Antioxidant, antibacterial and antibiofilm potential of green synthesized silver-zinc oxide nanocomposites from *Curcuma longa* extract against multi-drug resistant enteroaggregative *E.coli*

Presented by,
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Background of the study…

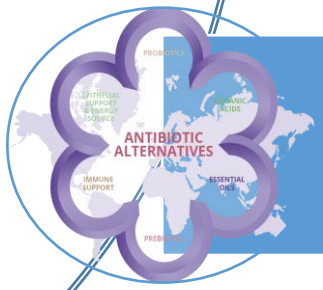
- Antimicrobial resistance (AMR) is a global health priority
- Antibiotic resistant infections (3 million) and 35,000 deaths in U.S and 33,000 deaths per year in Europe
- Food-borne illness: major public health challenge worldwide
- Food-borne illness in 2010 - 600 million cases
- Enteroaggregative *E. coli* (EAEC): major emerging enteric pathogen with increased detection in diarrheal episodes around the world
- Dissemination of MDR-EAEC pathotypes has been linked to high morbidity, case fatality and higher healthcare expenses

(Desai *et al.*, 2022; Ram *et al.*, 2022; Mohammed *et al.*, 2022)

Critical gaps identified…

Escalating trends in the emergence of drug resistant food-borne pathogens

Dwindling antibiotic discovery pipeline



Lack of suitable antibiotic alternative therapies

Study Objectives

Green synthesis & characterization of Ag/ZnO NCs using *C. longa* extract

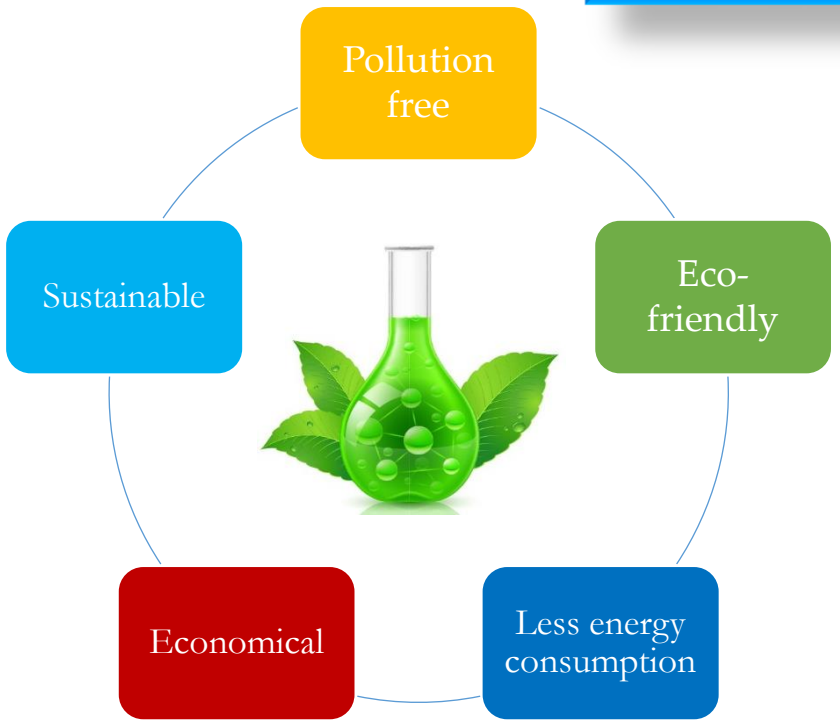
Evaluation of its antioxidant, antibacterial & antibiofilm potential against MDR-EAEC isolates

