials and Methods

Chemicals

Zinc Nitrate (Zn $(NO3)_2 \cdot {}_6H_2O$) 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), phosphate buffered saline (PBS), and Acridine orange/Ethidium bromide (AO/EB), and other chemicals were attained from Sigma Aldrich, USA. (Aeromonas hydrophila, E. coli-443, Staphylococcus aureus- 902 and *Propionibacterium acnes-1951*) was purchased from MTCC, Chandihar, India. Nutrient Agar medium, Nutrient broth, Oxytetracycline solution was purchased from Himedia, India. Test samples, petri-plates, test tubes, beakers conical flasks were from Borosil, India. Spirit lamp, double distilled water.

Collection of plant material and synthesis of ZnONPs

Initially, the *Tecoma stans* was collected from the PRIST Deemed to be University, Thanjavur, and then leaf was washed three times with distilled water and let it dried under shade condition. Then, 10 g of fresh *Tecoma stans* was mixed with 100 mL of distilled water and heat-macerated at 80 ^{0}C for 20 min. The resultant suspension was filtered using Whatman No.1 filter paper. 0.1Mof (Zn (NO3)26H2O) solute was mixed with 100 ml of *Tecoma stans* extract and stirred repeatedly at a temperature of 80 ^{0}C for 4-6 h. After that solution will become completely dried and the dried -ZnO-NPs were collected and the resultant NPs were calcined at 700 ^{0}C for 5 h (Seshadri 2021).

Zinc oxide nanoparticles from *Tecoma stans* showed the potent antimicrobial and in vitro anticancer activity against the osteosarcoma MG-63 cells Cell culture Human breast carcinoma cells (MCF7) and human normal cell line MCF10A were obtained from Pune. MCF7 and MCF10A cells were grown in DMEM, complemented with fetal bovine serum (10%) in a CO_2 incubator (5% CO_2, 95% air) at 37 ^{0}C

Preparation of the leaf extract

Fresh leaves were collected from *Tecoma stans* plants in the RVCE campus. The leaves were washed several times with water to remove the dust particles and then sun dried to remove the residual moisture. The extract used for the reduction of zinc ions (Zn^{2+}) to zinc nanoparticles (ZnO) was prepared by placing 50g of washed dried fine cut leaves in 250 ml glass beaker along with 100 ml of sterile distilled water. The mixture was then boiled for 60 minutes until the colour of the aqueous solution changes from watery to light yellow. The extract was cooled to room temperature and filtered using filter paper. The extract was stored in a refrigerator in order to be used for further experiments (Vidya *et al*., 2013).

Characterization of zinc oxide NPs

Structural features of plant mediated nanoparticles were characterized by advance techniques such as FTIR, EDX, XRD and SEM. All analysis was made in triplicates. Obtained data were analyzed by origin software (Mehar *et al*., 2019). Here, we report plant-based synthesis of zinc oxide nanoparticles using the aqueous extracts of *Tecoma stans*. Green synthesis of ZnO-NPs has eco-friendly aspects and various biomedical applications. The metabolites found in the aqueous extract of *Tecoma stans* act as an oxidizing, reducing, and capping agent for the synthesis of biogenic ZnO-NPs. The green synthesized nanoparticles will be characterized using modern techniques such as Fourier transform infrared (FTIR) spectroscopy, ultraviolet (UV) spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), dynamic light scattering (DLS) (Faisal *et al.*, 2021).

Antibacterial assays

The antibacterial assays were done on human pathogenic *Escherichia coli* and *Staphylococcus aurous*, Listeria monocytogenes and Salmonella typhi by standard well diffusion method. Antibacterial activities of ZnO NPs were evaluated against highly multiple drug-resistant bacterial strains. Well diffusion method was carried out to evaluate the antibacterial activity of ZnO NPs. For this (10 mg/ml) ZnO Nps dissolved in DMSO. The inoculums of bacterial strains were prepared by growing a single colony for overnight in nutrient broth. Petri plates containing media were swabbed with bacterial strains by using cotton buds' wells were made in nutrient agar 0.1M ZnS and aqueous plant extract (positive control), DMSO (negative control) and 20ml of each synthesized- ZnO NPs were added in wells. Petri plates were incubated for 24 hours at 37 °C and the inhibition zone was measured. This experiment repeated thrice (Mehar *et al.,* 2019).

