RJC

**RASĀYAN *J. Chem.***
Vol. 18 | No. 1 |285-294| January - March | 2025
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

# ANTIOXIDANT, ANTIMICROBIAL, AND CYTOTOXICITY EVALUATION OF BIOSYNTHESISED ZINC OXIDE NANOPARTICLES FROM *Elaeagnus conferta*

**C. Merlin Rose and H. Benita Sherine**
PG and Research Department of Chemistry, Thanthai Periyar Government Arts and Science College (Autonomous), Trichy- 620 023, Affiliated to Bharathidasan University, Tiruchirappalli (Tamil Nadu) India
✉Corresponding author: merlin@thanthaiperiyargasc.ac.in

## ABSTRACT

Nanotechnology and nanomedicine discoveries have undergone impressive modifications, pushing the techniques to a new level to get the best outcome in healthcare. Nanoparticles made by green methods are far superior to those synthesized by chemical as well as physical methods. The green technique avoids the utilization of exclusive chemicals, consumes less energy, and generates eco-friendly products. The goal of the present work is to explore ZnO NPs (zinc oxide nanoparticles) green synthesis through *Elaeagnus conferta* leaf extract (EC-ZnONPs). Characterization techniques including TEM (transmission electron microscopy), FTIR (Fourier-transform infrared spectroscopy), and XRD (X-ray diffraction), have been utilized to explain the structural, morphological, as well as chemical properties of *E.Conferta*-mediated ZnONPs. These analyses confirm the effective synthesis of crystalline ZnONPs with controlled size distribution and surface functionalization. The prepared nanomaterials demonstrated substantial antibacterial activity against several gram-positive as well as gram-negative bacterium along with fungi, thus, showing potential antioxidant properties. Moreover, EC-ZnONPs showed notable mortality in MCF-7 breast cancer cells thus revealing EC-ZnONPs potential in cancer therapy. This paves the way for the synthesis of EC-ZnONPs for different biological and nutraceutical applications.

**Keywords:** *Elaeagnus Conferta*, Biosynthesis, Zinc Oxide Nanoparticles, Characterization, Antimicrobial Activity, Antioxidant, Anti-Cancer.

RASĀYAN*J. Chem.*, Vol. 18, No.1, 2025

## INTRODUCTION

In the 21st century nanotechnology has a major impact on a global scale due to its size ranging from 1-100nm.[1,2] Nano-science and technology is an interdisciplinary field, which needs a scientific outlook from diverse areas of science and technology. The principles of physics, chemistry, biology, as well as engineering are integrated into the study of nanoscience.[3] The synthesis of nanomaterials has been done through many methods as the Chemical method, Physical method, and green method.[4] Many researchers focus on synthesizing inorganic nanoparticles, because of their hydrophilic, biocompatible, non-toxicity along extremely stable in comparison with organic nanoparticles.[5] Inorganic nanoparticles comprise metal oxides, elemental metals, as well as metal salts. Metal oxide nanoparticles are applicable in agriculture, material science, clinics, environmental management, etc.[6,7] Metal oxide nanoparticles have many unique features that have made them an ideal candidate for antimicrobial therapy in nanomedicine.[8,9,10] For example, metal oxide nanoparticles have been recognized and used as genotoxicity materials to study their genotoxicity potential and their possible health risks in man through human health.[11,12] Metal oxide nanoparticles are critically important for various modern technologies. Hence, due to their applications in a wide range, these metal oxide nanoparticles have drawn some potency in all spheres of life starting from agriculture to healthcare.[13] An alternate method to plant extract-mediated nanoparticle synthesis that is most biocompatible, efficient, affordable, and secure is biosynthesis, occasionally referred to as green synthesis.[14,15] Moreover, plant extracts function as reducing as well as stabilizing agents in nanomaterials synthesis.[16] Recently, studies have proven that green synthesized ZnO NPs utilizing several plant extracts have had a lot of success as they displayed significant biological activities.[17,18] Free radicals can also be scavenged by these nanoparticles and on the other hand, they perform anticancer action by enhancing apoptosis in cancer cells. Furthermore, a possible antidiabetic activity of ZnO NPs had been evidenced,


http://doi.org/10.31788/RJC.2025.1819186

considering enzyme inhibitory as well as cytotoxic effects on some cell lines, pointing them as potential medications to treat cancer as well as diabetes.[19] These demonstrated multilayered biological functionalities of these ZnO NPs, which reinforces their potential applicability in biomedical and nutraceutical materials. An efficient remedy for hepatitis, cancer, malaria, and other acute diseases is Plant extracts-mediated ZnO NPs.[20,21] The common name of *Elaeagnus conferta* is bastard oleaster, which belongs to the family *elaeaganaceae*. *E. conferta* has very good anti-microbial, anti-tumor, anti-fungal, anti-inflammatory, anti-diabetic, anti-viral, antioxidant, along cytotoxic activities, which are reported in many papers.[22] The medicinal activities are very high due to existence of carotenoids and ascorbic acid. The main advantage of using plant extract is to reduce the toxicity of nanoparticles and unnecessary usage of toxic chemicals. The current investigation thus attempted to biosynthesize and characterize ZnONPs mediated by leaf extract from *Elaegnus conferta* as well as their applications.

