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Synthesis of ZnO nanoparticles from *Alpinia purpurata* and their antimicrobial properties

Aswathi sreenivasan CV^{*}, Justi Jovitta C^{*}, S Suja

Department of Biochemistry, Dr.N.G.P.Arts and Science College, Kalapatti Road,Coimbatore-641 048

ABSTRACT

In this paper, ZnO nanoparticles have been synthesized from *Alpinia purpurata* with the use of surfactants under microwave condition. The size of the ZnO nanoparticles is about 50nm. So the study promising that the ZnO nanoparticles have antimicrobial activities. The Scanning Electron Microscope(SEM) was used to characterize the particle size and morphology.

Keyword: ZnO nanoparticles, Antimicrobial activity

**Corresponding author:*



INTRODUCTION

Nanoscience has been established recently as a new interdisciplinary science . It can be defined as a whole knowledge on fundamental properties of nanoscience objects. The prefix ‘ nano’ indicates one billionth or 10^{-9} units. The nature of this unit being determined by the word that follows .It is widely accepted of dimensions, rather than of any other unit of scientific measurements. It is widely agreed that Nanoparticles are clusters of atoms in the size range of 100nm[1]. Nanoparticles are defined as having at least one useful dimension less than 100nm. Properties of unique properties of nanoparticles stem from the vastly increased ratio of surface area to volume at this dimension .Due to their smaller size and large surface to volume ratio , exhibit interesting novel properties which include nonlinear optical behavior, increased mechanical strength , enhanced diffusivity , high specific heat, magnetic behavior and electric resistivity . There are wide range of scientific application of metal nanoparticles in the field of biotechnology , sensors , medical diagnostic , catalysis , high performance engineering material , magnetic recording media , optical devices , diagnostic , catalysis , DNA labeling and drug delivery[2].

Nanoparticles are of great scientific interest as they are effectively a bridge between bulk material and atomic or molecular structures . Size dependent properties are observed such as quantum confinement in semiconductor particles, surface Plasmon resonance in some metal particles and superparamagnetism in magnetic materials. The properties of materials change as their size approaches the nanoscale and as the percentage of atoms at the surface of a material becomes significant [3].

MATERIALS AND METHODS

EXPERIMENTAL

All chemical reagents such as $ZnSO_4 \cdot 7H_2O$, NaOH, HCl, ethyl acetate and acetone were of analytical grade (MERCK) and were used without further treatment.

Collection of plant samples

Alpinia purpurata was collected from “Freshcut” Agricultural farm Eranakulam , kerala.

Preparation of plant extract

Freshly collected rhizomes are used in this study. Primarily the rhizomes weighing 20g were washed and the cleaned rhizomes were dried in shade. Then it was powdered in to small fine particles and boiled for 30minute at $80^{\circ}C$. Then the rhizome extract were collected in separate conical flasks by standard filtration method.



Synthesis of a natural surfactant

Excess of an aqueous NaOH solution (pH-11) was poured into the crushed, dried and powdered plant material. The mixture was heated on a water bath with continuous stirring for 1h and then allowed to stand for 12h. The alkaline extract was filtered, acidified with 10ml aqueous HCl solution (PH-1), and allowed to stand for precipitation. The precipitate (containing natural surfactant and by products) was separated by filtration. The residue was washed with distilled water and pre extracted with ethyl acetate by refluxing for about 5-6 h. The pre extractant was distilled off and the residue was extracted with acetone. The acetone extract was allowed to stand overnight. The precipitate obtained was mixture of natural surfactant which was finally separated and dried.

Synthesis of Zinc oxide nanoparticles

Surfactant Assisted Synthesis: In each of the four reaction flasks 22ml aqueous solution containing $ZnSO_4 \cdot 7H_2O$ (1M) and natural surfactant (0.4M) was mixed with 12ml aqueous NaOH solution (4M). The resulting mixture in the four flasks was stirred vigorously under room temperature and then the respective reaction flask were exposed to reaction conditions by placing them in microwave oven (1min). The white precipitates were filtered, washed with distilled water and then dried at room temperature.

SEM analysis of Zinc oxide nanoparticles

SEM analysis was done using Hitachi S-4500 SEM machine. Thin film of sample were prepared on a carbon coated copper grid by just dropping a very small amount of sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid allowed to dry by putting it under a mercury lamp for 5minutes[6].