Antioxidant analysis of ZnO nanoparticles

The antioxidant activity (A_A%) of each substance was determined using the DPPH scavenging of free radicals assay. Various concentrations of ZnO-NPs were added to every vial excluding blank. Then, 3 mL of ethanol was got to add, along with 0.3 ml of 0.5 mM DPPH drastic approach in ethanol. The control solution was made by combining 3.5 ml of ethanol with DPPH radical solution (0.3 ml). After 30 minutes of reaction, absorbance was measured at its maximum wavelength. The total antioxidant activity portion (A_A%) was calculated with the following equation (Munandar *et al.*, 2022).

% AA = (abs. at blank – abs. at test) x 100% abs. at blank

Cytotoxicity study

Human breast cancer cell line, MCF-7, and human breast cell line, MCF10A, were procured from the National Centre for Cell Science, Pune, India. The cells were subculture in Dulbecco's modified Eagle medium (DMEM) with low glucose having phenol red supplemented with 10% fetal bovine serum and 1% penicillin/ streptomycin solution to minimize the microbial contamination. The cells were seeded at a density of 5×10^3 cells/well in a 96-well flat bottom microplate and maintained at 37 °C in 5% CO_2 incubator with 95% humidity overnight prior to the experiment. The cells were treated with different concentrations (500, 250, 125, 62.5, 32.75 μg/ml) of methanolic extracts by serial dilution method. Initially, cells were diluted with serumfree medium to achieve twice the desired maximum test concentration. The cells were incubated for another 48 h, and cytotoxicity was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Warake *et al.*, 2021).

Statistical analysis

The data were expressed as mean ± standard deviation of results obtained from three independent experiments. $P \leq 0.05$ was considered significant for all the represented results. All the graphs were plotted using Origin Pro 2025 software.

Results and Discussion

UV absorption analysis of ZnO nanoparticles

The Figure shows the UV-Vis absorption spectra of ZnO nanoparticles. The UV-Vis spectroscopic study shows the Plasmon resonance property, confirmed the reduction of metal ion and formation of nanoparticle peak at 272-290 nm. To determine the optical property of the synthesized ZnO NPs, small amount of the Nano powder was re-suspended in about 10 ml of de-ionized water and scanned between 300 and 700 nm in UV-Spectrophotometer (Shimadzu UV-1800) and measured the maximum absorbance (Umamaheswari *et al.*, 2021). peak at 377nm and absorption peak at 325 nm for Zinc oxide nanoparticles (Vaishnav *et al.*, 2017). The presence of secondary metabolites in plants reduces zinc ions in the solution to zinc oxide. The plant extract not only acts as reducing agents but as stabilizing agents as well. This was confirmed by taking the UV-visible spectrum analysis in the range of 280 nm-800 nm. The spectrum showed a peak at 320 nm, which is specific for ZnO nanoparticles. For ZnO nanoparticles, the absorbance peak is reported between 310 nm and 360 nm of wavelength (Jayachandran and Nair, 2021).