## EXPERIMENTAL

### Collection of Plant and Extract Preparation

Plant material *E.Conferta* was collected from Yercaurd Hills, Tamil Nadu. The disease-free plant part (leaf) was spread out and dried in the laboratory at room temperature for 8-10 days until they were easily broken by hand and ground to a fine powder using an electronic blender. About 20 g of dried and powdered plant material (leaves) were soaked separately with 250 mL of the solvent alcohol, in a soxhlet apparatus for 24 hrs until complete extraction of the materials. The extraction procedure must be carried out for 24 hours. The filtrate was refrigerated for subsequent qualitative analysis and synthesis of nanoparticles.

### Phytochemical Screening of Alcoholic Extract of *E.Conferta*

The presence of bioactive components in the plant extract must be identified. Various qualitative chemical experiments were carried out to find the phytochemical profile of the extract of *E.Conferta.* Standard screening experiments were performed upon extract to identify several phytocompounds that exist among them as shown in Fig.-1.[23,24]

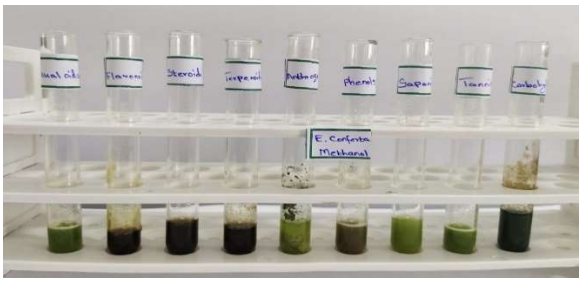


Fig.-1: Phytochemical Screening Test

### Biogenic Synthesis of ZnO Nanoparticles and its Characterisation

ZnO NP biogenic synthesis was done by adding 25mL of plant extract to 25mL of 1mM solution of zinc acetate dihydrate. Then for 1h, the reaction mixture was kept on a magnetic stirrer. After 1h, 20mL of NaOH was added to the solution, and it was placed in a magnetic stirrer for 3 hours for complete reduction of $Zn^{2+}$ ions as well as the formation of ZnO nanoparticles (white precipitate).[25,26] Prepared ZnO nanoparticles were collected, then washed using double distilled water, and subsequently oven-dried at 70ºC to get powdered ZnO NPs. For additional characterization and utilization, dried samples were kept in an airtight container at room temperature.[27,28,29] The bio-synthesized EC-ZnONPs had been verified with the assistance of SEM, UV-Vis spectroscopy, XRD, FT-IR spectral analysis, EDAX analysis as well as TEM.

### DPPH Assay

Antioxidant properties of biosynthesised EC-ZnONPs had been done by Diphenylpicrylhydrazyl (DPPH) Assay. The different concentrations of synthesized EC-ZnONps had been mixed with 1000 µm DPPH solution (1mL). Mixtures were stored in a dark place for half an hour. After 30 min the color change was observed in terms of absorbance at 517nm using a UV-Vis spectrophotometer.[30,31] The percentage of radical scavenging activity was computed using the standard formula.

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control-Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

**Invitro Antimicrobial Activity**

The antimicrobial activity of EC-ZnONPs was investigated against some selected pathogens by the disc diffusion method.[32,33] MTCC (Microbial Type Culture Collection), located at the Institute of Microbial Technology in Chandigarh, India, is where all of the bacterial as well as fungal cultures were acquired. Hi-media's (Mumbai) MHA (Muller Hinton Agar) was used to screen for *invitro* antimicrobial activity. A total of 15mL molten medium had been added to sterilized petri plates for preparing MHA plates. After 5 minutes of plate solidification, a uniform 0.1 percent inoculum suspension was swabbed onto the plates and the inoculum was dried. The extract's concentration is 40mg/disc, was loaded on to a 6mm sterile disc. The loaded disc was positioned on the medium's surface, then extract was permitted to diffuse for 5 minutes before incubating plates for 24 hrs at 37°C. Upon incubating for 24 hrs, the inhibition zones surrounding the disc were determined in millimeters utilizing a transparent ruler.

**Invitro Cytotoxicity Study of Synthesised EC-ZnONPs**

Invitro cytotoxicity study of biosynthesized EC-ZnONPs was studied with MCF-7 (Michigan Cancer Foundation-7).[34,35] Monolayer cells had been dissociated utilizing trypsin-EDTA to create a single-cell suspension. Cell solution was diluted using a medium comprising 5 percent FBS for achieving $1\times10^5$cells/mL density. Cell suspensions (100µL/well) were inoculated into 96 well plates at 10,000/well density as well as incubated with 5 percent $CO_2$ and 95 percent air at 37°C. Following a 24-hour period, cells were subjected to repeated doses of ZnO nano samples. Varying concentrations (50, 250, 500, 750, 1000µL/mL) of ZnO NPs were inoculated into grown cells comprising 100 µL medium. Plates were subsequently incubated for 48 hours at 37°C, with 5 percent $CO_2$, 95 percent air, and 100 percent relative humidity. A yellow color solution of 3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) (15 µL) was incorporated into phosphate buffered saline (5µg/mL) in every well. At 37°C temperature, plates were incubated for a 4 h time period for MTT reduction. Resultant purple formazan crystals were dissolved in 100µL DMSO, and absorbance was quantified at 570 nm utilizing a microplate reader ("EMR500, Labomed").

