Unveiling the bioactive components of corona responsible for the enhanced Antimicrobial and cytotoxic propensity of zinc oxide nanoparticle synthesised using extracts of *Eucalyptus globulus, Mangifera indica,* and *Tagetes erecta*

Kumari Subham[a*], ***Sonali Jena***[a], ***Suman Jha***[a]

[a]**Department of Life Science, National Institute of Technology, Rourkela, Odisha, 769008, India**
Email ID: 519ls1006@nitrkl.ac.in

INTRODUCTION: Medicinal plants have their own biological activity and act as a better reducing and capping agent for metal ions to turn into respective metal nanoparticles. Metal NPs synthesized by biological means show better antimicrobial propensity and anti-cancerous activity than their chemical counterparts. However, the exact reason behind this is still obscure, and the need of the time.

AIM AND OBJECTIVE: To unveil the bioactive components of corona responsible for the enhanced antimicrobial and cytotoxic propensity of zinc oxide nanoparticle synthesised using extracts of Eucalyptus globulus, Mangifera indica, and Tagetes erecta.

METHODS: Zinc oxide nanoparticles were biofabricated using different plant extracts and characterised using a combination UV-Vis spectroscopy, DLS-ZETA, FTIR, XRD, and TEM followed by phyto-corona characterization using GC-MS. Antibacterial efficacy was investigated against Bacillus subtilis and Escherichia coli through broth microdilution assay, with MIC determination. Anticancer activity was evaluated against adenocarcinoma human alveolar basal epithelial cells (A549) using Alamar blue dye reduction assay and cell viability was measured. To trace the mechanism behind antimicrobial and anti-cancerous activity internalization and ROS studies were conducted.

RESULTS: The characterization of biofabricated ZnONP showed spherical, crystalline NP of 20-40 nm size with well-defined corona of 2-4 nm with negative surface potential. GC-MS analysis gave the exact constituent of bioactive compounds comprising the phyto-corona. ZnONPs were found to have antimicrobial activity against Bacillus subtilis and Escherichia coli via predominantly enhanced intracellular ROS generation with increasing concentration. Interestingly, the nanoparticles showed higher cytotoxicity towards A549 cells than human keratinocyte cells (HaCaT) and was also found to co-localize with the nucleic acid.

CONCLUSION: The horizon for the forthcoming progress in nanomedicine is very promising. The biological corona characterization unveils the presence of bioactive compounds responsible for enhanced antimicrobial and anti-cancer potential, along with providing stability to nanoparticles.

UNVEILING THE BIOACTIVE COMPONENTS OF CORONA RESPONSIBLE FOR THE ENHANCED ANTIMICROBIAL AND CYTOTOXIC PROPENSITY OF ZINC OXIDE NANOPARTICLE SYNTHESISED USING EXTRACTS OF *EUCALYPTUS GLOBULUS, MANGIFERA INDICA, AND TAGETES ERECTA*

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***Kumari Subham*[a]*, *Sonali Jena*[a], *Suman Jha*[a]**

[a]Department of Life Science, National Institute of Technology, Rourkela, Odisha, 769008, India
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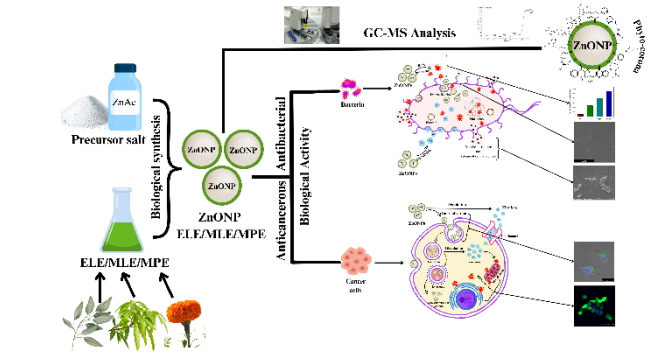

Fig. 1 Schematic representation of biologically synthesised ZnONP co-localisation and cytotoxicity in prokaryotic and eukaryotic cells.

RESULTS

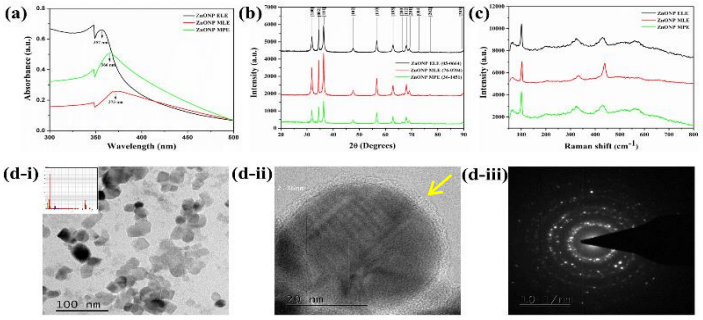

Fig. 2 Characterization of biologically synthesised ZnONPs, (a) UV-Visible spectra; (b) XRD diffraction pattern; (c) Raman spectra (d) TEM analysis of ZnONP MPE (i) TEM micrograph with inserted EDX spectra (ii) HR-TEM image, showing characteristic d-spacing in the crystalline ZnONP, and (iii) SAED pattern, showing crystal plane of ZnONP.