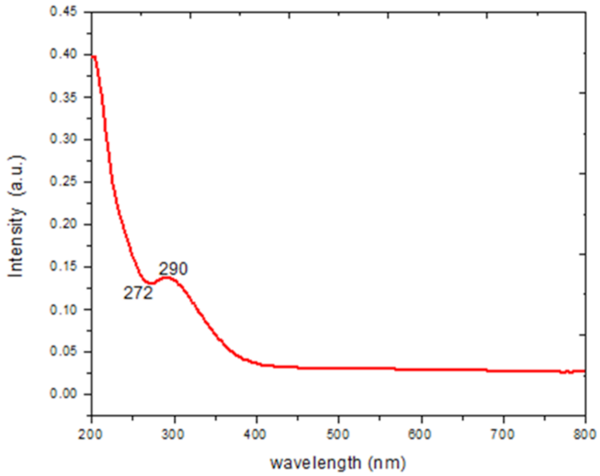

Fig. 1. UV–visible absorption spectrum of the ZnO nanoparticles

FTIR analysis of ZnO nanoparticles Wavenumber Values

The values represent the wavenumbers in inverse centimeters (cm^{-1}), which is the typical unit used in IR spectroscopy. The range from 4000 cm^{-1} to 400 cm^{-1} covers the mid-infrared region, which is commonly used to study molecular vibrations. Specific Wavenumbers (2938, 2925, 1514, 1529, 672, 693, 682 cm^{-1}) these specific wavenumbers likely correspond to particular vibrational modes of the molecules in the sample. For example, Peaks around 2938 cm^{-1} and 2925 cm^{-1} are often associated with C-H stretching vibrations. Peaks around 1514 cm^{-1} and 1529 cm^{-1} could be related to aromatic C=C stretching or N-H bending vibrations. Peaks around 672 cm^{-1}, 693 cm^{-1}, and 682 cm^{-1} might correspond to out-of-plane

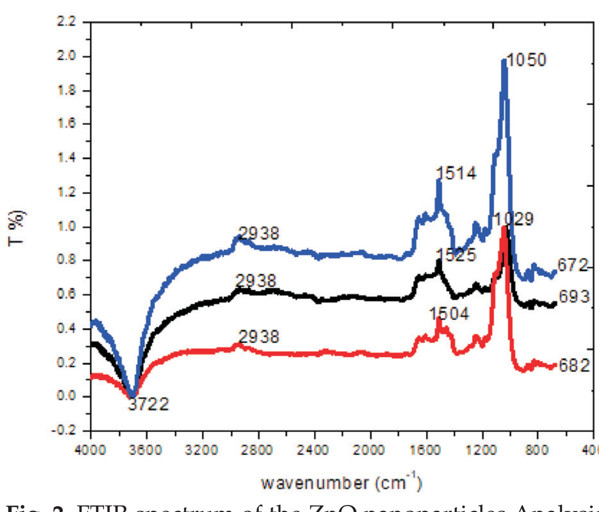

Fig. 2. FTIR spectrum of the ZnO nanoparticles Analysis

bending vibrations or metal-oxygen vibrations, depending on the sample. (Umamaheswari *et al.*, 2021). The spectra peaks between 1529.55^{-1} and 1500.31 cm^{-1} and 1260–1031.92 cm^{-1} indicates O-H in-plane and out-of-plane bonds, respectively. The broad peaks around 3700 - 3000 cm^{-1} is due to O-H stretch which corresponds to the hydroxyl groups which can be completely removed when the sample is sintered at temperatures >973 K as reported (Akintunde *et al.*, 2021).

XRD analysis of ZnO nanoparticles

The XRD pattern shows peaks at specific 2ε angles, which correspond to the diffraction from different crystal planes of the ZnO nanoparticles.

Crystal Planes

The peaks at 21.70°, 28.35°, 29.30°, 32.94°, and 35.29° can be matched to the crystal planes (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202) of ZnO. These planes are characteristic of the hexagonal wurtzite structure of ZnO. Hexagonal Wurtzite Structure ZnO typically crystallizes in the hexagonal wurtzite structure, which is a common crystal structure for many semiconductor materials. Standard Diffraction Peaks According to the Joint Committee on Powder Diffraction Standards (JCPDS No. 36-1451), the standard diffraction peaks for ZnO appear around 31.7° for the (100) plane, 34.4° for the (002) plane, 36.2° for the (101) plane, and 47.5° for the (102) plane. The width of the diffraction peaks can be used to estimate the crystallite size using the Scherrer equation.

Phase Composition

The presence of peaks corresponding to the hexagonal wurtzite structure indicates that the synthesized ZnO nanoparticles are predominantly in this phase. The absence of additional peaks suggests that the sample is free from significant impurities or secondary phases.