## RESULTS AND DISCUSSION

**Chemical Screening**

The secondary metabolites present in *E. Conferta* were studied by standard phytochemical screening tests. Results demonstrate the presence of flavonoids, alkaloids, terpenoids, phenols, steroids, carbohydrates, etc., which is displayed in Table-1.

Table-1: Phytochemical Screening of *Elaeagnus conferta* Leaf Extract

| Phytochemicals | Tests | Results |
|---|---|---|
| Alkaloids | Mayer's test<br>Wagner's test | ++ |
| Arthroquinone | Borntrager's test | - |
| Carbohydrates | Fehling's test | + |
| Flavonoids | Lead acetate test<br>$H_2SO_4$ test | ++ |
| Oils and Resins | Spot test | - |
| Phenols | Ferric chloride test<br>Lead acetate test | + |
| Saponin | Froth Test | - |
| Steroids | Liebermann-Burchard test | + |
| Tannin | Ferric chloride test | - |
| Terpenoids | Salkowski test | + |

Note: (+) Positive= Present, (-) Negative=Absent

**UV-Vis Analysis**

The UV-visible spectrum of alcoholic extract of *E.Conferta* and biosynthesised EC-ZnONPs were taken at the wavelength between 200 to 800 nm and it is shown in Fig.-2a and 2b. The spectrum of *E.Conferta*

shows the highest peaks at 354 and 408 nm. This indicates that the alcoholic extract contains Flavonoids and terpenoids. The peaks at the 509, and 534 nm indicate the existence of carbonyls, nitriles and azo compounds. The peak at 620nm indicates organic chromophore-like chlorophyll. These chromophores undergo both π- π* and n- π* transitions. In the spectrum of biosynthesized EC- ZnONPs, the maximum absorption peak was noted at 374nm. A sharp absorption peak indicates the monodispersed nature of the nanoparticle distribution.[36,37] This confirms the ZnO NPs formation.

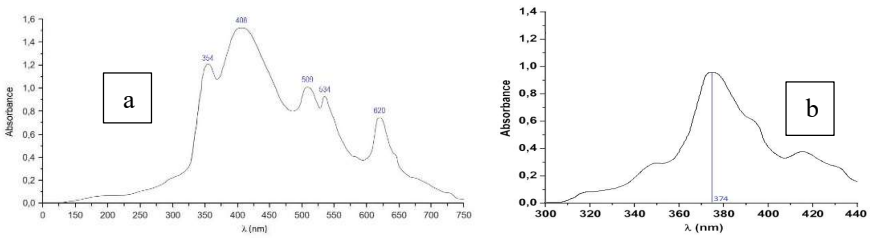



Fig.-2: (a)UV-Visible Spectrum of *E.Conferta* (b) EC Mediated ZnONPs

**FT-IR Analysis**

FT-IR spectrum of alcoholic extract of *E.Conferta* and biosynthesized EC-ZnONPs are shown in Fig.-3a and 3b. In Fig.-3a, Peaks are observed at 3874, 3642, 2837, 2562, 2232, 1111, and 678cm$^{-1}$ which are associated with OH stretching in alcohol, N-H stretching in amines, methyl and methylene groups, sulfur-containing compounds, C=O stretching, and aromatic ring. In the FT-IR spectrum of EC-ZnONPs (Fig.-3b), the Spectral peak at 618 nm confirms the strong metal-oxygen bond formation.

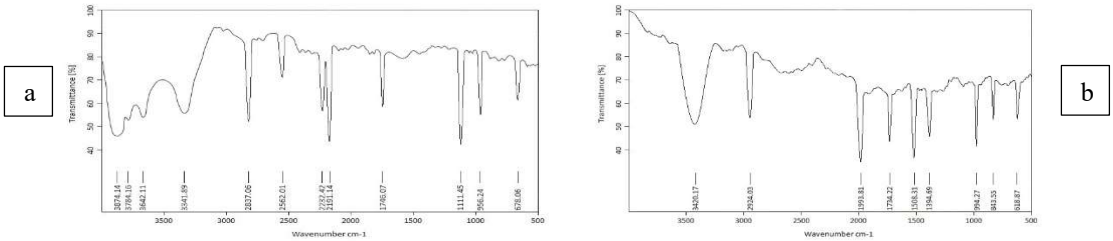



Fig.-3: FT-IR Spectrum of (a.) *E.conferta* (b.) EC-ZnONPs

**SEM Analysis**

The scanning electronic microscope of biosynthesized EC-ZnONPs is shown in Fig.-4a. SEM images were taken at various magnifications from 1µm-200 nm (Fig.-4a) which proves the spherical-shaped EC-ZnONPs with a mean average diameter of 75.14nm.[38] The SEM image confirms the spherical-shaped nanoparticles formed, inclined together because of the existence of a capping agent. This capping agent stabilizes the nanoparticles. The composition of elements in E.Conferta mediated ZnO nanoparticles has been studied by EDAX analysis as demonstrated in Fig.-4b. The composition of zinc as well as oxygen was found to be 64.89 % and 35.11 % respectively.