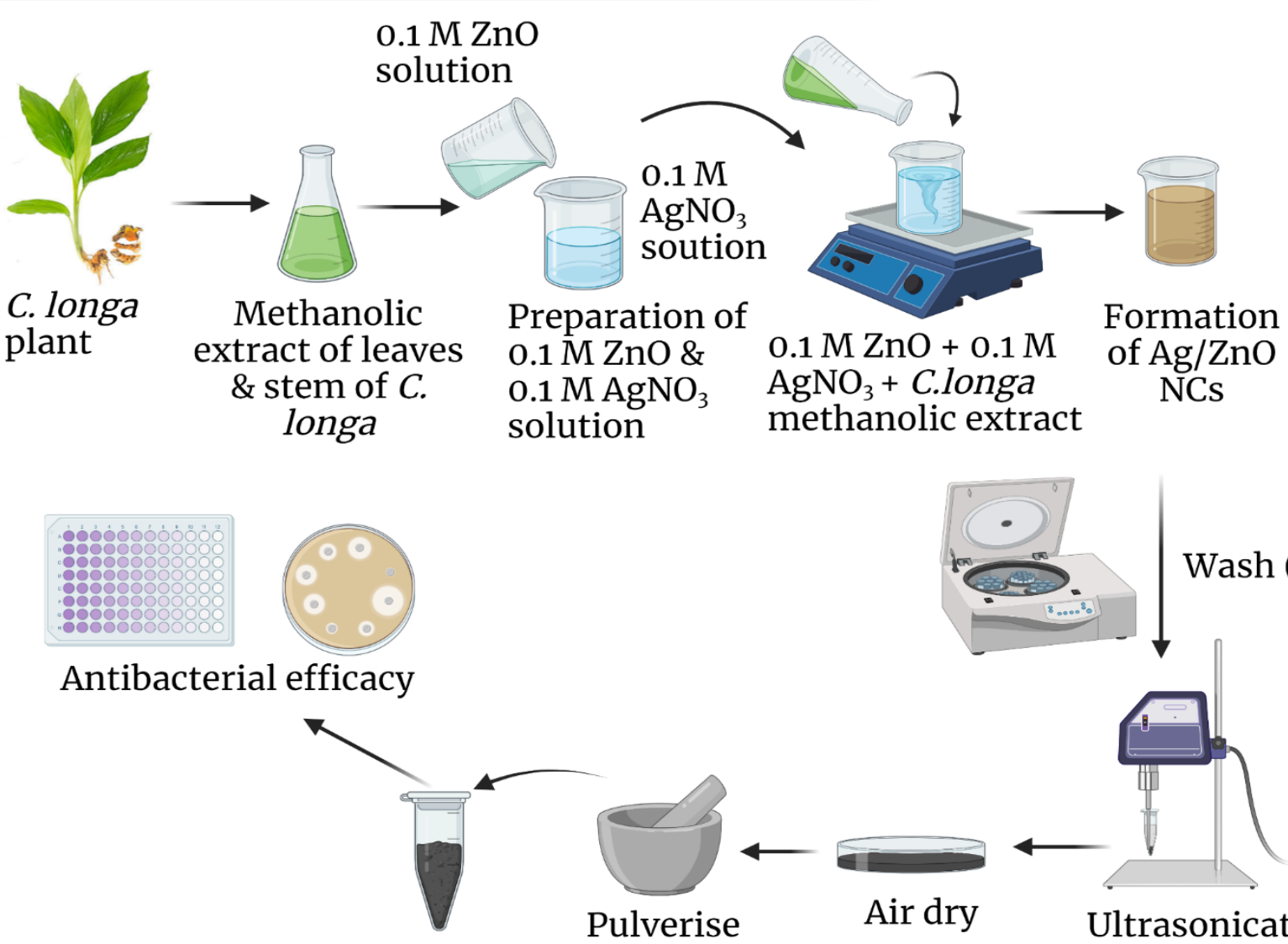
Re-validation of test strains

- **MDR-EAEC** isolates (n= 3) maintained in the Zoonoses laboratory of Department of Veterinary Public Health, CVAS, Pookode (from the ICAR-NASF project repository)

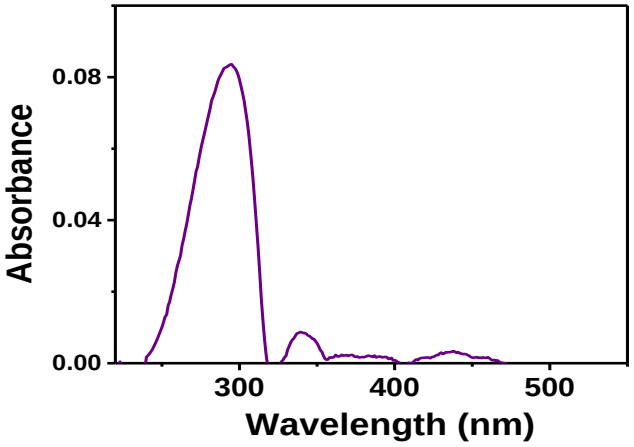
- **Revived and re-validated** using:
  - **Biochemical tests : IMViC test + + - - pattern (Cheesbrough, 1985)**
  - **PCR assay (Vijay *et al*., 2015)**
  - **Antibiotic susceptibility testing (CLSI, 2019)**

