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Green synthesis of ZnO Nanoparticles infused biofilm as a woundhealing sheet

D Shobana[1], S Reneta Siana[2] and S Justin Packia Jacob*
Department of Biotechnology,
St Joseph's college of Engineering, OMR, Chennai,
Tamil Nadu, India-600119

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ABSTRACT:
The high-cost and limitations of current wound healing treatments have led to the search for alternative approaches or drugs, particularly from medicinal plants. Leaves of Abutilon indicum is traditionally used by villagers to heal the wounds, which is difficult to heal by other medicines. Synthesis of Zinc oxide nanoparticles was performed using the extracts of Abutilon indicum leaves and it was characterized by using UV-Vis, SEM-EDX, XRD and Fourier-Transform Infrared Spectroscopy. The size of the nanoparticles was characterized using IMAGEJ software and it was found to be in the range of 54 nm. The Agar well diffusion assay was performed by inoculating Staphylococcus aureus to evaluate antimicrobial Activity. The above green synthesized Zinc oxide nanoparticles were infused with sodium alginate-based biofilm. The biofilm thus prepared possesses an anti-inflammatory effect; it will be used in the form of a band-aid, or the biofilm sheet as such can be used as a wound healing sheet.
Key words: Abutilon indicum, Zinc oxide nanoparticles, Anti-microbial and Anti-inflammatory activity, wound-healing sheet, Characterization.

I. INTRODUCTION:

For the past two decades, extensive research efforts have been made towards the preparation of cost-effective and eco-friendly nanostructured materials in the research fields of science, engineering and biotechnology. Due to the high 'surface-to-volume ratio', nanoparticles (NPs) show unique and fascinating features such as optical, catalytic, size, shape, self-assembly and conductivity features. Nanoparticles (NPs) are typically clusters 1–100 nm in size. Among the widely studied NPs, ZnONPs have gained significant attention and possess notable benefits in the production of pottery, transparent materials, elastic polymers, ointments, lubricants, dyes, adhesives.ZnONPs have shown different biological and clinical applications, including antimicrobial, anticancer, antioxidant,and enzyme inhibiting effects, as well as biocompatibility,The green synthesis of ZnO NPs using plant extracts has emerged as a sustainable and cost-effective alternative to conventional chemical methods.Abutilon indicum, commonly known as Thuthi, is a medicinal plant belonging to the family Maleaceau.It is commonly used as a stimulant purgative, anti-diabetic, and anti-inflammatory, as well as to cure leprosy, urinary infection, jaundice, menstrual infection, gonorrhea, piles, etc.The anti-oxidative, anti-microbial, anti-inflammatory, hepatoprotective, and anticancer properties of the plant extract were studied.In this study, we focus on the synthesis of ZnO Nanoparticles. Zinc oxide has gained much interest due to its photocatalytic activity, antibacterial activity, non-toxicity, and low-cost method.Zinc oxide nanoparticles conjugated with flavonoids, which are present in plant extract, provide a renewable source of bioactive compounds.These conjugated flavonoids could lead to the development of antimicrobial agents with enhanced efficacy.Wounds are a major global healthcare issue that have been defined as a "silent epidemic" for their prevalence and profound effects on global health and patients' lifestyles and psychological wellbeing.The bubbling community of microorganisms, consisting of diverse colonies encased in a self-produced protective matrix and playing an essential role in the persistence of infection and antimicrobial resistance, is often referred to as a biofilm.The nanostructure of the prepared ZnO nanoparticles were characterized using UV-Vis, SEM-EDX, FTIR, XRD analysis and anti-bacterial assay.

II. MATERIALS AND METHODOLOGY:

Materials:

The materials used for ZnO Nanoparticles Synthesis were Abutilon indicum leaves gathered from the local garden in Chennai,Zinc acetate (Zn(CH3CO2)2) and the biofilm was prepared by

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using Sodium Alginate, Sucrose and Mimosa Extract purchased fromSRL Chemicals Ltd., Chennai, Tamil Nadu. Solutions were prepared using deionized water.