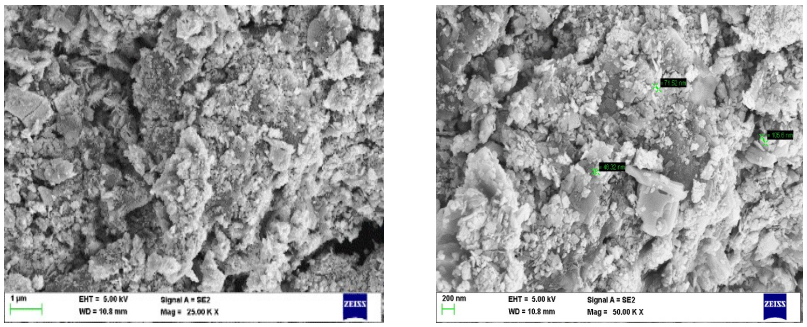



Fig.-4a: SEM Image of Biosynthesized EC-ZnONPs at 1µm and 200 nm

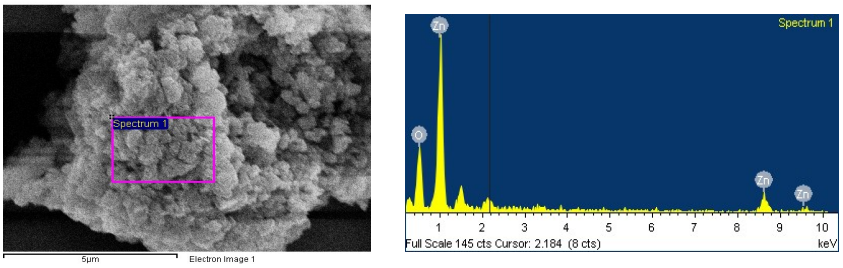



Fig.-4b: EDAX Spectrum of biosynthesized EC-ZnONPs

**XRD Analysis**

XRD study was done for biosynthesized EC-ZnONPs (Fig.-5). E.Conferta mediated ZnONPs demonstrated peaks with 2θ values at 31.64°(100), 34.30°(002), 36.18°(101), 47.73°(102), 56.41°(110), 63.08°(103), as well as 68.14°(112). This result is well associated with the data card (JCPDS-36-1451). XRD examination indicated that all diffraction peaks corresponded accurately to the hexagonal wurtzite structure of ZnO NPs, and this report is exactly matched with other articles.[39,40] The average Crystalline size was computed utilizing Debye Scherrer's equation and the value was 15.97nm.

Table-2: Average Crystalline Size of EC-ZnONPs

| S.No. | 2 theta | FWHM | D(Å) |
|---|---|---|---|
| 1 | 31.62869 | 0.54161 | 14.112731 |
| 2 | 34.3281 | 0.42145 | 18.810854 |
| 3 | 36.13537 | 0.49515 | 15.930995 |
| 4 | 47.44245 | 0.50528 | 15.033949 |
| 5 | 56.47866 | 0.61665 | 11.85406 |
| 6 | 62.79327 | 0.50336 | 14.070406 |
| 7 | 67.81468 | 0.50838 | 13.545625 |

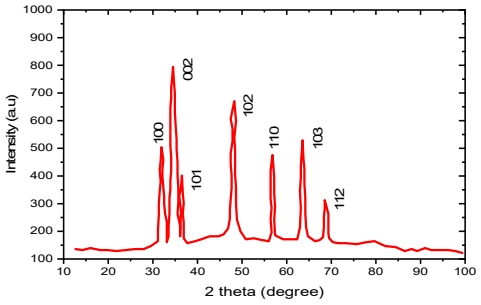



Fig.-5: XRD Spectrum of Biosynthesized EC-ZnONPs

**TEM Analysis**

TEM analysis gives knowledge of crystalline properties along with the size of synthesized NPs. The TEM image of EC-ZnONPs is shown in Fig.-6. This verifies that the synthesized nanoparticles are agglomerated, exhibiting spherical as well as hexagonal wurtzite structures, with 30.42nm average size of particles. This is substantiated by the XRD values and which also demonstrate the existence of spherical and hexagonal particles.

**Anti-oxidant Activity- DPPH Assay**

Anti-oxidants can be either man-made or naturally obtained. It may avoid or else delay the damage of cells resulting from some free radicals (unstable molecules). This quality is tested in *E.conferta* mediated ZnONPs with DPPH assay. The deep purple color of DPPH was changed to pale yellow by adding different concentrations of test samples and standard solution (Ascorbic Acid). Then sample absorbance was

observed using a UV-Vis Spectrometer at 517nm. The scavenging effect of *E.conferta* mediated ZnONPs and Ascorbic acid standard are demonstrated in Fig.-7. Outcomes verify that increasing the EC-ZnONPs concentration shows good anti-oxidant activity with the $IC_{50}$ value of 385µg/mL.