The spectra show the details of the crystal planes. Corresponding to the lattice planes (100), (002), (101), (102), (110), (103), (112), and (201) (Jayachandran and Nair, 2021). The sharp and narrow diffraction peak positions with 2è values of 31.78, 34.43, 36.27, 47.55, 56.62, 62.88, 66.40, 67.97, 69.11, 72.59, 76.99, 81.41, 89.64 were indexed as (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), (202), (104) and (203) hkl crystal planes. The peak intensity profiles were in good agreement

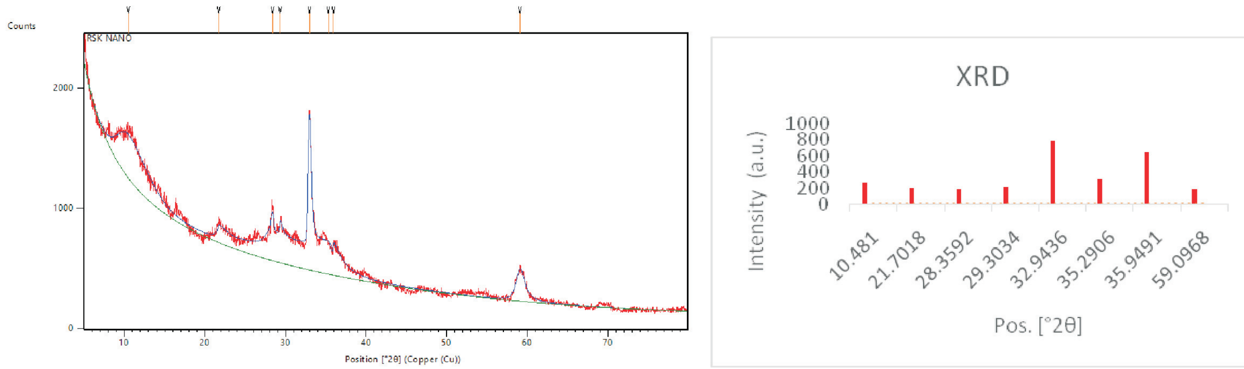

Fig. 3. XRD Analysis

with those of powder ZnO.

Scanning Electron Microscope (SEM) Analysis

The morphology of the biosynthesized ZnO-NPs loaded on silica gel was analyzed by an SEM microscope. The SEM images revealed that the ZnO nanoparticles were spherical with an average diameter of 38 nm Aggregation and Shape. The SEM images reveal that the ZnO nanoparticles tend to aggregate, with most particles exhibiting a flake-like shape at the Nano scale. This aggregation is a common phenomenon in nanoparticles due to their high surface energy, which drives them to minimize surface area by clustering together. Impact on Applications Although the aggregation, this characteristic does not significantly hinder the potential applications of ZnO nanoparticles. The flake-like morphology can still be beneficial for various uses, such as catalysis, sensing, and optoelectronics, where surface area and morphology play crucial roles. TEM

