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Synthesis of Zinc Oxide Nanoparticle from *Hibiscus rosa-sinensis* leaf Extract and Investigation of Its Antimicrobial Activity.

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ABSTRACT

In this study ZnO nanoparticles have been synthesized with the use of surfactant from leaves of *Hibiscus rasa-sinensis.* The particle size and morphology of the synthesized nanoparticle is characterized by UV spectrophotometer and SEM analysis. ZnO nanoparticles were found to inhibit bacterial growth in comparison to the plant extract and standard antibiotic disc.

Keywords: *Hibiscus leaves*, Scanning electron microscope, Spectrophotometer.

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INTRODUCTION

The field of Nanotechnology is one of the most active areas of research in modern material science. The word "nano" is used to indicate one billionth of meter. The term Nanotechnology was coined by Taniguchi a, researcher at the University of Tokyo, Japan. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. New applications of nanoparticles and nanomaterials are emerging rapidly [1].

ZnO nanostructures are the forefront of research due to their unique properties and wild applications. The advantage of using ZnO nanoparticles is that they strongly inhibit the action of pathogenic microbes when used in small concentration. Moreover these are durable and show great selectivity and heat resistance [3].

MATERIALS AND METHOD

Plant Material

The leaves of *Hibiscus rosa-sinensis* was collected from Kannur district, Kerala. It was shade dried and powdered.

Chemicals

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

Synthesis of a natural surfactant

The surfactant was isolated from the leaves of *Hibiscus rosa-sinensis, the* plant material under ambient conditions. In a typical synthesis, 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 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.

Surfactant assisted synthesis of Zinc oxide nanoparticles

In each of the four reaction flasks 22ml aqueous solution containing $ZnSO_4 7H_2O$ (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.

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Characterization of Zinc oxide nanoparticles

The shape, size and microstructures of the ZnO nanoparticles were characterized by using scanning electron microscopy. 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[2].

Antibacterial activity of ZnO nanoparticles

The Antibacterial activity of isolated plant zinc oxide based nanoparticles pellets were tested by paper disc method (Gulcin *et al.*, 1993) .The bacterial species were collected from Department of Microbiology lab , Dr.N.G.P. Arts and Science College, Coimbatore. The test organisms used for the assay are *Proteus vulgaris, Staphylococcus aureus, E.coli, Pseudomonas areoginosa, Klebsiella pneumonia.* The antibacterial activity of the synthesized ZnO nanoparticles was evaluated by measuring the zone of inhibition.

Disc diffusion assay

The disc of 4mm was 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 150^0C for 15min. The disc was impregnated with 10µl of concentrated crude extract and another disc were impregnated with 10 µl synthesized zinc oxide nanoparticles. The disc were evaporated at 37^0C for 24 h. Prepared discs containing the various fractions were carefully placed on the inoculated plates using a sterilized forceps in each case. The antibiotic tetracycline was used as control .The plates were then turned upside down and incubated at 37^0C for 24 h in an incubator. The results were taken by considering the zone of growth and inhibition of the bacteria by the test fractions. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone (IZ) around the disc.

RESULT AND DISCUSSION

Synthesis of ZnO nanoparticles

In the present study, ZnO nanoparticles were synthesized using surfactants under ambient conditions. The surfactant was isolated from the leaves of *Hibiscus rosa-sinensis.* Surfactant seems to play an important role in determining the size of the ZnO nanoparticles.

The size of the nanoparticles drastically decreases in the presence of the natural surfactant. It is well known that ZnO is a polar crystal, Zn^{2+} lies within a tetrahedral group of four oxygen ions. Zinc and oxygen atoms are a tetrahedral group of four oxygen ions. Zinc and oxygen atoms are arranged alternatively and the top surfaces are Zn terminated while the bottom surfaces are oxygen terminated. During the synthesis process, surfactants tend to adsorb on the active sites of ZnO nuclei and leads to the formation of ZnO nanoparticles[5].

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Charecterization of ZnO 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[2].

The SEM analysis clearly shows the distribution of ZnO nanoparticles prepared with the natural surfactant. The image show spherical shape and hexagonal nanoparticles. Owing to the uniform distribution of oxidized metal anions in the three dimensional polymeric network structures, the agglomerization could be induced by densification resulting in the narrow space between particles.

UV-Visible absorption spectra of the ZnO particles show absorption spectra with the absorption peak ranging from 358-375 nm due to its surface Plasmo resonance.