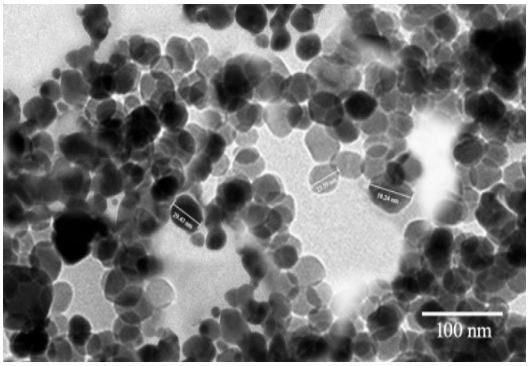



Fig.-6: TEM image of biosynthesized EC-ZnONPs

Table-3: Scavenging Effect of Ascorbic Acid and EC-ZnONPs

| Concentration µg/mL | Scavenging effect of Ascorbic acid (%) | Scavenging effect of EC-ZnONPs (%) |
|---|---|---|
| 100 | 25.34 | 24.42 |
| 250 | 33.56 | 29.80 |
| 500 | 41.78 | 38.84 |
| 750 | 50 | 51.92 |
| 1000 | 55.48 | 63.65 |

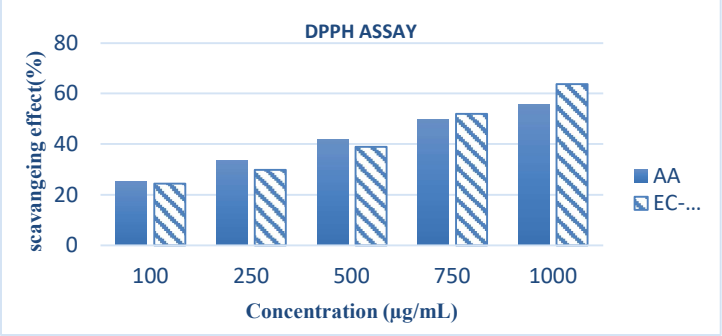
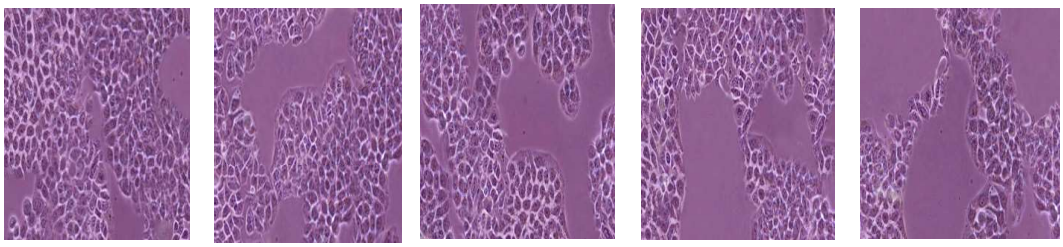



Fig.-7: Scavenging Effect of EC-ZnONPs

**Cytotoxicity Study of ZnO Nanoparticles**

The MTT assay was employed to measure the *in vitro* cytotoxic impact of ZnO NPs on cell lines of breast cancer (MCF-7) as well as cell inhibition (%). As seen in Fig-8, cytomorphological alterations caused by ZnO NPs on MCF-7 cell lines at varying concentrations (50, 250, 500, 750, and 1000µL) involve an intracellular suicide program with morphological changes that include shrinkage of cells, coiling, oxidative stress and biochemical reactions leading to apoptosis. The findings make it very clear that when ZnO NP concentration rises, consequently rises MCF-7 cell lines' rate of apoptosis (Fig.-8a and Fig.-8b).



50 µg/mL 250 µg/mL 500 µg/mL 750 µg/mL 1000µg/mL

Fig.-8a: Anticancer Effect of EC-ZnONPs against MCF-7 Cell Line

After 48 hours of exposure, a dose-dependent rise in cell inhibition is noticed. From the result (Table-4) obtained, EC-ZnONPs showed $CTC_{50}$ value in MTT assay is 582.27µg/mL. These findings demonstrate that cytotoxicity rises in a dose- as well as time-dependent manner. Increasing ZnO NPs concentration decreases the cell viability. The plant-mediated nano ZnO is predicted by the CTC50 value to be a potential medication for the treatment of chemotherapy. Complete apoptosis was detected when ZnO NPs were present at 1000 µg/mL.