Green Synthesis of ZnO nanoparticles:

The leaves of Abutilon Indicum were cleaned with tap water and deionised water to eliminate impurities before being dried. Freshly collected Abutilon indicum leaves were shade-dried and powdered.10g of dried leaves from Abutilon Indicum were boiled for 10 mins in 100 ml deionized water and filtered.The filtrate was allowed to cool down to room temperature and then stored at 4^{0}C for further experimentation.The plant extract of Abutilon Indicum was added dropwise to 11g of zinc acetate solution, which was stirred at 1200 r min^{-1} and 50^{0}C, in order to synthesize ZnO NPs.The reaction was monitored using color and turbidity change in the solution.Preparation of 1M NaOH solution using 7g of NaOH and 200ml of deionized water and a few drops of 1 M NaOH were added to elevate the pH, and the solutions were stirred at room temperature for 2 hours, resulting in notable color changes. After this, the white precipitate wasseparated by centrifugation at 8000 rpm for 10 minutes, and the NPs were recovered. The recovered NPs were washed twice with deionized water, once with ethanol, and then centrifuged again. The pellet was dried in a hot air oven at 60^{0}C, and the powdered ZnO nanoparticles were stored for experimental study.

Preparation of biofilm:

The formation of biofilm utilising Abutilon indicum, zinc oxide nanoparticles, and sodium alginate entails multiple procedures. Prepare the leaf extract by dissolving Abutilon indicum in 30 ml distilled water, Then, add 1.5 grams of sodium alginate to the mixture. After combining these solutions, add 100 milligrams of zinc oxide nanoparticles to it. Finally, add the sucrose after thoroughly mixing. Pour this mixture into a mould and let it dry until it forms a solid biofilm. To increase the robustness of the biofilm, cross-link it with a Mimosa extract. Once dry, the biofilm can be used for a variety of applications, including wound healing and drug delivery.

Characterization of ZnO NPs:

UV-Vis Spectrophotometer:

ZnO NPs were initially analysed using a UV-Vis spectrophotometer. UV-Vis spectra of leaf extract and synthesized ZnO NPs were measuredusing the UV-Vis Double

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BeamSpectrophotometer Systronics 220 between 200 and 800nm to determine the maximum absorbance or surface plasmon resonance peak value.

Fourier transformed infrared spectroscopy (FTIR):

Bruker Alpha Platinum ATR-IR FTIR spectroscopy was used for the present study. FTIRwas used to examine the involvement of functional groups in the reduction of Zinc Acetate into ZnO Nanoparticles.

X-ray Diffraction (XRD):

TheXRD results of ZnO NPs were obtained using copper Ka radiation (k = 1.5406 A˚) in the scanning range of 20^0 to 80^0.The Debye-Scherrer equation was used to determine the nanoparticles' crystallite size.

Scanning Electron Microscope (SEM):

SEM images were used to examine the nanoparticles' surface morphology, texture, and particle size. The purity and elemental composition of the synthesized ZnO NPs were examined using an energy-dispersive x-ray spectrometer (EDX). The SEM images of ZnO NPs exhibited rock-shaped spheroid-like particles whose range and size were not equally distributed.

Anti-bacterial Assay:

Agar well diffusion assay was applied to evaluate the antimicrobial activities of ZnO NPs synthesized using the leaf extract of Abutilon Indicum against microorganisms, that is, Staphylococcus aureus.

III. RESULT AND DISCUSSION:

UV-Vis Spectrophotometer: The Absorption peak of the synthesized ZnO Nanoparticles was found at around 398 nm.

