Eco-friendly one pot synthesis of zinc oxide nanoparticles using catkin extract of *Piper longum*: *In vitro* antibacterial, antioxidant and antibiofilm potential against multi drug resistant enteroaggregative *E.coli*

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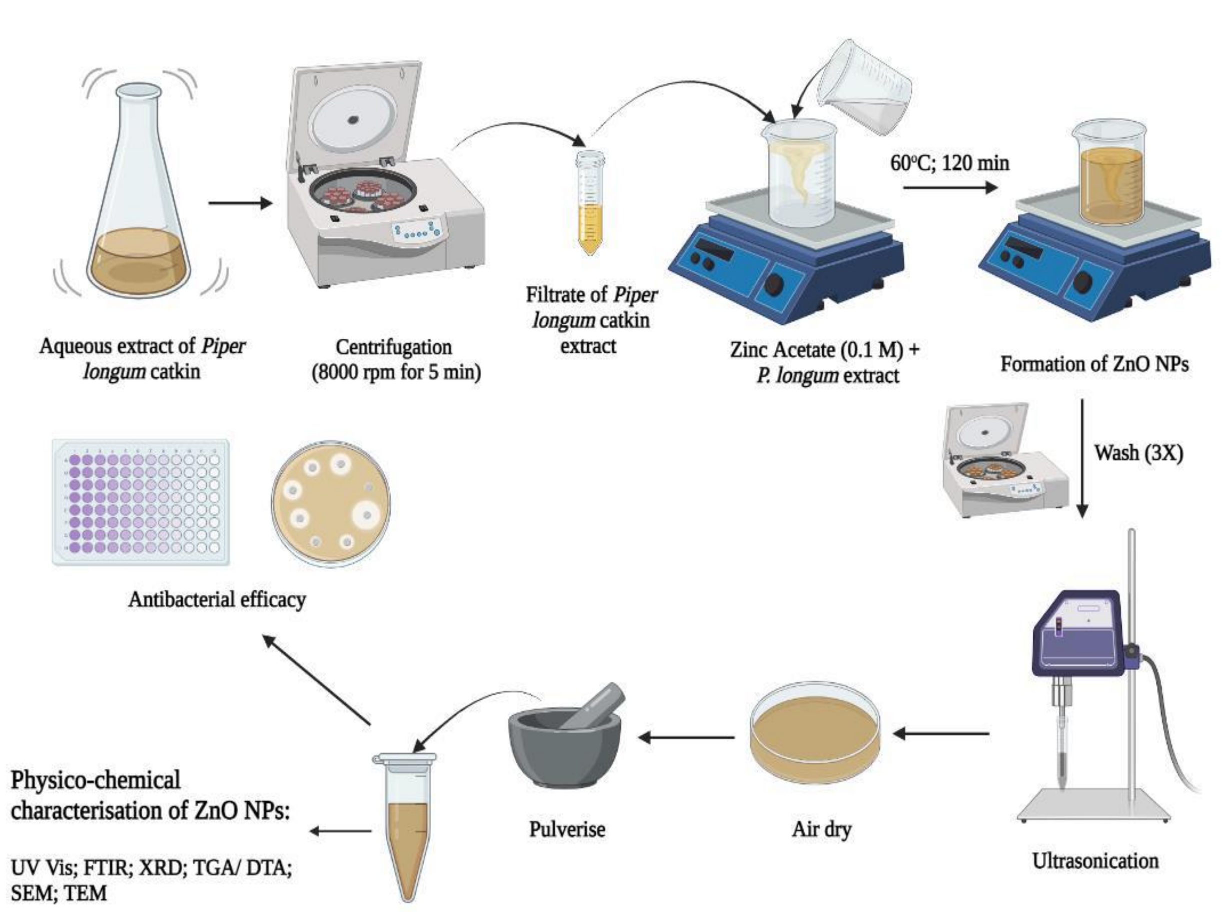
INTRODUCTION

