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GREEN SYNTHESIS OF ZNO NANOPARTICLES USING LEAVES EXTRACT OF *ANISOMELES MALABARICA* AND ITS ANTIBACTERIAL ACTIVITY

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Abstract: Zinc oxide (ZnO) has broad applications in various areas. Nanoparticle synthesis using plants is an alternative to conventional physical and chemical methods. It is known that the biological synthesis of nanoparticles is gaining importance due to its simplicity, eco-friendly and extensive antibacterial activity. The present study report the synthesis of ZnO nanoparticles using *Anisomeles malabarica* leaves extract. XRD confirmed the crystalline nature of the nanoparticles of 62 nm size. The XRD peaks at 31◦, 34◦, 36◦ and 47◦can be indexed to the (100), (002), (101) and (102) Bragg's reflection of cubic structure of metallic ZnO, respectively. The FTIR result clearly exhibit that the extract containing C-N as a functional group act in capping the nanoparticles synthesis. SEM images revealed that ZnO nanopartides were spherical shape was carried out at the different magnification range as 5000x and 20000x. X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), and Scanning electron Microscopy (SEM) with Energy dispersive X-ray analysis (EDX).analysis releated the stability of ZnONps synthesized from *A.malabarica* leaves Besides, this the study determines the antibacterial efficacy of the synthesized ZnO nanoparticles against clinical strains of *Staphylococcus aureus* and *Escherichia coli*.

Keywords: *Anisomeles malabarica*, UV, FTIR, XRD, SEM and EDAX, Antibacrial activity.

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INTRODUCTION

Nanotechnology has been successfully applied for disease diagnosis, *in vivo* molecular imaging and as an improved therapeutic platform. With the development of nanotechnology, nanostructures are recognized to have drawn remarkable potential applications in biology, medicine, therapeutic, sensors, electronics, optics, proteomics and bioanalytical fields because of their larger surface area, enhanced chemical reactivity and easier penetration into cells [1]. ZnONPs are widely used in industrial applications such as pigments [2]. A wide variety of physical and chemical processes for the synthesis of ZnO nanostructures have been developed including laser ablation[3]. Nanoparticles produced by plants are more stable and more varied in shape and size in comparison with those produced by other organisms [4].According to the use of eco-friendly biosynthesized nanoparticles as an alternative to the chemically synthesized ones would help to control chemical toxicity in the environment[5] .One of the most considered methods is the production of metal NPs using biological systems such as microbes, fungi and several plant extracts. NPs produced by plants are more stable and the rate of synthesis is faster in the case of microorganisms. Moreover, the NPs are more various in shape and size in comparison with those produced by other organisms[6].

ZnONPs are of unique material that exhibits semiconducting, piezoelectric and pyroelectric properties, it has versatile applications in transparent electronics, ultraviolet (UV) light emitters, chemical sensors, spin electronics, personal care products, catalyst, coating and paints. In fact, ZnO is non-toxic and chemically stable under exposure to both high temperatures and UV. Several physical and chemical processes have been used for the synthesis of large quantities of metal NPs in a relatively short period of time. Chemical method leads to the presence of some toxic chemicals adsorbed on the surface that may have adverse effects in medical applications and environment. In order to minimize the toxicity, we have a need for the development of simple and eco-friendly methods for the synthesis of NPs [7] .To reduce metal ions into zero-valancemetal nanoparticles. In addition, these bioreduction solutions contain natural capping agents that act to hinder aggregation of the synthesized metal nanoparticles and control their particle sizes.

Plant extract based bioreduction processes that utilize the plant leaf, flower, bark, or tuber have been used extensively for the synthesis of silver nanoparticles [8] Chemicals are less hazardous to human health and the environment are less toxic to organisms, ecosystems and inherently safer with respect to handling and use. Synthesis of inorganic metal oxide NPs using biological things has been great interest due to their unusual optical, chemical, photoelectrochemical and electronic properties[9] . Plant have been an integral part of life in many local for food and medicine both. India has more than 3000 years of medicinal heritage based on medicinal plants. Medicinal plants are widely used by all sections of the population

either directly as folk remedies are indirectly in the preparation of pharmaceuticals. Out of nearly 17,000 higher plants recorded in India, 7500 are reported to be in medicinal use by the rural and tribal communities[10] .

