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## SYNTHESIS AND CHARACTERIZATION OF ZNO NANOPARTICLES USING LEAF EXTRACT OF RHIZOPHORA MUCRONATA AND EVALUATION OF THEIR ANTIMICROBIAL EFFICACY

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### ABSTRACT

Nanotechnology is a developing interdisciplinary field of research interspersing material science, bionanoscience, and technology. Nanoparticles are studied extensively for their specific catalytic, magnetic, electronic, optical, antimicrobial, wound healing and anti-inflammatory properties. The main aim of the present study was to synthesize ZnO nanoparticles using the aqueous extract of Rhizophora mucronata leaves and to evaluate their antimicrobial efficacy against some selected microbes. The synthesis Zn nanoparticles were characterized by UV/VIS spectroscopy, particle size analyzer and Scanning Electron Microscopy. The synthesized Zn nanoparticles showed significant antimicrobial activity against Gram-positive and Gram-negative bacteria as well as against a fungal strain. A clear zone inhibition was measured; 40.05mm±0.137 for *Staphylococcus aureus*, and 36.15 mm ± 0.304 for *Escherichia coli* that comparably better result than a standard antibiotic. Thus from this study, it can be concluded that rhizophora mucronata extracts can be effectively used for synthesizing ZnO nanoparticles. This study also suggests that green synthesized Zn nanoparticles can be used as an alternative to existing antimicrobial agents.

**Key Words:** Green-synthesis, Zinc nanoparticles, Rhizophora mucronata, Scanning Electron Microscopy.

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### INTRODUCTION

Nanotechnology is a developing interdisciplinary field of research interspersing material science, bionanoscience, and technology<sup>1</sup>. Metal nanoparticles are known to exhibit various functions which are otherwise not observed in bulk phases. These nanoparticles are studied extensively for their specific catalytic, magnetic, electronic, optical and antimicrobial wound healing and anti-inflammatory properties<sup>2</sup>. Plant-mediated synthesis of nanoparticles are preferred over chemical synthesis due to its simplicity, eco-friendliness and extensive antimicrobial activity, non-toxic by-products and large-scale synthesis Zinc oxide nanoparticles are known to be one of the multifunctional inorganic nanoparticles with effective antibacterial activity. Antibacterial and antifungal activities of ZnO nanoparticles are observed even at very lower concentrations and also the antifungal activity does not affect soil fertility compared to the conventional antifungal agents<sup>3</sup>.



**Rhizophora mucronata**

### AIM

The main aim of the present study was to synthesize ZnO nanoparticles using the aqueous extract of rhizophora mucronata leaves and to evaluate their antimicrobial efficacy against some selected microbes.

### MATERIALS AND METHODS

#### PREPARATION OF AQUEOUS LEAF EXTRACT

Fresh leaves of Rhizophora mucronata were collected and washed in running tap water followed by double distilled water. The aqueous extract of the sample was prepared by boiling the freshly collected leaves (10g), with 100 ml of distilled water, at 60°C for about 20 minutes, until the colour of the aqueous solution changes from watery to light yellow. Then the extract was cooled to room temperature and filtered using filter paper and used for further experiments<sup>4</sup>.

#### PREPARATION OF ZINC NANOPARTICLES

For the synthesis of nanoparticle, Zinc acetate was dissolved in the extract and the solution was stirred constantly using magnetic stirrer. After complete dissolution of the mixture, the solution was kept under vigorous stirring for 5-6 h at about 150°C. The solution was then cooled at room temperature and the supernatant was discarded. The pale white solid product obtained was centrifuged twice at 4500 rpm for 15 min after thorough washing and dried at 80°C for 7-8 hours<sup>2,3</sup>.

## CHARACTERIZATION OF ZN NANOPARTICLES

The synthesis of Zn nanoparticle was evaluated by taking the absorbance in the range of 300-500 nm using the UV/VIS spectrophotometer<sup>5</sup>. The particle size of synthesized nanoparticles was obtained by particle size analyzer. The dried Zn nanoparticles were also subjected to SEM analysis for characterization<sup>6</sup>.