- **Antimicrobial resistance poses a major threat to public health globally.**
- **Of late, an unusual emergence of drug resistance among EAEC strains has been recognized worldwide; hence the AMR research paradigm should be shifted towards novel alternative tactics.**
- **Recently, nanotechnology employing ZnO NPs have revealed great attention due to their unique physicochemical characteristics.**
- **The green route of NP synthesis has replaced the conventional physical and chemical methods, as they produce unintended effects such as potential health hazards and environmental pollutions.**
- **The present study has been envisaged to attempt the green synthesis of ZnO NPs using *P.longum* catkin extract and further evaluate its *in vitro* antimicrobial, antioxidant and antibiofilm activity against MDR-EAEC strains.**

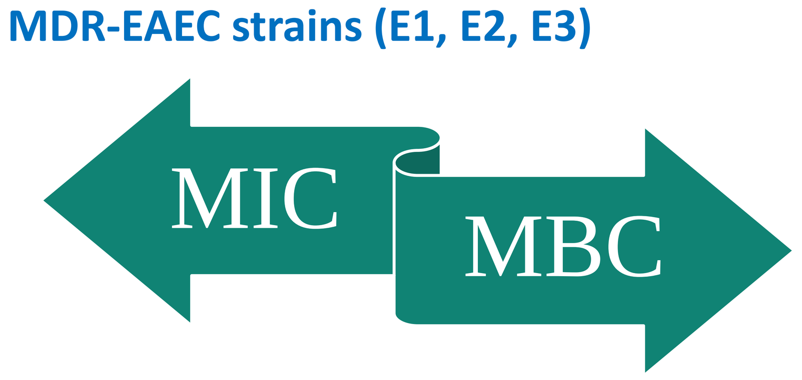
MATERIALS AND METHODS

1. Green synthesis and characterisation of ZnO NPs

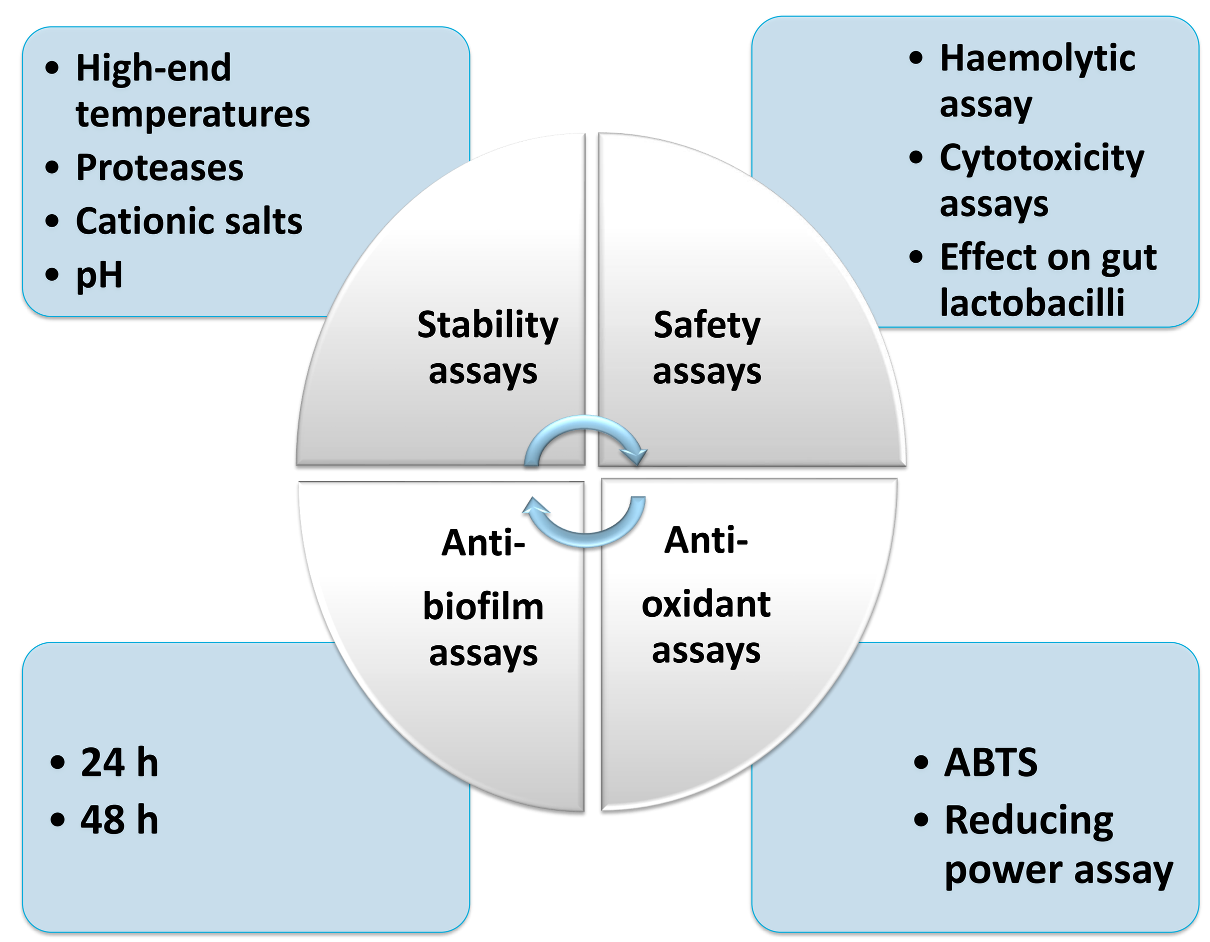


(Unni *et al*., 2022)

2. *In vitro* antibacterial activity of green synthesised ZnO NPs against MDR-EAEC strains (E1, E2, E3)



3. *In vitro* assays



RESULTS

1. Green synthesis and characterisation of of ZnO NPs

- **The catkin extract of *P. longum* reduced the aqueous solution of 0.10 M Zinc acetate dihydrate (at a ratio of 1:4) to ZnO NPs at 60 ◦C under constant stirring for 2 h.**
- **The appearance of a brown-colored precipitate at the bottom of the beaker indicated the formation of ZnO NPs**