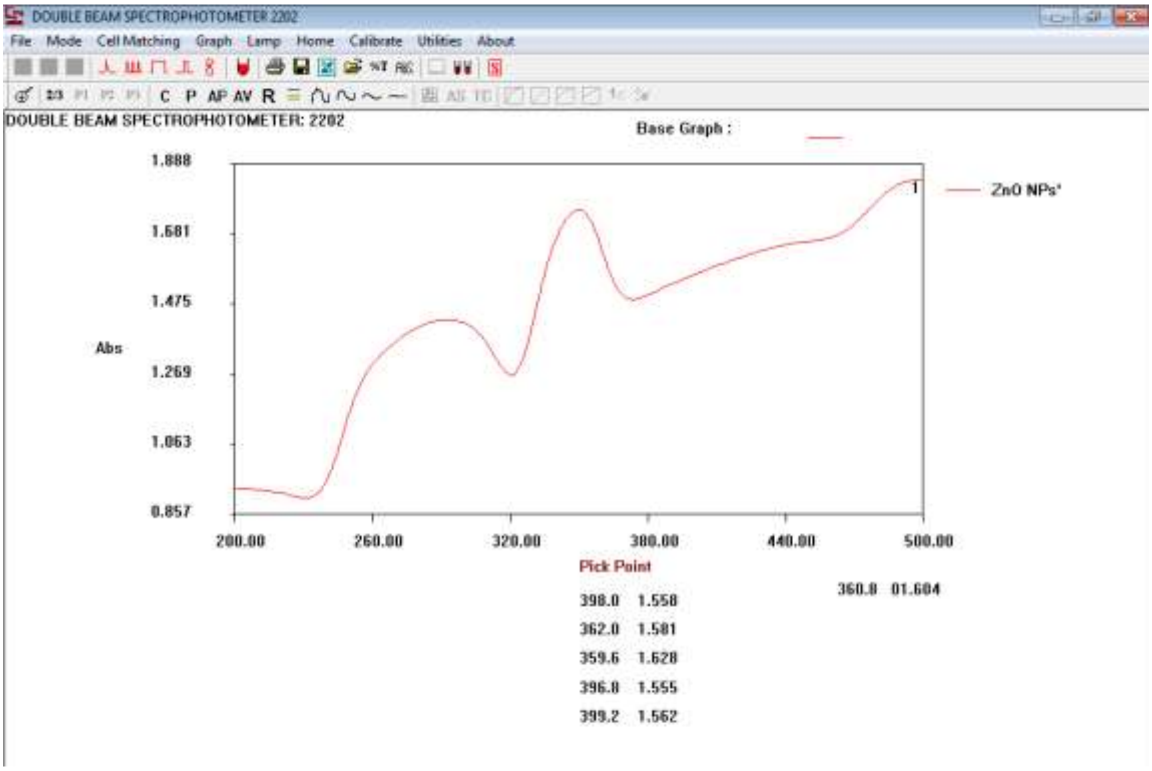

Fig1 UV-Vis Spectra of synthesized ZnO NPs

Scanning Electron Microscope(SEM):

Morphology of synthesized ZnO nanoparticles was analysed using SEM. The size of nanoparticle. The size of nanoparticles was measured using a scanning electron microscope. The particle size ranged from 50-150 nm, as seen in the SEM micrograph. We used ImageJ software to analyse the particle size, which was found to be 54 nm.

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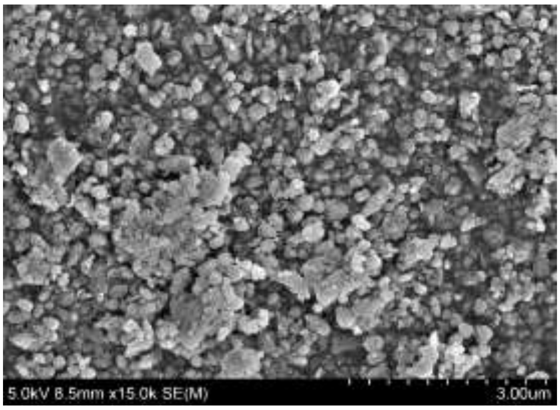

Fig2 SEM Image of synthesized ZnO NPs

Fourier transformed infrared spectroscopy (FTIR):

Synthesized zinc oxide nanoparticles were subjected to FT-IR and to detect the functional group.It was inferred that the samples have absorption peaks in the range of 1559.33cm-1,1507.70cm-1,1396.08cm-1.The absorption peak at 498.70 cm-1 corresponds to metal oxygen (ZnO stretching vibrations). So, FT-IR shows the confirmation of ZnO Nanoparticles Functional Groups.The peak at 1341 cm-1 corresponds to the stretching vibration of the primary amine's C-N bond or the primary alcohol's C-O bond. The peak at 3370 cm-1 was identified as primary alcohol in-plane bend or vibration. The peaks at 3853 cm-1, 3629 cm-1 and 3370 cm-1 correspond to the stretching vibration of hydroxyl molecules.