Green synthesis of Ag/ZnO NCs

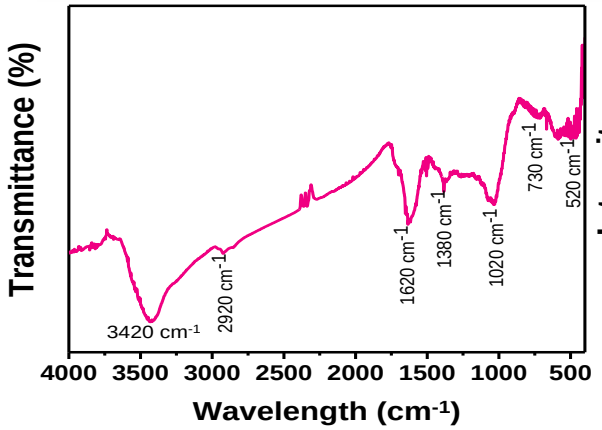




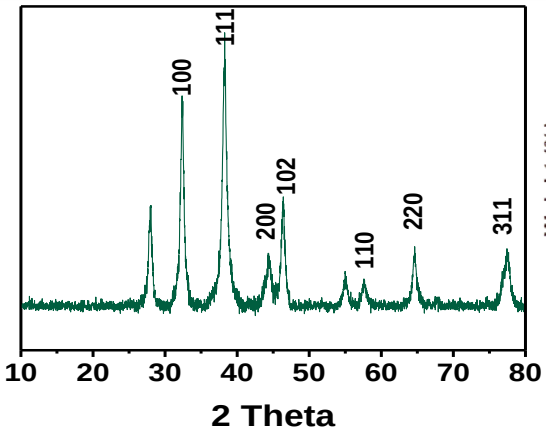
Characterization of Ag/ZnO NCs



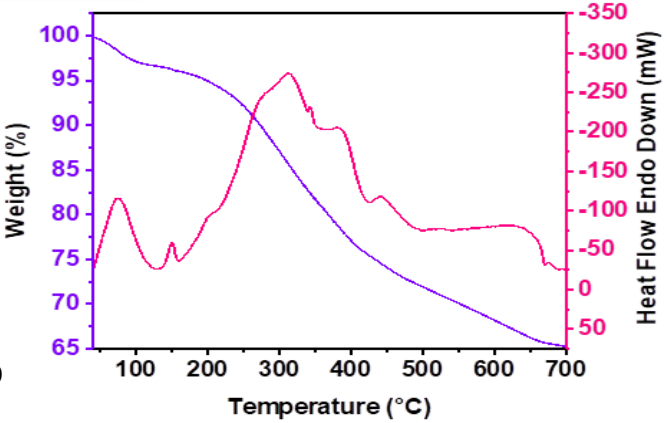
UV Vis



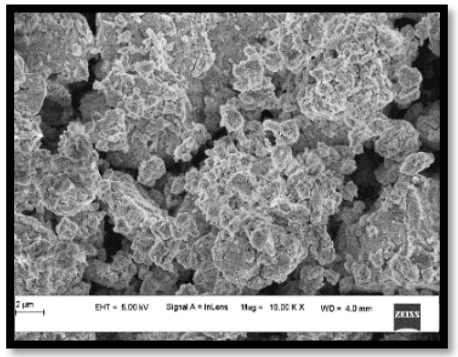
FTIR

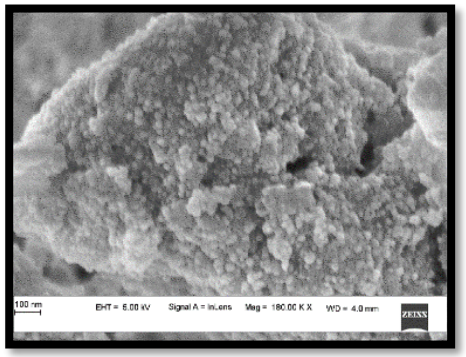


PXRD

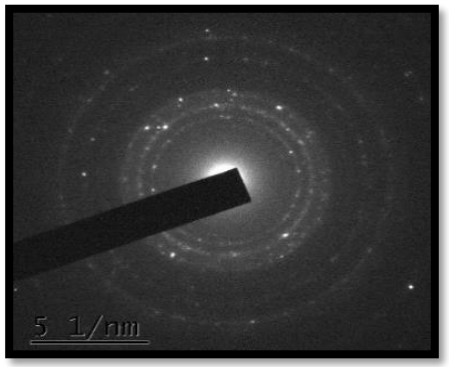


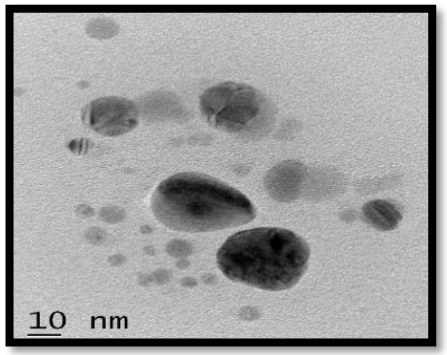
TGA/DTA