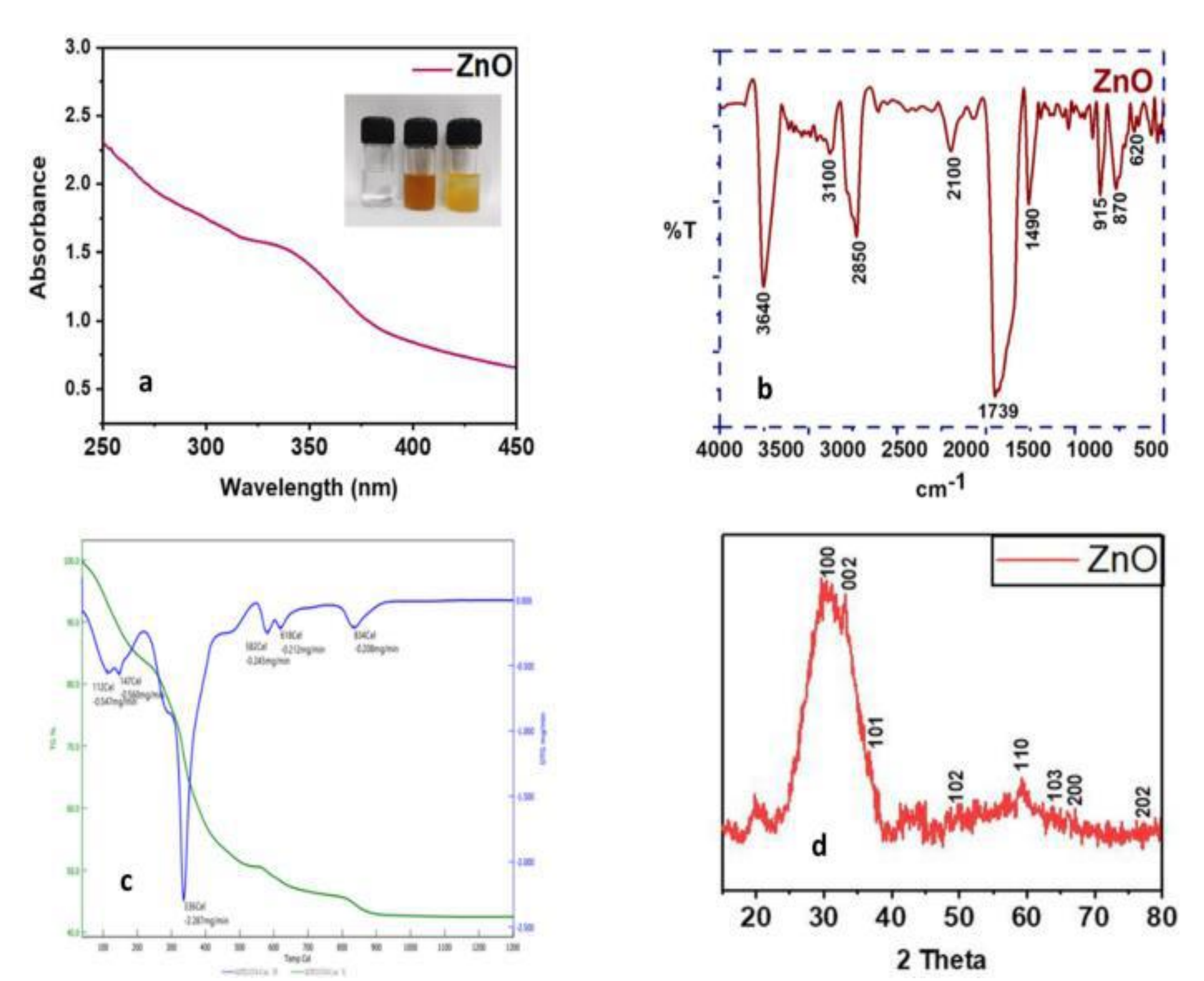


Figure 1. Physicochemical characterisation of Green synthesised ZnO NPs a) UV- Vis spectroscopy b) FTIR analysis c) TGA-DTA analysis d) P-XRD plot