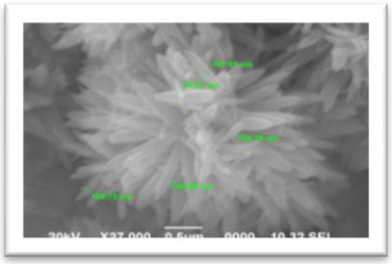

Fig 1

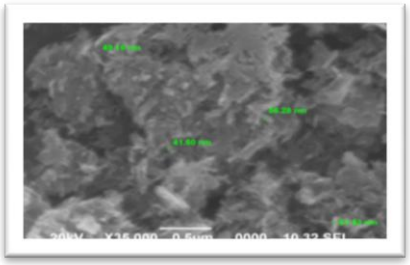

Fig 2

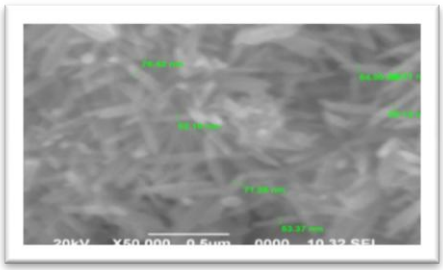

Fig 3

Antibacterial potential of ZnO nanoparticles

The antibacterial activity of ZnO by nanoparticles was evaluated by disc diffusion method. The majority of studies suggest that nanoparticles cause disruption of bacterial membranes probably by the production of reactive oxygen species (ROS) such as superoxide and hydroxyl radicals. As a particle approaches near the membrane, a potential called zeta potential is generated. This is different for various nanoparticles. ZnO nanoparticles have positive zeta potential at their surface. This depends on the nature of the surface of different bacteria. The antibacterial activity is also reported to be dependent on the

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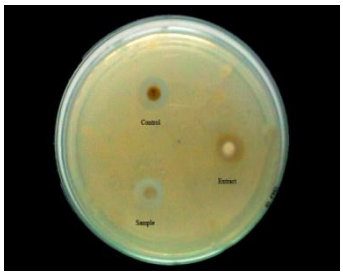
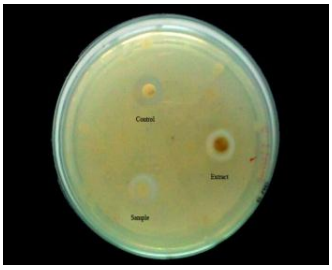
concentration of the ZnO nanoparticles and impact of the type of surfactant used. Inhibition of bacterial growth by ZnO nanoparticles could be attributed to damage of the bacterial cell membrane and extrusion of the cytoplasmic contents thereby resulting in the death of the bacterium [4].

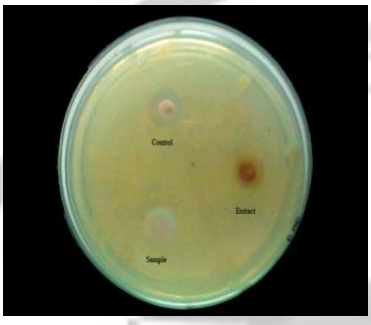
Fig 4: ANTIBACTERIAL ASSAY



Stephylococcus aureus



Escheria coli



Pseudomonas aeuroginosa

Klebsiella pneumonia

	Zone of inhibition (mm)		
Name of bacteria	ZnO nanoparticls	Plant extract	Antibiotic disc (Gentamicin)
Staphylococcus aureus	11	8	19
Escherichia coli	13	10	18
Pseudomonas aeuroginosa	22	7	25
Klebsiella pneumonia	10	6	15

Antibacterial activity of ZnO Nanoparticle

The antibacterial activity of ZnO nanoparticle was tested. The best activity obtained against *Pseudomonas aeurogenosa* and the least activity was showed against *Klebsiella pneumonia*. ZnO nanoparticles were found to be more abrasive than plant extract and thus contribute to the greater mechanical damage of the cell membrane and the enhanced bactericidal effect.

CONCLUSION

In the present study, ZnO nanoparticles have been synthesized with the use of surfactant which was isolated from the leaves of *Hibiscus rosa-sinensis.* The synthesized ZnO nanoparticles have been characterized using SEM, which could provide information with regard to surface area of the nanoparticles. The ZnO nanoparticle shows particle size ranges from 40-56 nm.

Moreover the synthesized nanoparticles have shown antibacterial potential against pathogenic bacteria. ZnO nanoparticles were found to inhibit bacterial growth in comparison to the plant extract and standard antibiotic disc. Inhibition of bacterial growth by ZnO nanoparticle can be attributed to damage of the bacterial cell membrane and extrusion of the cytoplasmic contents there by resulting in the death of the bacterium.

Since concentrations of ZnO nanoparticles have impact on the antimicrobial activity, therefore concentration dependent studies of nano ZnO structure synthesized under different reaction condition can be of great significance from technology point of view. The explored eco-friendly, high efficient ZnO nanoparticles prepared from *Hibiscus rosa-sinensis* leaves are expected to have more extensive application in biomedical fields and in cosmetic industries.

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