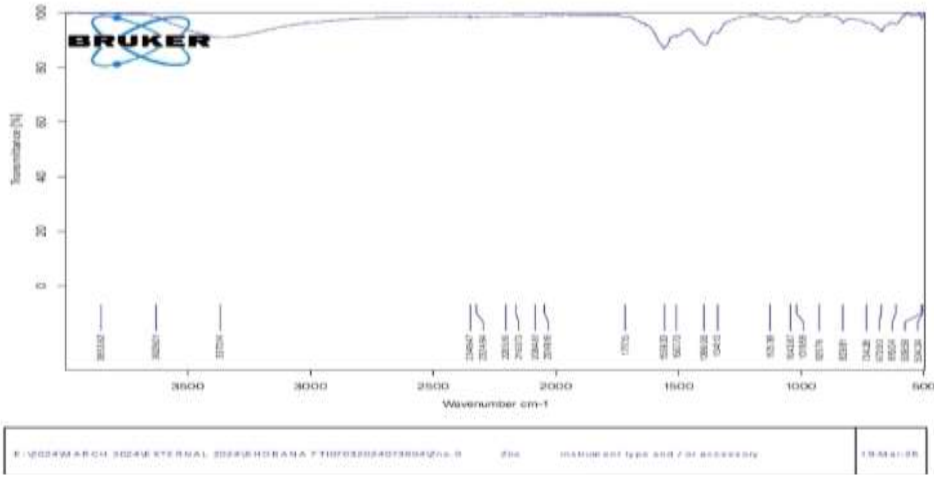

Fig3.1 FTIR spectrum of synthesized ZnO NPs

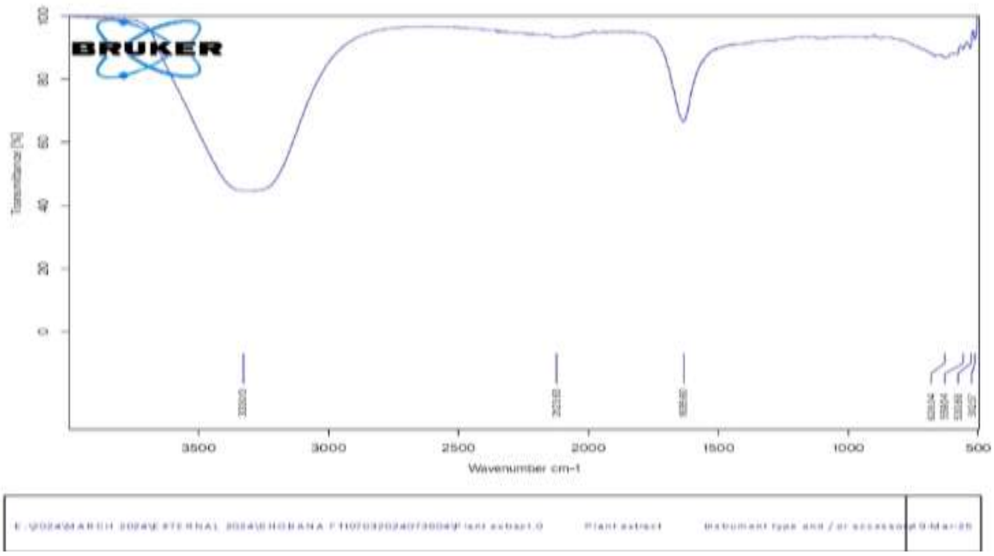

Fig3.2 FTIR spectrum of synthesized plant extract

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X-ray diffraction (XRD): The observed ZnO spectra in various phases confirmed the excellent purity of the ZnO produced utilising the green synthesis method. ZnO's remarkable crystallinity was shown by the presence of strong and sharp diffraction peaks in the XRD pattern. The crystallite size of ZnO NPs was calculated using the Debye-Scherrer equation and found to be 2.65 nm.