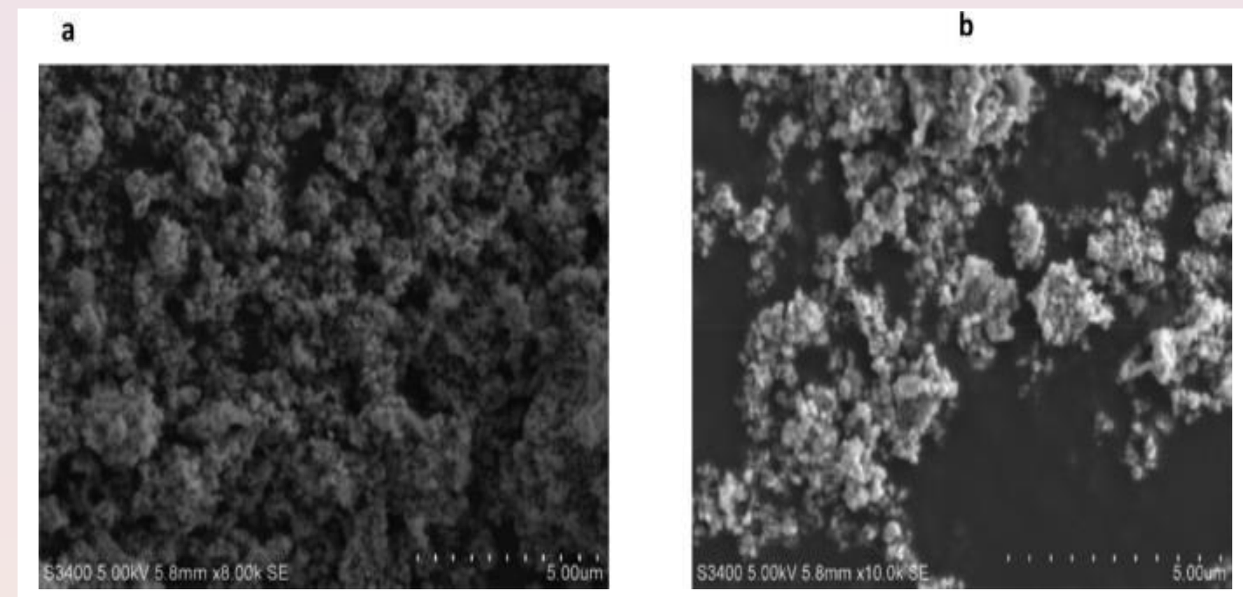


Figure 2. SEM imaging of green synthesized ZnO NPs. Scanning electron micrographs (a; b) exhibiting hexagonal and cubic shape of green synthesized ZnO NPs