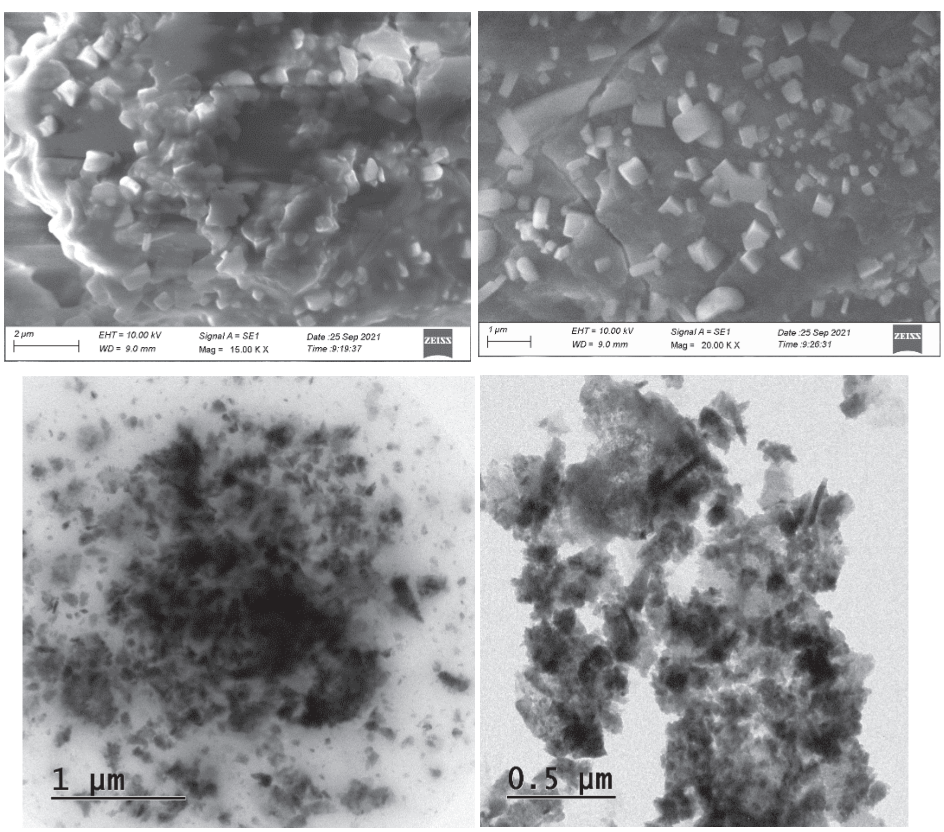

Fig. 4. SEM&TEM Analysis

Analysis Shape and Uniformity the TEM images corroborate the findings from the SEM analysis, showing that the ZnO nanoparticles lack a uniform shape. The flake-like structures observed in SEM are also visible in TEM, providing a more detailed view of the individual nanoparticles. Crystallinity. TEM can also provide information about the crystallinity of the nanoparticles. The images can reveal lattice fringes and defects, which are important for understanding the material's properties and performance in various applications.

Elemental Composition Analysis

The Energy Dispersive X-ray (EDX) analysis is a powerful technique used to determine the elemental composition and purity of materials, such as zinc oxide (ZnO) nanoparticles. Here's a detailed interpretation of the EDX results you provided. Elemental Composition: Zinc (Zn) 78.65%, Oxygen (O) 21.35% High Purity ZnO Nanoparticles the EDX analysis confirms that the sample primarily consists of zinc (Zn) and oxygen (O), with no significant presence of other elements.

This indicates high-purity ZnO nanoparticles. The atomic ratio of Zn to O is approximately 78.65:21.35, which is close to the stoichiometric ratio of ZnO (1:1). This further supports the formation of pure ZnO nanoparticles. The histogram distribution of the elements shows that the mean range of the elements in the ZnO nanoparticles is around 25 nm. This suggests a relatively uniform size distribution of the nanoparticles, which is important for consistent performance in various applications.

The antimicrobial activity

The antimicrobial activity test confirms that ZnO nanoparticles synthesized using *Tecoma Stan* extract exhibit significant antibacterial activity against Staphylococcus aureus. Their broad-spectrum antimicrobial properties, combined with eco-friendly synthesis, make them a promising alternative to conventional antimicrobial agents. Further research is needed to optimize their efficacy and explore their potential in medical, industrial, and environmental applications.

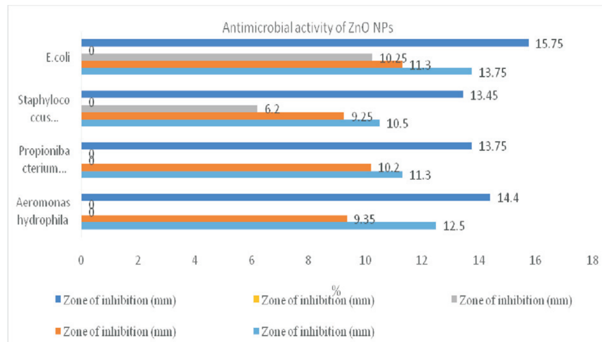

Fig. 6. Antimicrobial activity of ZnO NPs

Most Susceptible Organism: *E. coli* (Gram-negative) shows the highest sensitivity to ZnO NPs, with a ZOI of 13.75 mm at 200 µg/ml and 10.25 mm at 50 µg/ml. Least Susceptible Organism: *Aeromonas hydrophila* and *Propionibacterium acnes* show no activity at concentrations below 100 µg/ml. Moderate Susceptibility: *Staphylococcus aureus* (Gram-positive) shows moderate activity at 50 µg/ml, indicating intermediate sensitivity. The results demonstrate that ZnO NPs exhibit significant antimicrobial activity against a range of bacterial species, with E. coli being the most susceptible. The activity is concentration-dependent, with higher concentrations showing greater efficacy. These findings highlight the potential of ZnO NPs as a broad-spectrum antimicrobial agent for medical, industrial, and environmental applications.