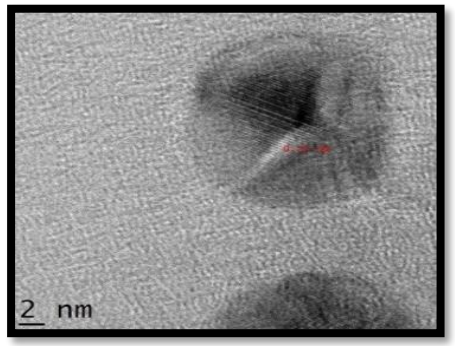




FE-SEM





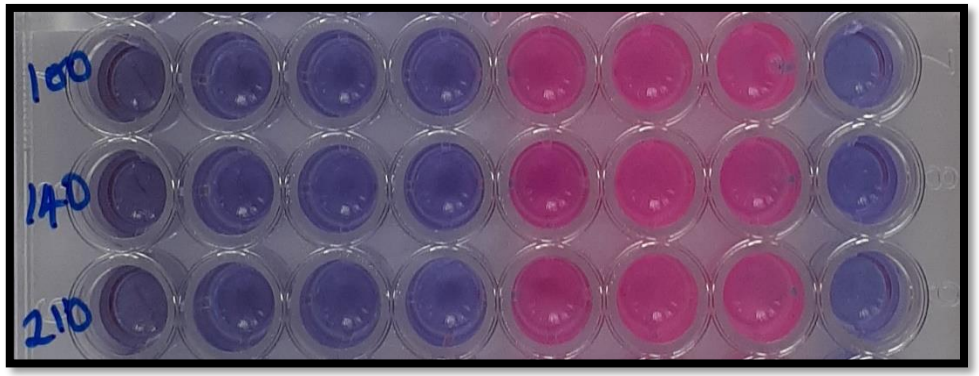


TEM

(Kyomuhimbo *et al*., 2019; Zare *et al*., 2019; Shreema *et al*., 2021; Berhanu *et al*., 2022 Pavithra & Jessie, 2022)

Assessment of antibacterial potential of Ag/ZnO NCs

- Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of Ag/ZnO NCs against MDR-EAEC isolates was evaluated.