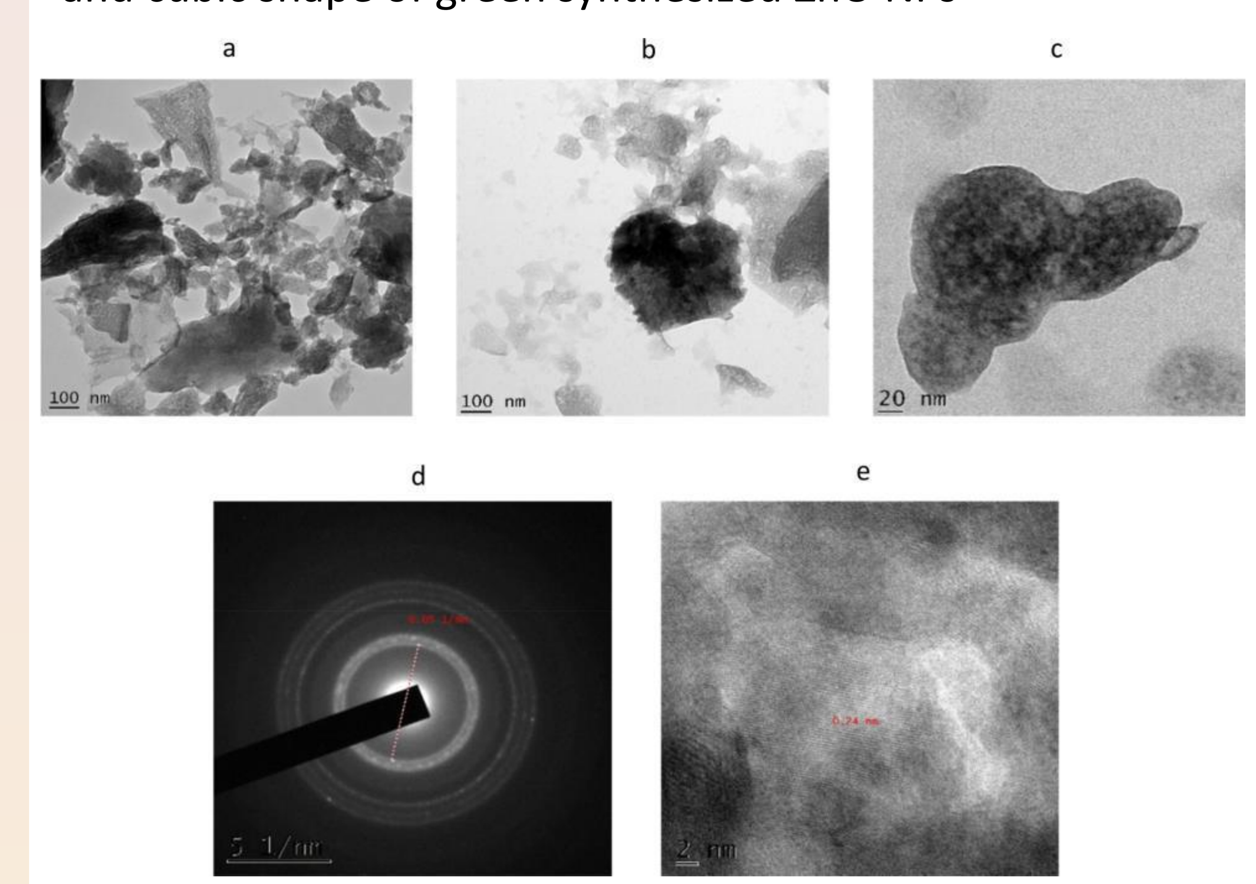


Figure 3. TEM imaging of green synthesized ZnO NPs

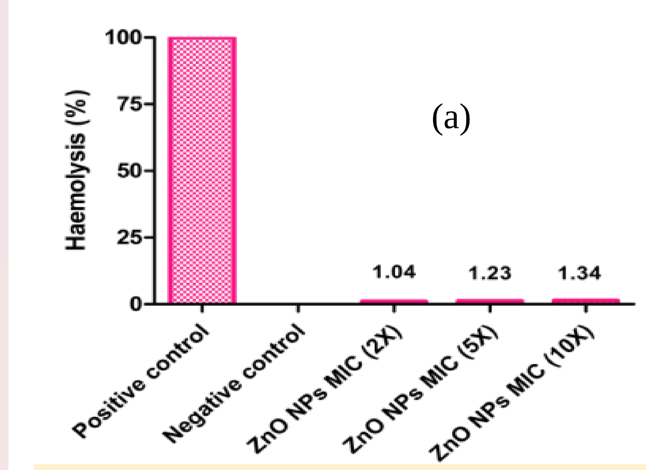
2. *In vitro* antibacterial activity of green synthesised ZnO NPs against MDR-EAEC strains (E1, E2, E3)

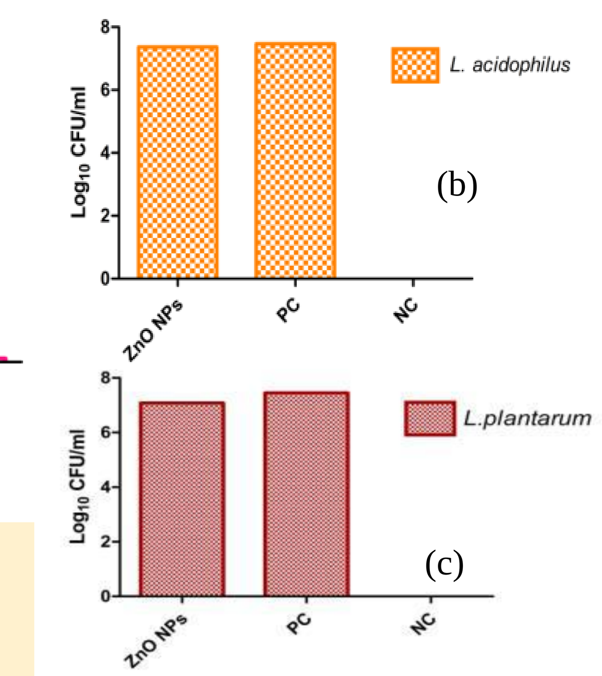
ISOLATES		MIC/MBC (µg/mL)
EAEC	E1	125/250
	E2	125/250
	E3	125/250

Table 1. MIC and MBC values of green synthesised ZnO NPs against MDR-EAEC isolates

- **The green synthesised ZnO NPs were found to be variably stable to high end temperatures (70 °C and 90 °C)**
- **MIC values of green synthesised ZnO NPs were halved on exposure to protease enzymes, and MBC values remained constant throughout the incubation period.**
- **The ZnO NPs maintained their antibacterial activity irrespective of the cationic salts**
- **The green synthesised ZnO NPs tested were found to be stable at different pH, as they retained their antimicrobial activity (MIC and MBC values)**

3. *In vitro* safety assays





❖ No cytopathic effect observed on HEK cell lines at 1X, 16 5X and 10X MIC levels.

Figure 4. (a) *In vitro* haemolytic activity of green synthesized ZnO NPs on poultry RBCs **(b)** *In vitro* efficacy of green synthesized ZnO NPs on *L. acidophilus* MTCC 10307 **(c)** *In vitro* efficacy of green synthesized ZnO NPs on *L. plantarum* MTCC 5690 ; PC: Positive control, NC: Negative control .