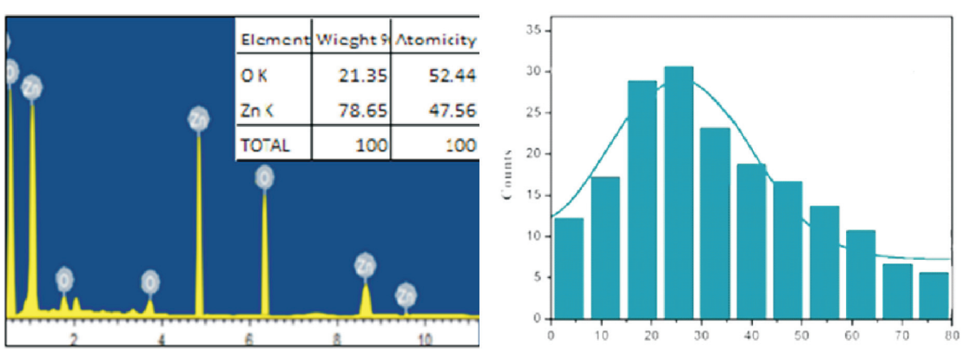

Fig. 5. Elemental Composition Analysis & histogram distribution of the elements

Antioxidant Activity DPPH radical scavenging

The DPPH scavenging assay is a commonly used method for assessing the antioxidant potential of a material. DPPH is a stable nitrogen-centered free radical that changes color from violet to yellow when reduced through hydrogen reduction or electron donation. The scavenging activity of the prepared ZnO nanoparticles is presented in Table 1. The results indicate that the scavenging percentage increases with an increase in the concentration of *Tecoma stans.* The *in-vitro* antioxidant activity of ZnO nanoparticles (ZnO-NPs) synthesized using *Tecoma stans* leaf extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. This assay is widely used to measure the antioxidant potential of substances by their ability to scavenge free radicals. Here's a detailed interpretation of the results. DPPH Radical Scavenging Activity the DPPH assay measures the ability of antioxidants to neutralize free radicals by donating hydrogen atoms or electrons. The IC value (half-maximal inhibitory concentration) indicates the concentration of the sample required to scavenge 50% of the DPPH radicals. Lower IC values indicate higher antioxidant activity. IC, ZnO-NPs (leaf extract): 36.58 µg/ml, Ascorbic acid (standard): 34.89 µg/ml, The leaf extract-based ZnO-NPs showed higher antioxidant activity (lower IC) compared to the root extract-based ZnO-NPs, and their activity was close to that of the standard antioxidant, ascorbic acid. Dose dependent activity both the *Tecoma stans* root and leaf extracts exhibited dose-dependent antioxidant activity, meaning the DPPH radical scavenging activity increased with increasing concentration of the extracts. The leaf extract demonstrated notable antioxidant activity, closely approaching the activity of the standard (ascorbic acid).

Table 1. *In vitro* Antioxidant Activity of *Tecomo stan* ZnO-NPs leaf and roots extract

Concentrations (µg/ml)	ZnO-NPs leaf	Standard as Ascorbic acid
20	17.27	24.11
40	32.6	40.46
60	53.68	61.37
80	68.92	80.55
IC_{50} Value	36.58	34.89

Cell viability assay

The Michigan Cancer Foundation-7 (MCF-7) cell line is indeed one of the most extensively used and well-characterized human breast cancer cell lines in biomedical research. Here's an overview of its key characteristics and its significance in breast cancer studies. MCF-7 cells are a cornerstone of breast cancer research due to their estrogen receptor expression and hormone-responsive nature.