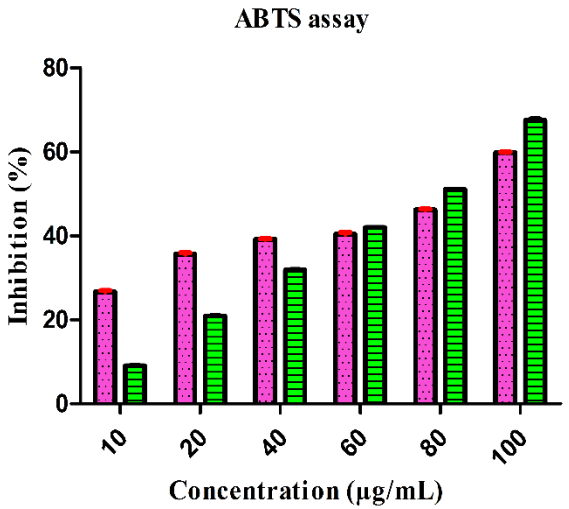


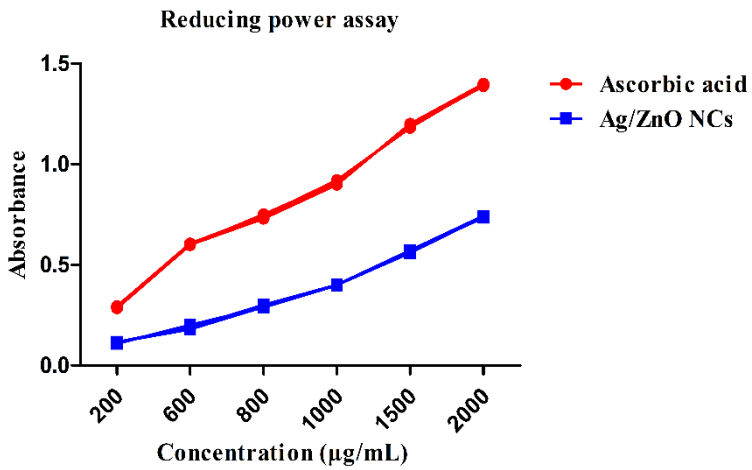
Sl No.	Isolate ID	MIC (µg/mL)	MBC (µg/mL)
1.	E1	31.25	62.5
2.	E2	31.25	125
3.	E3	31.25	125

MBC values of Ag/ZnO NCs was found to have a two to three-fold increase than their MIC values

In vitro antioxidant activity of Ag/ZnO NCs

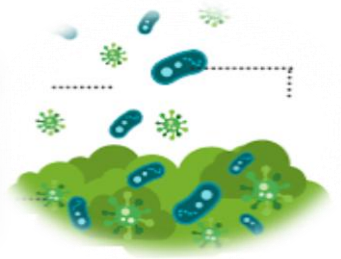
- *C. longa* has been found to harbor high concentrations of polyphenols, flavonoids, tannins, and ascorbic acid.
- ABTS and reducing power assays
- Observed dose-dependent increase in antioxidant potential both in ABTS and reducing power assays;
- Antioxidant activity of green synthesised Ag/ZnO NCs was lower than compared to the ascorbic acid standard.
- Combination of silver and zinc oxide together forming plant-based nanomaterials have been found to exhibit an increased antioxidant capacity and anti-proliferative action, which eliminates free radicals