Table-4: Cell Viability and CTC50 Value of EC-ZnONPs

| Concentration µg/mL | %$CTC_{50}$ | $CTC_{50}$ | Cell Viability |
|---|---|---|---|
| 50 | 25.97 | 582.27 | 74.03 |
| 250 | 40.90 | | 59.10 |
| 500 | 44.58 | | 55.42 |
| 750 | 56.03 | | 43.97 |
| 1000 | 67.69 | | 32.31 |

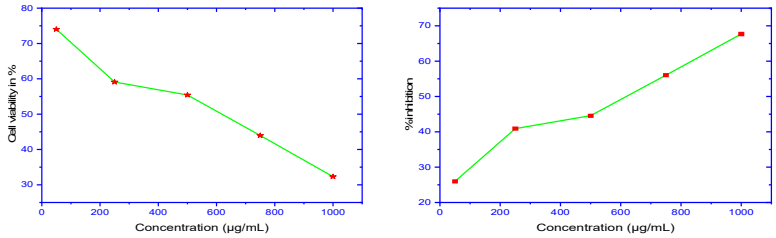



Fig.-8b: Graphical Representation of Anticancer Potential of EC-ZnONPs against MCF-7 Cell Line

**Antimicrobial Activity**

Antimicrobial activity of *E.conferta* mediated ZnONPs was studied against some pathogens like *S. aureus, A. niger, E.coli,* as well as *C. albicans* using the disc diffusion method[41,42] as shown in Fig.-9 and the graphical representation also given in Fig.-10. The biosynthesized ZnO nanoparticle from *Elaeagnus conferta* shows a better inhibition effect against *E. coli* compared to *staphylococcus* at 60 µL. Also, it shows good antifungal activity against *A.niger* even at lower concentrations (30 µL) compared to *C. albicans*. Interestingly, *E.conferta* mediated ZnONPs revealed efficient inhibition on gram-positive as well as gram-negative bacteria along with the fungus.

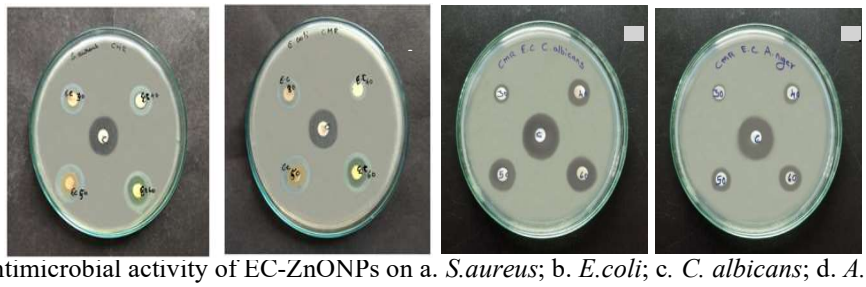


Fig.-9: Antimicrobial activity of EC-ZnONPs on a. *S.aureus*; b. *E.coli*; c. *C. albicans*; d. *A.niger*

Table-5: Antimicrobial activity of EC-ZnONPs

| Organism | Organism | Concentration in µg/mL | | | |
|---|---|---|---|---|---|
| | | 30 | 40 | 50 | 60 |
| *Escherichia coli* | 19 | 14 | 16 | 20 | 21 |
| *Staphylococcus aureus* | 20 | 15 | 17 | 19 | 20 |
| *Aspergillus niger* | 24 | 20 | 15 | 18 | 19 |
| *Candida albicans* | 21 | 17 | 14 | 18 | 13 |

## CONCLUSION

Nanoparticles have a wide range of applications in many fields, particularly in the medical field, because of their unique attributes for example surface area, reactivity, and the ability to combine with living systems

at the molecular level. However, nanoparticle synthesis by conventional procedures often contains the utilization of toxic chemicals. These chemicals are not only responsible for health risks but also cause environmental pollution. To avoid such conditions, there has been an increasing shift toward exploring eco-friendly and sustainable alternatives with a certain focus on plant-based green synthesized products. This prompted me to conduct my research using plant leaves and aiming to synthesize nanoparticles without environmentally hazardous chemicals.