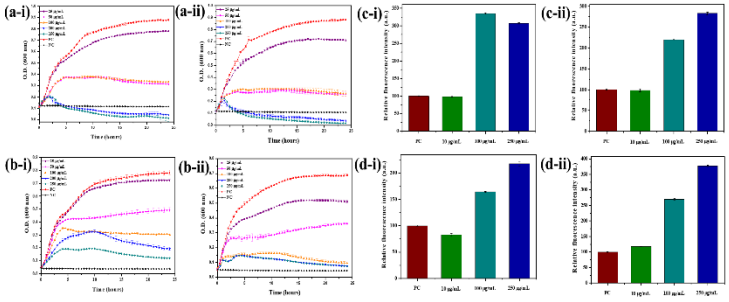

Fig. 3 Growth kinetics and ROS detection in (i) *B. subtilis* and (ii) *E. coli* in presence of varying concentrations of ZnONP CHE (a, c) and ZnONP MPE (b, d). Red line in the growth kinetics represent untreated cells in a and b.

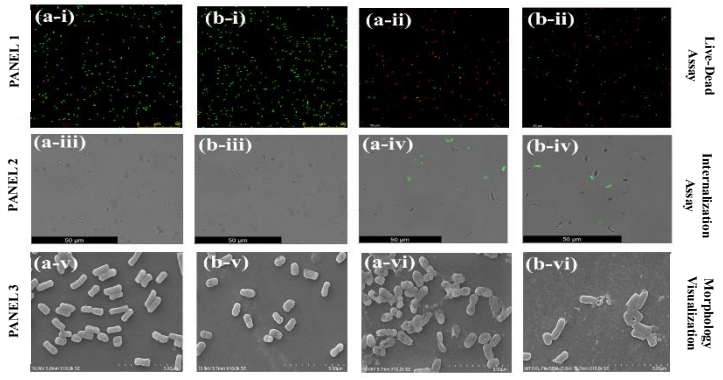

Fig. 4 PANEL 1: LIVE/DEAD BacLight assay of *B. subtilis (a)* and *E. coli (b)*: (i) untreated, and (ii) treated with 250 µg/mL ZnONP MPE; PANEL 2: Confocal imaging of FITC-conjugated ZnONP MPE and its sub-cellular localization: (iii) untreated, (iv) treated with FITC-conjugated ZnONP MPE (100 µg/mL), respectively; PANEL 3: Visualization of bacteria morphology using SEM, (v) untreated cells, (vi) treated with ZnONP MPE (100 µg/mL) showing membrane rupture, deformation, clumping and leakage of cytoplasmic content.

ACKNOWLEDGEMENT: **The author wish to thank the Department of Chemical Engineering, Department of Biomedical engineering and Department of Metallurgy and Materials, National Institute of Technology Rourkela, India, for providing TEM, Confocal Microscopy and XRD facilities.**

Table 1. List of representative phytochemicals obtained from GC-MS analysis of ZnONP MPE corona.

Sl. No.	RT	Compound Name	Chemical Structure	Biological Activity
1	5.56	3,3',7,8-Tetramethoxyflavone (1017083-18-9)		Antibacterial Anticancer
2	6.52	Demecolcine (477-30-5)		Anti-inflammation Anticancer
3	9.70	Octadecanoic acid (56599-88-3)		Antibacterial Anticancer
4	10.63	Gibberellic acid (GA3 ME) (77-06-5)		Antibacterial Anticancer
5	30.10	Bithionol (97-18-7)		Anticancer Antibacterial Anthelmintic Algaecide

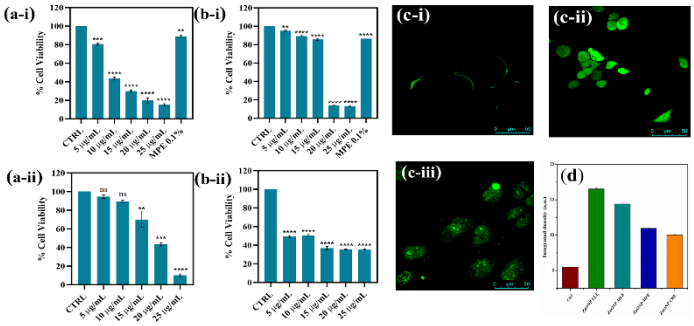

Fig. 5. ROS mediated cell cytotoxicity in (a) A549 and (b) HaCaT cells upon treatment with (i) ZnONP MPE and (ii) ZnONP CHE; using Alamar Blue; (c) Confocal imaging of DCFH-DA-mediated ROS detection in A549 cells (c-i) control (untreated), (c-ii-iii) upon treatment with ZnONP MPE and ZnONP CHE, respectively, and (d) integrated density plot.

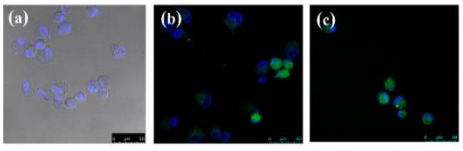

Fig. 6 Confocal imaging of FITC-conjugated ZnONP and its sub-cellular localization in A549 cells: (a) control (untreated) cells, and (b, c) cells treated with ZnONP MPE and ZnONP CHE, respectively (Scale bar 25 µm).

INFERENCE

1. Biosynthesis of ZnONP was achieved using *Eucalyptus globulus, Mangifera indica,* and *Tagetes erecta* leaves/petal extract owning to its reducing and stabilizing potential and further characterized.

2. The corona of biosynthesized ZnONPs were preliminary characterized by FT-IR and further the chemical composition of the corona was deciphered using GC-MS analysis.

3. Enhanced antimicrobial and cytotoxic propensity of biosynthesized ZnONP MPE compared to ZnONP CHE was attributed to the phytochemicals present in the Phyto-corona.

4. The mechanism behind biological activity was found to be co-localisation leading to oxidative stress mediated DNA-damage in cells resulting in reduced viability.

5. To this end, therapeutic potential of biosynthesized ZnONP could help to opt for alternative antibiotics against other pathogenic bacteria and base for development of anticancer agents.