Antimicrobial activity

The Antimicrobial activity of isolated plant zinc oxide based nanoparticles pellets were tested by paper disc method[10]. The microorganism were collected from Department of Microbiology lab, Dr.N.G.P. Arts and Science College, Coimbatore. The antimicrobial activity of the synthesized ZnO nanoparticles evaluated using bacterium as per the colony count method.

Test organisms used

- i. *Staphylococcus aureus*
- ii. *Proteus vulgaris*
- iii. *Escherichia coli*
- iv. *Pseudomonas*
- v. *Klebsiella pneumonia*
- vi. *Aspergillus niger*
- vii. *Aspergillus flavus*



viii. *Fusarium*

ix. *Rhizopus*

Preparation of medium

- **For bacterial culture**

2.8g of nutrient agar was weighed correctly and dissolved in 100ml of sterile distilled water. P^H was adjusted to 7.2 and was autoclaved at 121⁰C for 15 minutes. 20ml of molten agar was poured in to the sterile petriplate and allowed to solidify.

- **For fungal culture**

3.9g of potato dextrose agar was weighed correctly and dissolved in 100ml of sterile distilled water. P^H was adjusted to 7.2 and was autoclaved at 121⁰C for 15 minutes. 20ml of molten agar was poured in to the sterile petriplate and allowed to solidify.

Disc diffusion assay

The disc of 4mm were prepared using a what-man No:1 filter paper. The discs were obtained by punching and putting in vials bottles and sterilizing in an oven at 150⁰C for 15min. The disc were impregnated with 10 μ l of concentrated crude extract and another disc were impregnated with 10 μ l synthesized zinc oxide nanoparticles .The disc were evaporated at 37⁰C for 24 h. Prepared discs containing the various fractions were carefully placed on the inoculated plates using a sterilized forceps in each case[6].The antibiotic tetracycline and penicillin was used as control .The plates were then turned upside down and incubated at 37⁰C for 24 h in an incubator. The results were taken by considering the zone of growth and inhibition of the bacteria and fungus by the test fractions. Antimicrobial activity was evaluated by measuring the diameter of the inhibition zone (IZ) around the disc.

RESULT AND DISCUSSION

Synthesis and characterization of nonmaterial have become an area of intense research over the last few years. Several material scientists have reported the preparation and antimicrobial activity of ZnO nanoparticles. Nanocrystalline ZnO particles have found tremendous application in the field of high sensitivity biomolecular detection and diagnostics[28], antimicrobials and catalysis and micro-electronics. However, there is still need for economic, commercially viable as well environmentally clean synthesis route to synthesis ZnO nanoparticles.

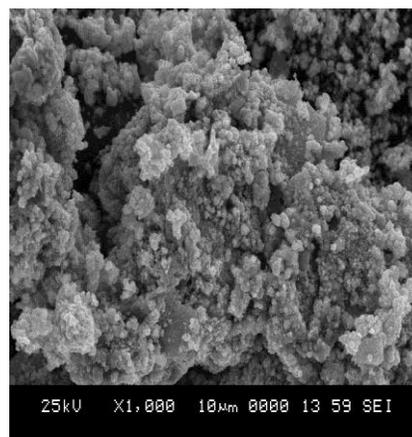
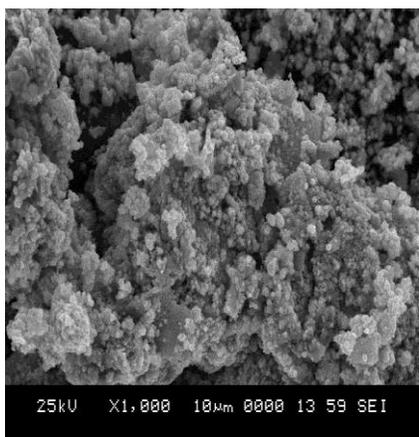
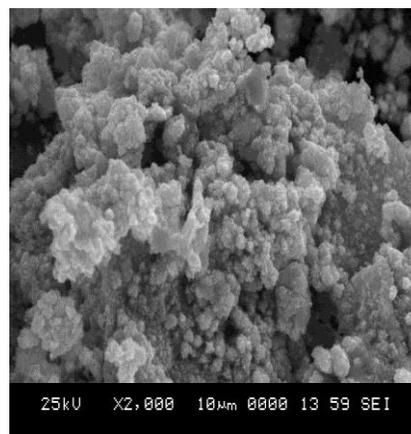
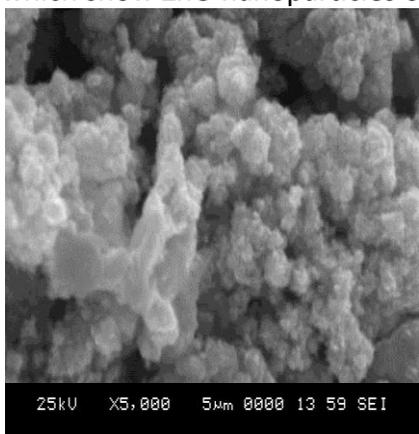
The use of plant extracts has opened a new awareness for the control of disease, besides being safe and non- phytotoxic . It is found that the plant extracts are effective against various microorganisms including pathogens . The search for antimicrobial agent has continued