4. *In vitro* antioxidant assays

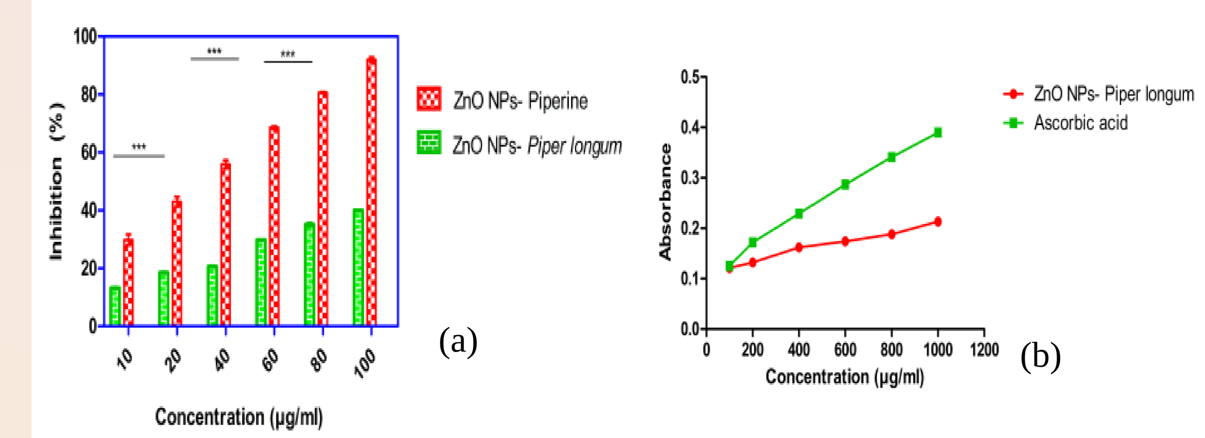


Figure 5. *In vitro* antioxidant activity of green synthesized ZnO NPs **(a)** ABTS assay **(b)** Reducing power assay

5. *In vitro* antibiofilm activity of ZnO NPs

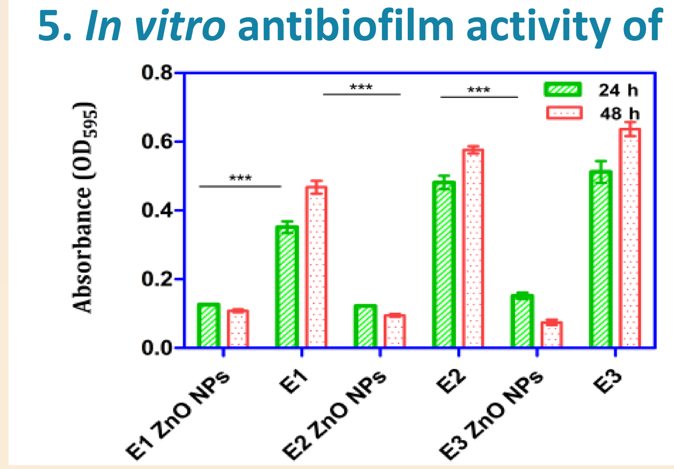


Figure 6. Inhibition of MDR-EAEC biofilm at 24 h and 48 h when treated with green synthesized 7 ZnO NPs

CONCLUSION

- **We successfully synthesised ZnO NPs employing the aqueous extract of *Piper longum* catkin.**
- **The *in vitro* assays revealed that the synthesised ZnO NPS possess excellent antibacterial and antibiofilm activity against MDR- EAEC strains with antioxidant potential.**

REFERENCES

Unni, V.; Abishad, P.; Prasastha Ram, V.; Niveditha, P.; Yasur, J.; John, L.; Prejit, N.; Juliet, S.; Latha, C.; Vergis, J.; Kurkure, 4 N.V. Green synthesis, and characterization of zinc oxide nanoparticles using Piper longum catkin extract and its in vitro 5 antimicrobial activity against multi-drug-resistant non-typhoidal Salmonella spp. *Inorg. NanoMet. Chem.* **2022**

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