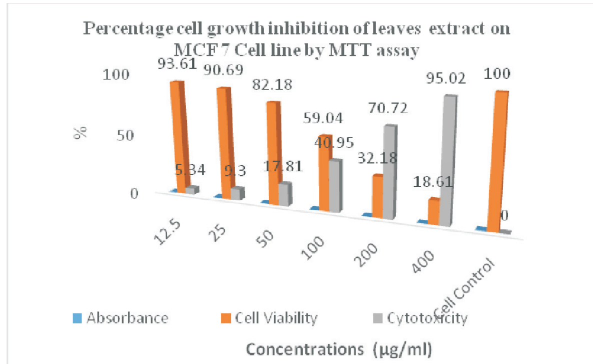

Fig. 7. Percentage cell growth inhibition of leaves extract on MCF 7cell line by MTT assay activity of ZnO NPs

They are particularly valuable for studying hormone-dependent breast cancer and evaluating therapies targeting estrogen signaling. However, researchers often complement MCF-7 studies with other cell lines to account for the diversity of breast cancer subtypes and behaviors. The anticancer potential of *Tecoma leaf* extracts was assessed against MCF-7 cells at varying concentrations (12.5, 25, 50, 100, 200, and 400 µg/ml). The cytotoxic effects of these extracts were compared to paclitaxel, a standard chemotherapeutic agent. *Tecoma stans* Extracts on MCF-7 Cell Growth Inhibition Leaves extract Lowest inhibition: 6.39% at 12.5 µg/ml, highest inhibition: 81.38% at 400 µg/ml, lowest inhibition: 6.05% at 12.5 µg/ml, highest inhibition: 80.94% at 400 µg/ml. Trend as the concentration increases, cell growth inhibition also increases, indicating a dose-dependent cytotoxic effect. Comparison with Standard Drug (Paclitaxel) Paclitaxel inhibition: 8.97% at 12.5 µg/ml to 93.61% at 400 µg/ml. IC-50 Values (Concentration required to inhibit 50% of cells) *Tecoma stans* Leaves Extract 186.61 µg/ml Paclitaxel (Standard): 147.42 µg/ml. The cytotoxic activity of *Tecoma stans*-mediated ZnO-NPs was evaluated by MTT assay against MCF-7 breast cancer cell lines, which confirmed that ZnO-NPs exhibit cytotoxic activity (Mahmoud *et al.*, 2021).

Conclusion

In conclusion, zinc oxide nanoparticles (ZnO NPs) were successfully biosynthesized using *Tecoma stans* leaf extract, which acted as an effective reducing and stabilizing agent. Comprehensive characterization confirmed the formation of pure, crystalline nanoparticles with a hexagonal wurtzite structure. UV-Vis spectroscopy revealed a characteristic absorption peak at 377 nm, while XRD analysis confirmed the crystalline nature and phase purity. FTIR identified the functional groups involved, and electron microscopy (SEM/TEM) showed the nanoparticles' morphology and size, which was further supported by EDX analysis confirming high elemental purity. The synthesized ZnO NPs demonstrated significant biological activity. They exhibited potent, dose-dependent antimicrobial effects against a range of pathogenic bacteria, particularly E. coli. Furthermore, the nanoparticles showed strong antioxidant capabilities in the DPPH assay and promising cytotoxic activity against MCF-7 breast cancer cells, highlighting their potential for biomedical applications. This eco-friendly synthesis method produces ZnO NPs with multifaceted properties, making them a strong candidate for use in pharmaceutical, antibacterial, and anticancer therapies. Future work should focus on in-vivo studies to further explore their therapeutic efficacy and safety.

Credit authorship contribution statement

The contributions of all authors to this research were as follows: Mrs. T. Durgadevi was responsible for conceptualization, investigation, resources, and the writing of the original draft as well as its review and editing. Dr. G. Anburaj contributed through data curation, provision of resources, and overall supervision of the project. Dr. C. Thillaiyadi Valliammai was involved in conceptualization and visualization. Dr. M. Mahadevi contributed to the writing of the original draft and the subsequent review and editing process.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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