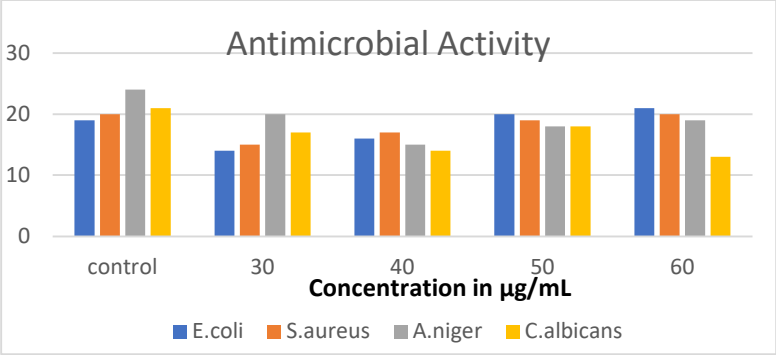



Fig.-10: Graphical Representation of Antimicrobial Activity of EC-ZnONPs

For this purpose, A green synthesis method was employed to produce *Elaeagnus conferta* leaf extract-mediated ZnO nanoparticles (EC-ZnONPs). The leaf extract from *E.Conferta* functions as both a reducing along stabilizing agent, simplifying the formation of ZnO nanoparticles in a safe and environmentally friendly manner. The characterization methods for example FT-IR, UV-Vis, SEM, XRD, and TEM confirmed crystalline ZnO NPs synthesis with spherical morphology having a 30.42nm average diameter. *E.conferta* mediated ZnO nanoparticles biological activity was also calculated with promising results. EC-ZnONP antioxidant activity was examined via the DPPH (2,2-dipicryl-1-picrylhydrazyl) assay, which exhibited significant antioxidant activity. This antioxidant activity suggests their potential application in combating factors against various diseases and aging processes. Additionally, EC-ZnO's *in vitro* cytotoxicity was tested against MCF-7 breast cancer cells, with a $CTC_{50}$ value of 582.27µg/mL. This denotes that the nanoparticles have a moderate level of cytotoxicity. Also, the EC-ZnONPs displayed promising antimicrobial activities against a broad spectrum of microorganisms. These findings highlight its potential effect in an extensive diversity of biological as well as nutraceutical utilization. Future investigation is required to understand their mechanisms of action, optimize their healing potential, and evaluate their safety through *in vivo* studies. This would help to develop EC-ZnONPs as a variable alternative to traditional synthetic nanoparticles for medicinal and environmental sustainability.

## ACKNOWLEDGEMENTS

Authors are thankful to the authorities of their institutions for providing the basic facilities.

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest

## AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

C. Merlin Rose https://orcid.org/0000-0002-4319-6862
H. Benita Sherine http://orcid.org/0009-0009-9741-4261

## REFERENCES

1. G. Ali Mansoori, *Nanoscience and Plant-Soil Systems*, **48**, 1(2017), https://doi.org/10.1007/978-3-319-46835-8
2. A. Mandal, E. Ray Banerjee, *Nanomaterials and Biomedicine*, 1 (2020).
3. P. Moriarty, A Very Short Introduction, Nanotechnology, Oxford University Press, (2022).
4. A. Zahrah, *Molecules*, **28(7)**, 3086(2023), https://doi.org/10.3390/molecules28073086
5. H. Abid, M. Javaid, S. Ravi Pratap, Shanay Rab, and S.Rajiv, *Global Health Journal*, **7(2)**, 70(2023), https://doi.org/10.1016/j.glohj.2023.02.008
6. Md Abdus Subhan, *RSC advances*, **12(51)**, 32956(2022), https://doi.org/10.1039/D2RA02005J.
7. A.G Kaningini, A. Nelwamonondo, S. Azizi, M. Maaza, and K.C. Mohale, *Coatings,* **12(10),** 1586(2022), https://doi.org/10.3390/coatings12101586
8. H. Kotrange, A. Najda, A. Bains, R. Gruszecki, P. Chawla and M.M. Tosif, *International Journal of Molecular Sciences*, **22(17)**, 9596(2021), https://doi.org/10.3390/ijms22179596
9. J. Mitra and Joyee Mitra, *Metal Oxides for Biomedical and Biosensor Applications*, **2022**,183(2022), https://doi.org/10.1016/B978-0-12-823033-6.00006-5
10. M.V. Nikolic, Z.Z. Vasiljevic, S. Auger, and J. Vidic, *Trends in Food Science and Technology*, **116**, 655(2021), https://doi.org/10.1016/j.tifs.2021.08.019
11. R. A. de Jesus, G. C. de Assis, R. J. de Oliveira, M. Bilal, R. N. Bharagava, M.N. Iqbal, L. R. Ferreira, and R. T. Figueiredo, *Biodegradation and Biodeterioration at the Nanoscale*, **2022**, 529(2022), https://doi.org/10.1016/B978-0-12-823970-4.00025-7
12. P. Nikolova, and M. S. Chavali, *Biomimetics,* **5(2)**,27(2020), https://doi.org/10.3390/biomimetics5020027
13. S. K. Das, M. R. Khan, A. K. Guha and N. Naskar, *Green Chemistry*, **15(9)**, 2548(2013), https://doi.org/10.1039/C3GC40310F
14. Iravani and Siavash, *Green Chemistry*, **13(10)**, 2638 (2011), https://doi.org/10.1039/C1GC15386B
15. M.F. Tassew, G. Chouhan, *International Journal of Health Sciences*, **6(S4)**, 6059(2022), https://doi.org/10.53730/ijhs.v6nS4.9529
16. A. Agila, J. Rosaline Vimala, M. Stella Bharathy, G. Dayana Jeyaleela, and S. Margrat Sheela, *Biomedical and Biotechnology Research Journal,* **6(3)**, 341(2022), https://doi.org/10.4103/bbrj.bbrj_136_22
17. S. N. Mohamad Sukri, K. Shameli, S.Y. Teow, J. Chew, L.T. Ooi, M. Lee-Kiun, N.A. Ismail, and H. Moeini, *Frontiers in Microbiology,* **14**, 1194292(2023), https://doi.org/10.3389/fmicb.2023.1194292
18. B. Naiel, M. Fawzy, and M.W.A. Halmy, *Scientific Report,* **12**, 20370(2022). https://doi.org/10.1038/s41598-022-24805-2
19. A. Alkaladi, A.M. Abdelazim, and M. Afifi, *International Journal of Molecular Sciences*, **15(2)**, 2015(2014), https://doi.org/10.3390/ijms15022015
20. P. Kuppusamy, M. M. Yusoff, G. P. Maniam, and N. Govindan. *Saudi Pharmaceutical Journal,* **24(4)**, 473(2014), https://doi.org/10.1016/j.jsps.2014.11.013
21. M. Y. Darwesh, S. Ibrahim, and M. Abbood, *Results in Chemistry*, **7(5)**, 101368(2024), https://doi.org/10.1016/j.rechem.2024.101368
22. N.Gill Singh, and M. Gupta. *Research Journal of Pharmacy and Technology*, **11**, 2667(2018), https://doi.org/10.5958/0974-360X.2018.00494.8
23. M. Gupta, M. Gulati, and K. Bhupinder, *Medical Hypotheses,* **175**, 111075(2023), https://doi.org/10.1016/j.mehy.2023.111075
24. M. Gupta and S. Naresh, *Plant Archives,* **21**, 547(2021), https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.084
25. P. Jamdagni, P. Khatri, and J.S. Rana, *Journal of King Saud University-Science,* **30(2)**, 168(2018), https://doi.org/10.1016/j.jksus.2016.10.002
26. H. Hameed, A. Waheed, M.S. Sharif, M. Saleem, A. Afreen, M. Tariq, A. Kamal, W.A. Al-onazi, D.A. Al Farraj, S. Ahmad and R.M. Mahmoud, *Micromachines*, **14(5)**, 928(2023). https://doi.org/10.3390/mi14050928