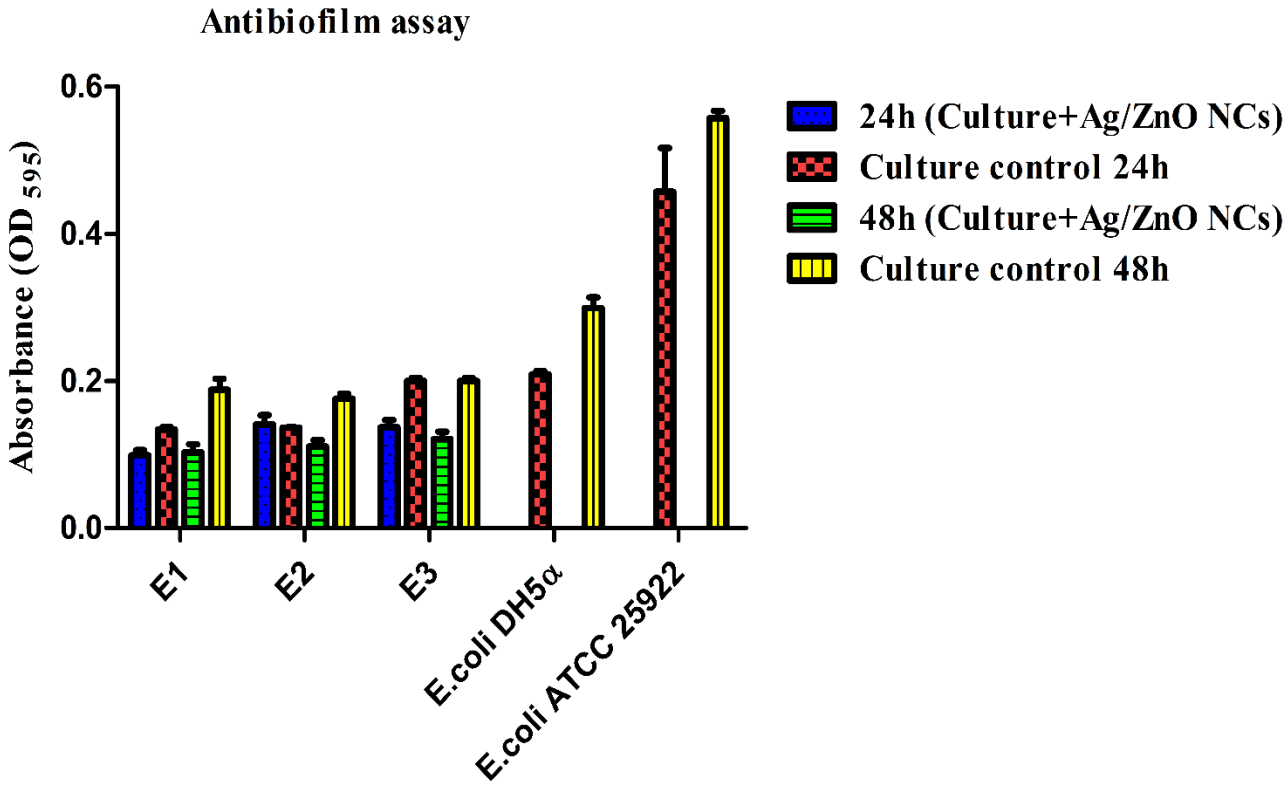


(Ehsan *et al.*, 2022; Memarzia *et al.*, 2022)

In vitro anti-biofilm activity of Ag/ZnO NCs



- Evaluated *in vitro* antibiofilm efficacy of Ag/ZnO NCs against MDR-EAEC isolates by employing crystal violet staining assay at 24 and 48 h
- Highly significant (***P*< 0.001**) biofilm inhibition was exhibited by Ag/ZnO NCs after 24 h and 48 h
- The antibiofilm activity of green synthesised Ag/ZnO NCs might be due to the increased ROS generation along with the suppression of exopolysaccharides of MDR-EAEC, which are a crucial component of bacterial biofilms



(Akhil *et al.*, 2016; Abishad *et al*, 2022)

Summary

C. longa plant

C. longa extract

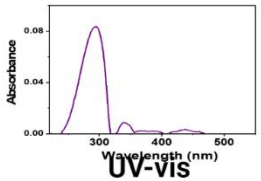
Green synthesis

0.1 M $AgNO_3$ +
0.1 M ZnO_3
solution

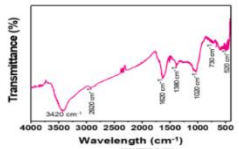
Reduction/
Stablilization

Capped Ag/ZnO NCs

Characterisation



UV-VIS



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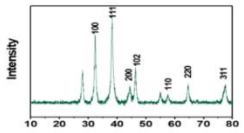
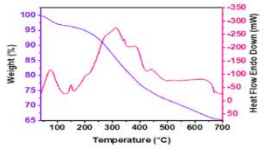


PXRD



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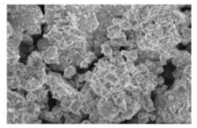

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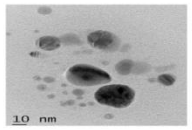
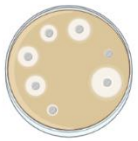


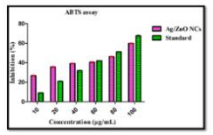
TEM

Antibacterial/
antioxidant/
antibiofilm assays

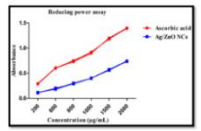


ABST

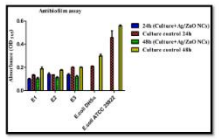
MIC/MBC



ABTS



Reducing power



Antibiofilm

Antibacterial

Ag/ZnO NCs

Antioxidant

Antibiofilm

Acknowledgement

- Dean, CVAS, Pookode
- Advisory Committee Members
- ICAR- NASF Funding; Investigators & Project Staff of NASF project
- KVASU funding
- Staff & Students, Department of VPH
- STIC/ SAIF, CUSAT


Thank
you