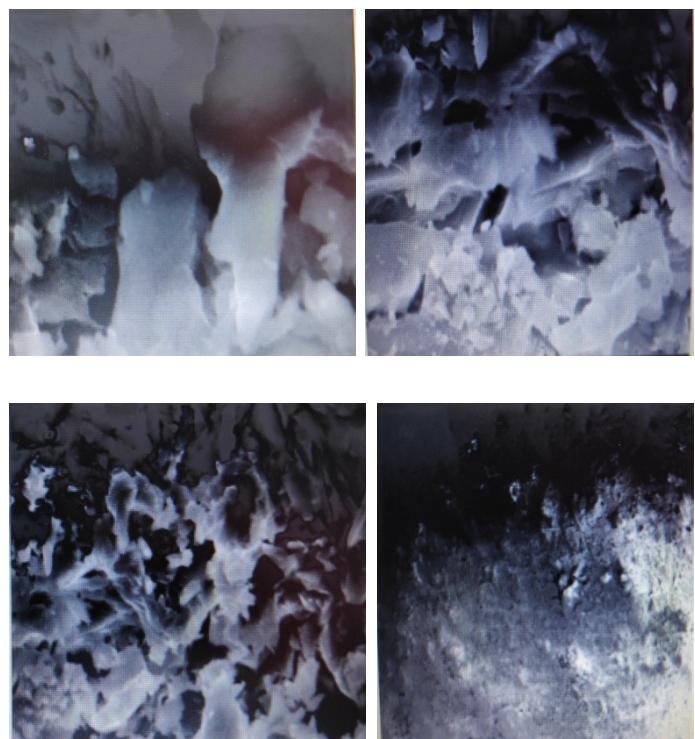
## TEST ORGANISMS

Microorganisms were procured from Microbial Type Culture Collection and Gene bank<sup>14</sup>. The antimicrobial activity was carried out against both Gram positive and Gram negative bacteria viz. *Staphylococcus aureus* (Gram positive, MTCC 87), *Bacillus cereus* (Gram positive, MTCC 1305), *Escherichia coli* (Gram negative, MTCC 10312). The bacterial strains were maintained on Nutrient Agar and fungi on Potato Dextrose Agar. The microbes were sub cultured at an interval of one month<sup>11</sup>.

Microbial strain	Ag. extract of Rhizophora mucronata leaves (100 mg/mL)	ZnO NPs of Rhizophora mucronata leave (100mg/mL)±SD	Gentamicin (std.antimicrobia l) (100 mg/mL)	MIC± SD
<i>Staphylococcus aureus</i>	No zone	40.05 mm ± 0.137	25 mm	9.765 µg ± 0.00
<i>Bacillus cereus</i>	No zone	36.15 mm ± 0.304	26 mm	19.531 µg ± 0.00
<i>Escherichia coli</i>	No zone	40.10 mm ± 0.05	-	5000 µg ± 0.00