Anisomeles malabarica (Lin) is a traditional medicinal plant found in tropical and subtropical regions of India, belongs to family Lamiaceae. It is erect shrub commonly known as 'Malabar catmint', commonly found in Western Ghats from Maharashtra to Kerala in India [11]. The herb is reported to possess anticancer, allergenic, antihelmintic[12], antiallergic, antianaphylactic, antibacterial,[13-15] anticarcinomic, anti-inflammatory,[16] antileukemic, antinociceptive, antiplasmodial, and antiseptic, antiperotic properties[17],[18] .*Anisomelic* acid, is one of the major compounds in *A. malabarica (L.) R. Br.,* is a cembrane type diterpenoid, which can be synthesized chemically. The present tudy describes antibacterial effects of biosynthesized ZnO nanoparticles using an leaves extract of *A. malabarica*.

MATERIALS AND METHODS

Sample Collection

The healthy plant samples were collected from the Kolli hills (lattitude 11.2485' E and longitude 78.3387' N) near Sethamangalam, dry rocky region of Namakkal district, Tamilnadu. The leaves were separated from the collected plant and dried under room temperature. After drying it was pulverized to powder in a mechanical grinder for further studies.

Preparation of leaf extracts

The collected fresh, healthy leaves were washed thoroughly with double distilled water. 10 gm of plant powder was weighed and mixed with 100 ml of distilled water. The extraction was carried out in a orbital shaker for 24 hours. The solution was filtered through Whatman no.1 filter paper. The filtered samples were collected in a sterile conical flask. The obtained extract was used for the synthesis of ZnO nanoparticles.

Green synthesis of ZnO nanoparticles using leaf extract

Green synthesized ZnO nanoparticles were prepared by sol- gel method. In a typical experiment, 0.2 M solution of Zinc nitrate hexahydrate ($Zn(NO_3)_2 6H_2O$) was prepared with 50 ml of plant extracts (*A. malabarica*). The reaction mixtures were stirred using a magnetic stirrer for 4 h at room temperature. After that, the stirred process was complete in precipitation of Zinc oxide was recognized by the brown color colloidal particles at the bottom of the flask. It is believed that the color change indicates the development of the hydrolysis of $Zn(OH)_2$. Then this solution was taken in centrifuge tube and it was centrifuged at 10,000 rpm for 15 minutes. The pellets were taken after centrifugation and it were air dried.

Antibacterial activity of ZnO nanoparticles

The antibacterial activity of ZnO NPs was determined by agar well diffusion method. The ZnO NPs were screened against *S. aureus* and *E. coli* (Gram negative) organisms. The bacteria was grown in Luria Bertani broth medium. Test organisms were seeded over the surface of MHA agar plates. An aliquot plant sample 40 µl (ZnO NPs) sample was loaded in MHA agar plate. The plates were incubated at 37^{o}C for 18–24 hrs. The antibacterial activity of NPs was demonstrated by the diameter of the zone of inhibition developed around the sample. The zone of inhibition were measured.

Characterization of ZnO NPs

X-ray diffraction (XRD) analysis of ZnO powder samples of glass substrates was carried out on the JEOL LDX 8030 instrument operating at 40 kV with a current of 30 mA using Cu Ka radiation k = 1.5405Å over a wide range of Bragg angles(10. 6 2h 6.75). The surface morphology of ZnO nanoparticle was measured using a JEOL JSM 6390 model. An elemental analysis of the sample was studied by energy dispersive analysis of X-rays. Fourier transform infrared spectroscopy (FTIR) analysis of the samples was performed by Perkin Elmer make Model Spectrum RX1 (Range 4000–400 cm^{-1}). The sample was dried at room temperature and then KBr pellets were prepared. The transmittance of the samples was measured. The morphology of ZnO nanoparticles was examined by means of scanning electron microscopy (SU5000, Hitachi with spectral imaging system Thermo Scientific NSS (EDS), the tape of detector (BSE-3D), acceleration voltage (20 kV), working distance (5 µm), pressure (in the case of variable vacuum conditions.

Stastistical analysis

All the data were performed in triplicate (n=3) with standard error (SD±) using microsoft excel ver 7.0.

RESULTS AND DISCUSSION

Uv visible spctroscopy

The UV-Vis spectrum of ZnO Nps is Confirmation of the synthesized ZnO product in nano-scale was exhibited by the highly blue-shifted absorption maximum occurring around 325 nm. For bulk ZnO the absorption maximum usually occurs around 385 nm approximately.