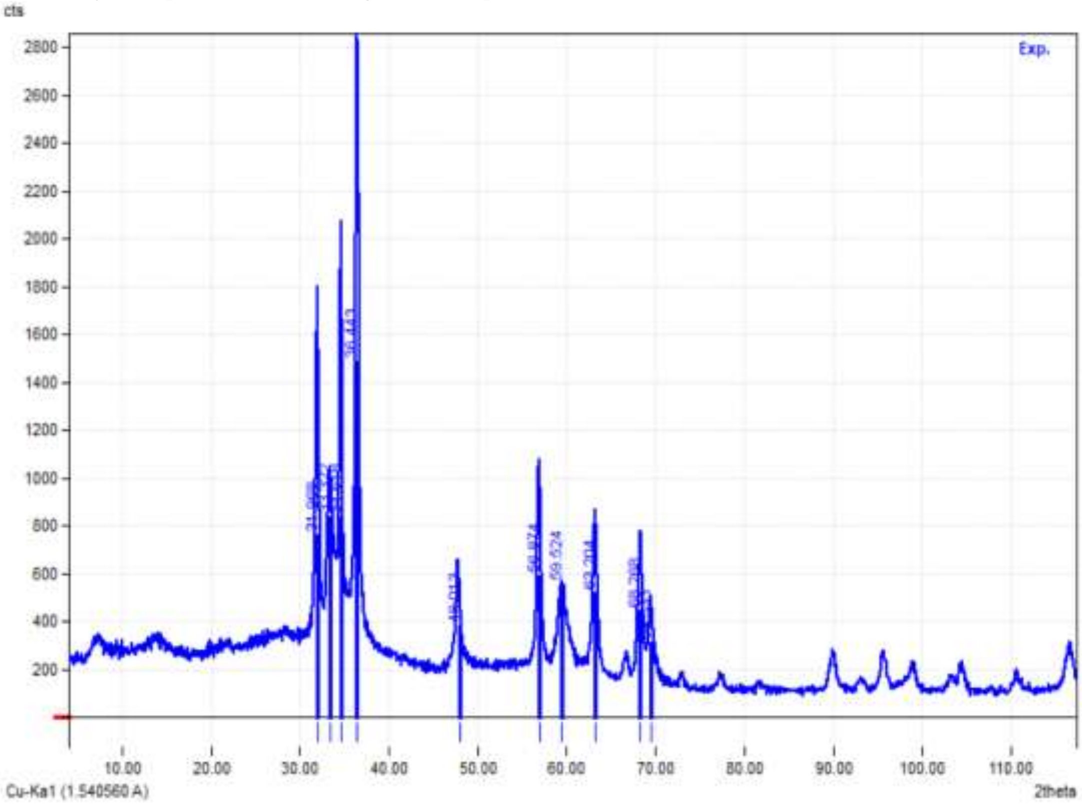

Fig4 XRD pattern of synthesized ZnO NPs

Antibacterial Assay:

A. Inoculum preparation

A loopful of bacterial species (Staphylococcus aureus) wasinoculated in the sterile nutrient broth and incubated overnight at 37°C.

B. Well diffusion assay

The agar Well diffusion assay was used to determine the growthinhibition of bacteria by the sample. Nutrient broth agar was preparedand poured into a sterile petri plate and allowed to solidify. Theovernight nutrient broth culture of Staphylococcus aureus species wasinoculated in Nutrient agar using sterile cotton swab. The given sampleswere placed in the well. Incubated for 24 hrs. tetracycline25 µgwas used as a standard.

S. No.	Organism	STD	Zone of inhibition (mm)		
			1000 µg	500 µg	250 µg
1.	Staphylococcus aureus	15	13	12	-

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Fig5 ZnO nanoparticles at different concentrations

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