## ANTIMICROBIAL ACTIVITY

The microbicidal activity of synthesized NPs (100mg/mL) was measured against pathogenic strains and found 40.05 mm ± 0.137 zone of inhibition against *S. aureus*, 36.15 mm ± 0.304 for *Bacillus cereus* and 40.10 mm ± 0.050 for *Escherichia coli*. These results documented better antibacterial activity of produced ZnO NPs than the standard antibiotic; Gentamicin (100 mg/mL) that showed zone of inhibition 25 mm against *S. aureus* and 26 mm for *E. coli*. The biocidal action of ZnO NPs revealed their mechanism that involve the disruption of cell membrane with the action of Zn<sup>+2</sup> on its surface that ultimately cause the death of microbes<sup>15,16</sup>. Further standard protocols were followed to measure MIC for the above mentioned strains and observed concentrations for *S. aureus* was 9.765 µg ± 0.00, *E. coli* was 19.531 µg ± 0.00 and *A. niger* was 5000 µg ± 0.00. This minimum concentration of ZnO NPs required for antimicrobial activity as given in Table 1 depicted the cost effectiveness of initially green synthesized ZnO NPs (91 g/100 mL) and its application in antimicrobial activity. Some researchers also studied mode of inhibitory action of ZnO NPs for microbial growth, as Mishra et al documented cell damage caused by these NPs with the presence of protein and nucleic acid of nutrient agar, Femi, Prabha, Sudha, Devibala, and Jerald<sup>9</sup> demonstrated the surface binding of NPs with thiol group of glycoproteins on the cell wall of microbes and decreases the permeability with subsequently lysis of cell to inhibit cell growth. Gunalana also explained the damage of cell membrane with leakage of protein, minerals and genetic material by the interaction of ZnO NPs with microbial strains<sup>12</sup>.



SEM images of Rhizophora mucronata mediated ZnO nanosheets: (a) surface image of nanosheets (b-d) numerous thinner sheets accumulated to form a nanosheet networks.

## GRAPHS

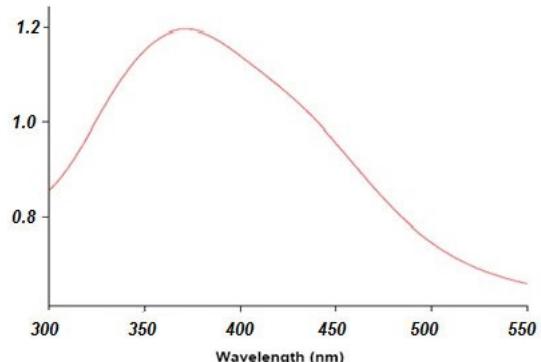


Fig.1. UV-vis spectra of ZnO nanoparticles solution

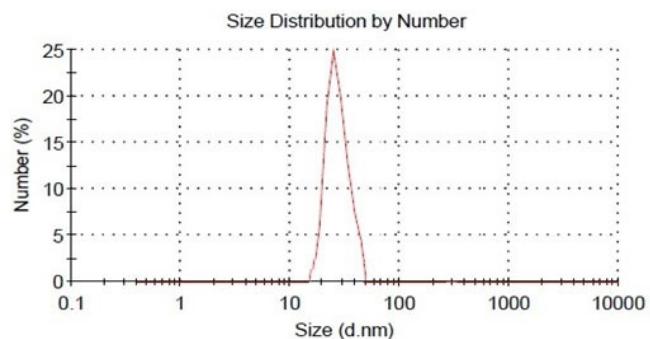
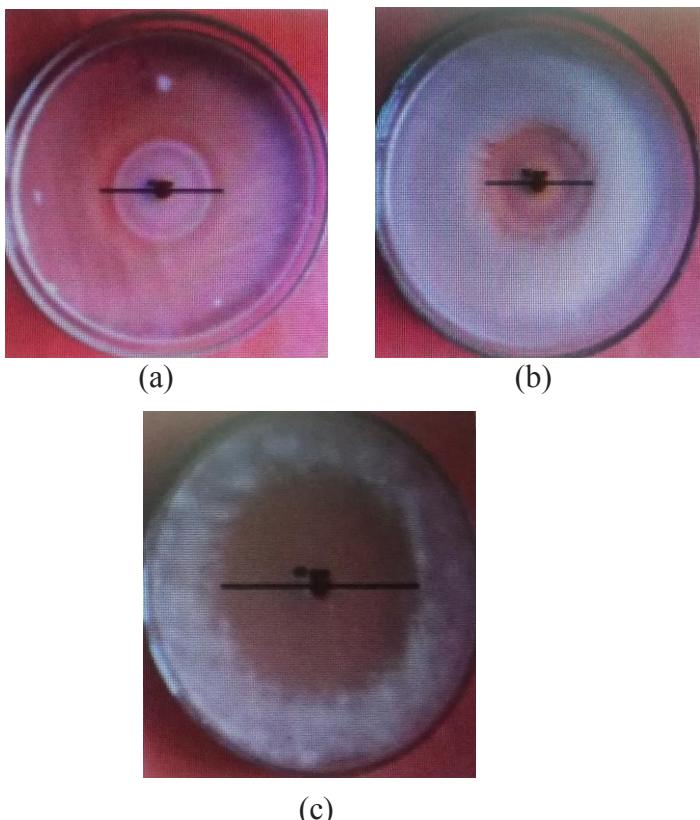