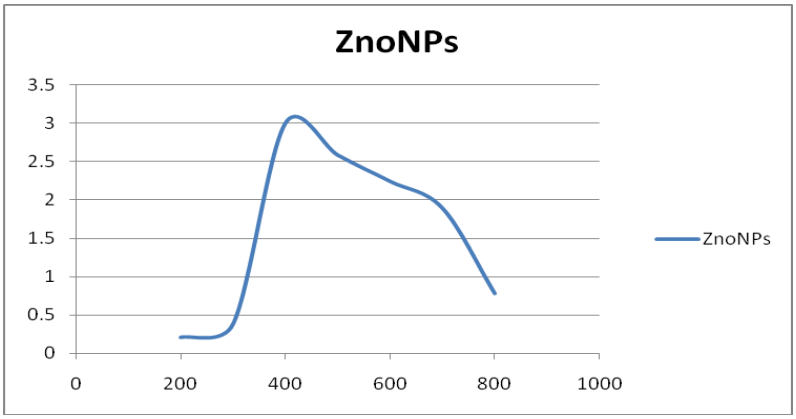

Fig1. UV-Vis spectrum of synthesized ZnO NPs

XRD pattern of Zn nanoparticles

The purity, crystallinity, average particle size of ZnO NPs was confirmed by X-ray diffraction analysis technique. The XRD pattern of calcinated ZnO NPs is recorded in the range of 20–60. The sharp peak indicates the purity and crystallinity of NPs. The XRD peaks are consistent with the JCPDS data card 36-1451 hexagonal phase and primitive geometry of Zn NPs. The detected peaks corresponded with those of hexagonal phase zincite were found at the lattice planes of (100), (002), (101), (102), and (110) in the 2θ value: 31.68, 34.35, 36.17, 47.47,and 56.52, respectively. The crystalline size of the most intense plane (101) was 36.17 nm, determined by employing Debye–Scherrer's equation. Nanosized value of ZnO suggests that the *A. malabarica* leaves extract can be employed as the hydrolytic and precipitating agent for the formati on of ZnO NPs.

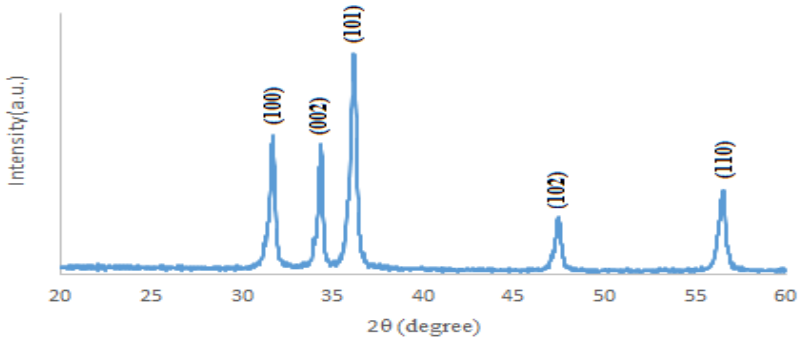

Fig2.XRD pattern of ZnO nanoparticles of heated sample.

Fourier transform Infrared Spectroscopy (FTIR)

FTIR measurements were carried out in order to determine identify the biomolecules for capping and efficient stabilization of the metal nanoparticles synthesized by *A. malabarica*

leaf. The FTIR spectrum of ZnO nanoparticles. The band at 3442 cm^{-1} corresponds to O-H stretching H-bonded alcohols and phenols. The peak at 2918 cm-1 corresponds to O-H stretch carboxylic acids. The assignment at 1627 cm-1 corresponds to N-H bend primary amines. The peak at 1382 cm-1 corresponds to C-N stretching of aromatic amine group and the bands observed at 1514, 1267, 1039 cm-1 corresponds to C-N stretching alcohols, carboxylic acids, ethers and esters. Therefore the synthesized nanoparticles were surrounded by proteins and metabolites such as terpenoids having functional groups of alcohols, ketones, aldehydes and carboxylic acids. From the analysis of FTIR studies we confirmed that the carbonyl group from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly from the metal nanoparticles.