27. S. Mourdikoudis, M.P. Roger, and T. Nguyen, *Nanoscale,* **10(27)**, 12871(2018), https://doi.org/10.1039/C8NR02278J
28. P.P. Mahamuni, P.M. Patil, M.J. Dhanavade, M.V. Badiger, P.G. Shadija, A.C. Lokhande and R. A. Bohara, *Biochemistry and Biophysics Reports*, **17**, 71(2018), https://doi.org/10.1016/j.bbrep.2018.11.007
29. H. S. Jayawardena, S.H. Liyanage, K. Rathnayake, U. Patel and M. Yan, *Analytical Chemistry*, **93(4)**, 1889 (2021), https://doi.org/10.1021/acs.analchem.0c05208
30. M. N. Emmanuel, Medicinal Plants, Antioxidant Potential, and Cancer, *Cancer(Second edition)*, Academic Press, pp. 349-357(2021), https://doi.org/10.1016/b978-0-12-819547-5.00031-6
31. S. Baliyan, R. Mukherjee, A. Priyadarshini, A. Vibhuti, A. Gupta, R. P. Pandey, and C. M. Chang, *Molecules*, **27(4)**, 1326(2022), https://doi.org/10.3390/molecules27041326
32. H. Khalili, R. Soltani, S. Negahban, A. Abdollahi, and K. Gholami, *Iranian Journal of Pharmaceutical Research*, **11(2)**, 559(2012), https://doi.org/10.22037/ijpr.2012.1094
33. A. Rivera, B. Viñado, N. Benito, F. Docobo-Pérez, F. Fernández-Cuenca, J. Fernández-Domínguez, J. Guinea, A. López-Navas, M.A. Moreno, M.N. Larrosa, A. Oliver and F. Navarro, *Enfermedades Infecciosas y Microbiologia Clinica*, **41(9)**, 571(2023), https://doi.org/10.1016/j.eimc.2022.04.015
34. O.S. Aslantürk, In Vitro Cytotoxicity and Cell Viability Assays: Principles, Advantages, and Disadvantages, InTech, pp. 1-17(2018).
35. C.C. Anajwala, R.M. Patel, S.L. Dakhara, and J.K. Jariwala, *Journal of Advanced Pharmaceutical Technology and Research,* **1**, 245(2010).
36. E. Gurgur, S.S. Oluyamo, A. O. Adetuyi, *SN Applied Science,* **2**, 911(2020), https://doi.org/10.1007/s42452-020-2269-3
37. S. Talam, S.R. Karumuri, and G. Nagarjuna, *International Scholarly Research Notices,* **2012**, 372505(2012), https://doi.org/10.5402/2012/372505
38. T. Nethavhanani, A. Diallo, R. Madjoe, K. Lebogang and M. Malik, Proceedings of the 28th World Conference of The International Nuclear Target Development Society (2018).
39. K.Z. Monsef, M. Sarfaraz, and M. Asadi Asadabad, *Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry*, **41(7)**, 814(2011), https://doi.org/10.1080/15533174.2011.591308
40. H. Sowa, and H. Ahsbahs, *Journal of Applied Crystallography* **39(2)**, 169(2006), https://doi.org/10.1107/S0021889805042457/ko5022zno87sup6.hkl
41. M. Vijaylaxmee and S. Richa, *International Journal of Pharma Research and Health Sciences,* **3(3)**, 694 (2015).
42. Y. Xie, Y. He, P.L. Irwin, T. Jin, and X. Shi, *Applied and Environmental Microbiology*, **77(7)**, 2325(2011), https://doi.org/10.1128/AEM.02149-10

[RJC-9186/2025]