Fig.2. The size distribution of ZnO nanoparticles by number

This reported no zone of inhibition for *E. coli*,  $5.300 \pm 0.570$  for *S. aureus* against *C. sinensis* ZnO NPs. Antimicrobial activity of black tea extract was performed by Vasudeo and Sonika for different pathogen bacterial strains and found zone of inhibition  $14 \pm 2$  for both *E. coli* and *S. aureus*. The MIC calculated for chloroform tea extract was  $25 \mu\text{g/mL}$ . the antibacterial activity of *rhizophora mucronata* against some famous pathogenic strains and reported the significant biocidal property of seeds<sup>8</sup>. During this study *C. sinensis* NPs depicted better antimicrobial activity than other researchers' findings and with low MIC. The high zone of inhibition with MIC was in agreement with documented literatures that ZnO NPs rupture lipid bilayer of bacterial and fungal cell wall with the ultimate death of microbes<sup>4</sup>.



Zone of inhibition (mm) measured for antimicrobial activity of *rhizophora mucronata* ZnO NPs evaluated on (a) *Staphylococcus aureus* (b) *Escherichia. coli* (c) *Bacillus cereus*.

## RESULT AND DISCUSSION

When the leaf extract incubated with Zn nitrate, the colour changed from pale yellow to pale brown after one hour of incubation at room temperature. The change in colour indicates the formation of nanoparticles. UV spectroscopy analysis showed maximum absorption at about 330 nm. The size of the particles was determined by particles size analyzer. The sizes of particle in diameter were found to be 853 nm. SEM characterizations of the synthesized Zn nanoparticles are which reveals much lesser diameter as compared to particle size analyzer. The nanoparticles were examined under various magnifications. SEM image has showed individual zinc particles as well as a number of aggregates. The synthesized Zn nanoparticles showed significant antimicrobial activity against Gram positive and Gram negative bacteria as well as against a fungal strain. The results are expressed as zone of inhibition (mm)  $\pm$  SD.

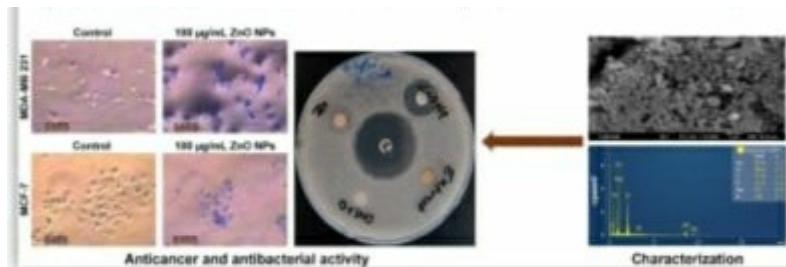
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*Rhizophora mucronata*



Zinc acetate



## CONCLUSION

Thus ZnO nanoparticles were synthesized by using *rhizophora mucronata* leaves extract that effectively inhibit microbial growth. Moreover during characterization UV-visible spectral peak at 350 nm confirmed the purity of ZnO NPs and FTIR results documented clearly the capping, reducing and stabilizing phytochemicals found in green tea. XRD diffractogram revealed characteristic peak of ZnO NPs with size range 30–40nm those coalesced to organize in nanosheets as depicted in SEM images.

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