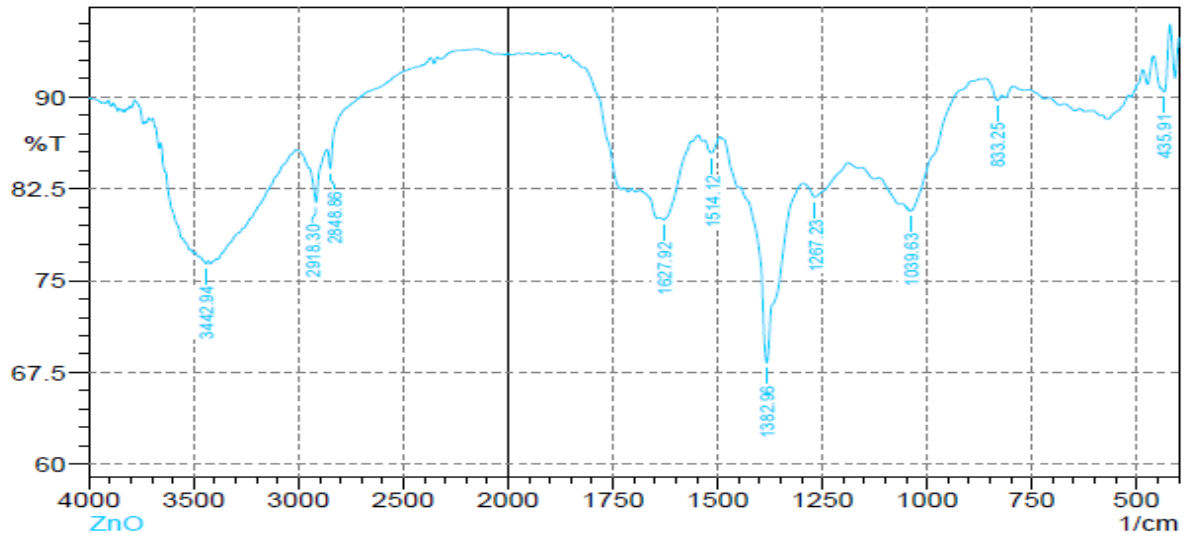

Fig3. FTIR analysis of ZnO nanoparticles.

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) analysis to study the surface morphology of the ZnO nanoparticles was observed in spherical structure. The SEM image of the green synthesized of nanoparticles was carried out at the different magnification such as 5000x and 20000x The synthesized nanoparticles showed some agglomeration due to the polarity and electrostatic attraction of ZnO nanoparticles.

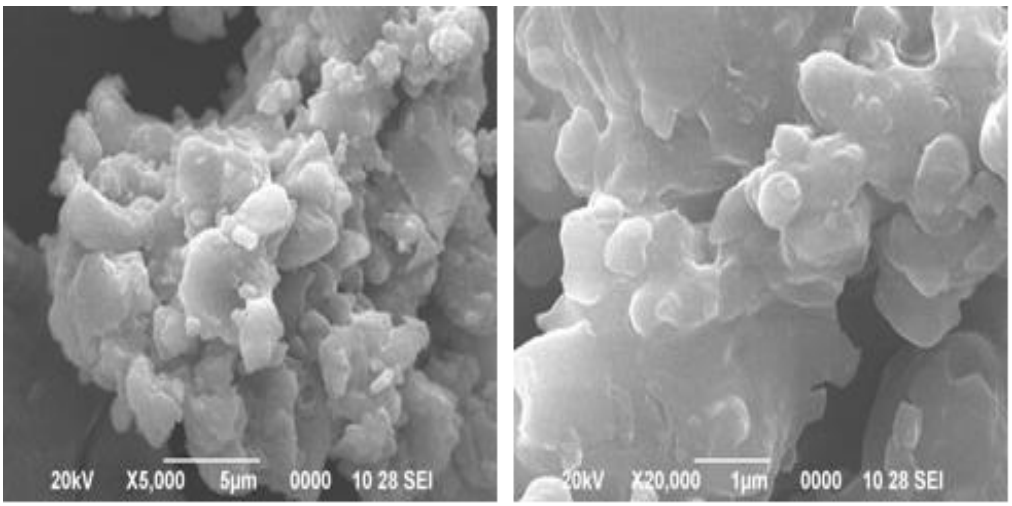

Fig4. Surface morphology of ZnO nanoparticles at different magnifications

EDX analysis of ZnO nanoparticles

Energy dispersive X-ray spectroscopy (EDX) was employed to establish the element identity of the observed particles. In the analysis by energy dispersive X-ray spectroscopy (EDX) of the ZnO nanoparticles, the presence of elemental metal signal (Zinc and oxygen) was confirmed.