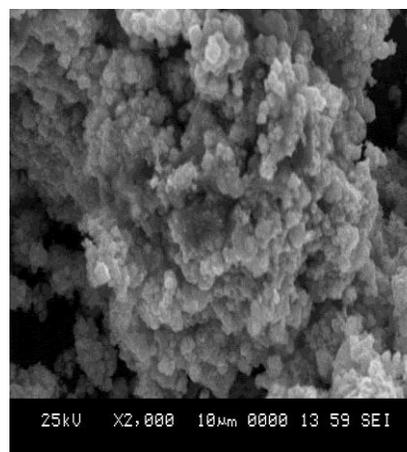
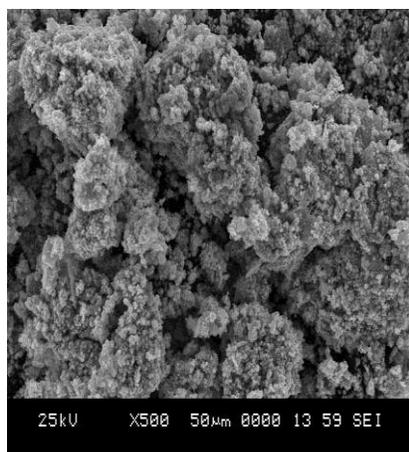
to be concentrated on lower plants ,fungi and bacteria[12]. The present study was carried out to the comparative evaluation of the antimicrobial activity of *Alpinia purpurata* extract and nanoparticles synthesized from plant in respect to standard antibiotics.

ZnO has long been recognized as having inhibitory effect on microbes present in the medical and industrial process . The most important application of ZnO and ZnO nanoparticles is in medical industry such as topical ointments to prevent infection against burn and open wounds . Further these biologically synthesized nanoparticles were found highly toxic against different multi drug resistant human pathogens[17].

The SEM images of ZnO nanoparticles

It is important to know the exact nature of the ZnO nanopartacles formed and this can be deduced from the SEM image of the sample. Two small significant peaks are observed at 376-480 and the absorbance is at 1.47-1.50.A SEM micrograph of the dry mass is presented which show ZnO nanoparticles of size 50nm.





The antibacterial activity of ZnO nanoparticles

Table: 1

Name of bacteria	Zone of inhibition by Nanoparticles(mm)	Zone of inhibition by plant extract(mm)	Zone of inhibition by tetracycline(mm)
<i>Staphylococcus aureus</i>	6.5	7.9	20
<i>Proteus vulgaris</i>	7.1	7.8	11
<i>Escherichia coli</i>	6.8	8.6	-
<i>Pseudomonas</i>	7.6	7.7	26
<i>Klebsiella pneumonia</i>	6.9	7.9	12

The antifungal activity of ZnO nanoparticles

Table: 2

Name of fungus	Zone of inhibition by Nanoparticles(mm)	Zone of inhibition by plant extract(mm)	Zone of inhibition by penicillin(mm)
<i>Aspergillus niger</i>	8.0	8.8	20
<i>Aspergillus flavus</i>	8.4	8.4	24
<i>Fusarium</i>	8.0	8.0	20
<i>Rhizopus</i>	7.0	7.6	22

CONCLUSION

The study included the synthesis of ZnO nanoparticles from the plant rhizome of *Alpinia purpurata* and their antimicrobial activity. From the study, it was concluded that the ZnO nanoparticles have been synthesized with the use of surfactant which was isolated from the plant source under ambient condition. Moreover, the ZnO nanoparticles have shown antimicrobial activity. Since, concentration of ZnO nanoparticles have impact on the antimicrobial activity, therefore, concentration dependent studies of nano-ZnO structures synthesized under microwave condition can be of great significance from technology point of view.

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