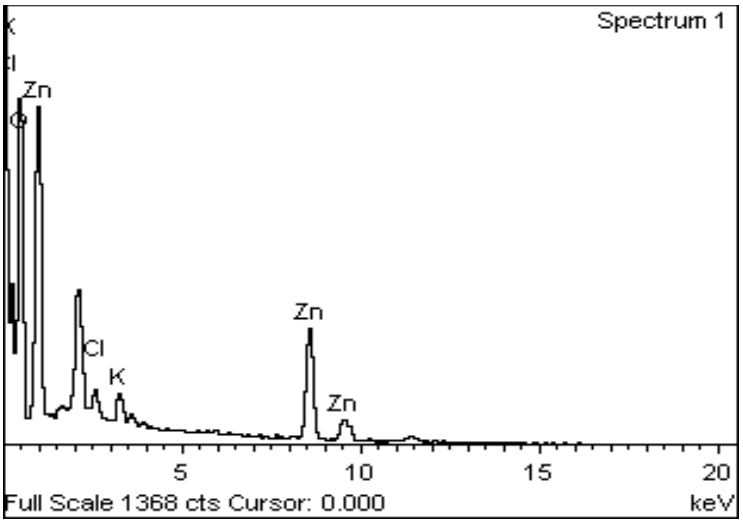

Fig5. EDX images of ZnO nanoparticles

Antibacterial activity of ZnO nanoparticles

Pathogenic bacteria collected from the MTCC were used in this study. The Gram-negative bacterial species, *Escherichia coli* and the Gram-positive *Staphylococcus aureus* had been used in the assay. 24 h fresh cultures were prepared and the standardized. The antibacterial activities of ZnO NPs against the studied pathogenic strains are shown in Fig. 5. The values of

zone of inhibition obtained from the assay are presented in Table 1. All Gram-negative bacteria had shown good sensitivity towards the green synthesized ZnO NPs for the concentration 20 µl . It is quite interesting to note that all bacterial species tested in this study showed resistance to the synthetic antibiotic drug which in turn indicates the better antibacterial activity of the ZnO NPs than the commercially available synthetic drug.

Table: 1 Zone of inhibition of ZnONPs nanoparticles against bacterial pathogens

S.No	Test Sample	Zone of inhibition (mm)					
		S.aureus			*E.coli*		
		10 µl	20 µl	30 µl	10 µl	20 µl	30 µl
1	Zn nanoparticles	18.0±1.0	21.1±07	24.3±2.1	16.5±1.5	19.0±1.8	23.2±2.0
2	Cholora mphenicol	23.0±1.0	28.0±07	32.1±2.0	22.0±1.5	25.5±1.7	33.8±2.5

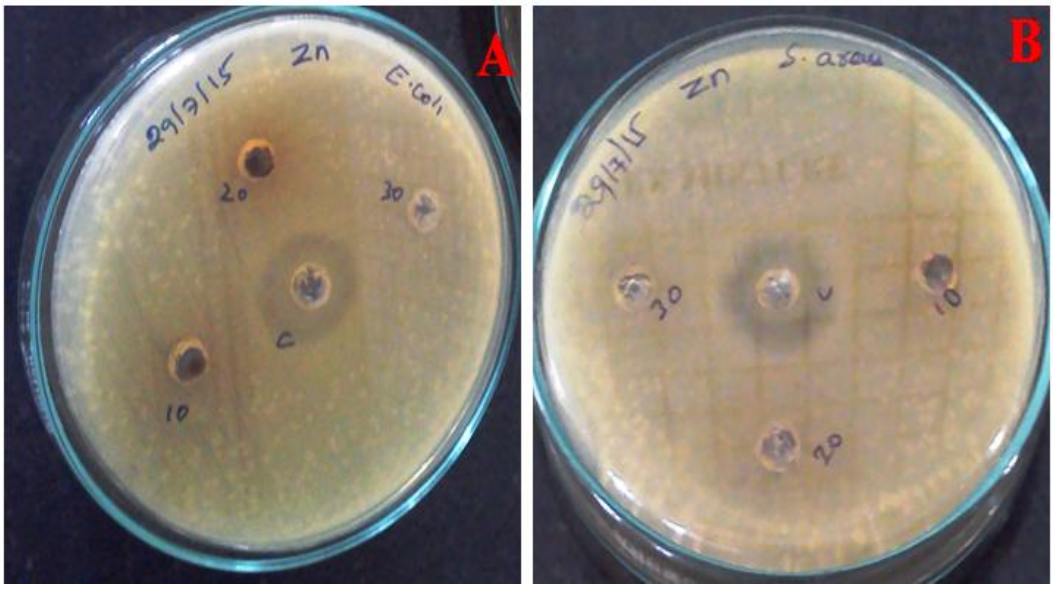

Fig6. Antibacterial activity of *A. malabarica* ZnONPs against *E.coli* and *S.aureus*.

CONCLUSION

ZnO particles functionalized with ZnO nanoparticles were successfully fabricated using a *A. malabarica* leaves extract mediated bioreduction process. UV–visible spectroscopic analysis

revealed the ZnO nanoparticles were rapidly reduced by the *A. malabarica* leaves extract with in 90 min. FTIR, XRD and SEM–EDS analysis confirmed the formation of stable ZnO nanoparticles on the surface of the ZnO particles with an average size of 5–20 nm. This also confirmed the effectiveness of the *A. malabarica* leaves extract mediated bioreduction for the Zn into ZnONPs .

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