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ZnO NANOPARTICLES FROM FUCOIDAN: GREEN SYNTHESIS, CHARACTERISATION AND ANTI BACTERIAL ACTIVITY

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ABSTRACT

In this study, fucoidan, a polysaccharide derived from *Sargassum wightii* Greville ex J. Agardh, a brown seaweed, was used in the production of zinc oxide nanoparticles employing an eco-friendly,low cost-efficient process. Fucoidan served as a reducing, capping, and stabilizing agent. The Zinc oxide nanoparticles synthesized were subjected to analysis by Fourier transform infrared spectroscopy (FT-IR) and ultraviolet–visible spectroscopy (UV–VIS). The UV–Vis spectra exhibited a prominent absorption peak at approximately 370 nm. Furthermore, strong antibacterial activity was demonstrated by the phyto mediated-synthesized nanoparticles. To ascertain the potential of the produced nanoparticles to provide effective natural nano-medicine active and against microbial infection, the antibacterial activity was assessed, particularly against Gram harmful bacteria, *Escherichia coli,* and Gram-positive bacteria, such as *Staphylococcus aureus and Bacillus subtilis.*

INTRODUCTION

The rise in proliferation of microbes that are resistant to drugs is significant for the field of science in the continuous successful advancement of efficient therapeutics. There has been an increase in demand for ecologically sound methods of synthesizing metal nanoparticles without the use of hazardous chemicals since the notion of green nanoparticle synthesis was developed.(Chen *et al* ., 2008; Thakkar *et al* ., 2010; Mubarak Ali *et al* ., 2011; Hemalia Padalia *et al* ., 2015; ; Thovhogi *et al* ., 2015).The potential of algae-based nanoparticles (NPs) to mitigate and control diseases caused by antibiotic-resistant pathogenic bacteria brings about a lot of interest in research today. With the use of substances like ZnO, CdO, and Sm2O3, among others, green synthesis has been used to synthesize nanoparticle-based noble metals with the lowest possible sizes and without the use of hazardous chemicals. (Diallo *et al.,* 2015; Sone *et al.*, 2015; Thema *et al.*, 2015; Thovhogi *et al.*, 2015).

Fucoidans, sulfated polysaccharides found in the cell walls of brown algae, are being extensively researched as a natural source of compounds with biological activity. These biopolymers are distinctive because they have a variety of biological activities, including antiviral, antibacterial, anticoagulant, immunostimulating, anti-inflammatory, and anticancer properties (Cumashi *et al.,* 2007;Morya *et al.*,2012).Their application in the food sector was made possible by their bacteriostatic qualities and lack of toxicity, which prolong product shelf life without destroying the natural human microbiota upon ingestion.The aims of the present investigation

are to synthesize zinc oxide nanoparticles through greener approach from seaweed polysaccharide fucoidan which is extracted from the brown marine algae *Sargassum wightii*, to characterize the zinc oxide nanoparticles that have been biosynthesized and to assess the antibacterial activity of the zinc oxide nanoparticles.

MATERIALS AND METHODS

COLLECTION AND PREPARATION OF SEAWEED EXTRACT

Seaweed was harvested by hand picking only in the low-tidal and subtidal areas of Hare Island, Gulf of Mannar, down to a depth of one meter. To get rid of the epiphytes and silt particles, the materials were carefully cleaned in the field using marine water.In order to begin the process of extracting the bioactive components from the seaweed samples, the dried seaweed materials were ground using an electric kitchen blender.

CRUDE FUCOIDAN EXTRACTION:

A successive extraction procedure (Rioux *et al.*,2007) was used to extract fucoidan and alginate with a few alterations. To eliminate the pigments, 100 g powdered material was immersed in 500 ml of a 1:1 v/v chloroform/methanol solution for a whole night. Subsequently, the residue was extracted in three different solvents for three hours at 70°C: 2% CaCl2, 0.01 N HCl and 3% Na2CO3. Crude fucoidan was produced by combining and concentrating extracts obtained in 2% CaCl2 and 0.01 N HCl using a freeze dryer. Sodium alginate, the final residue extracted in aqueous 3% Na2CO3, was precipitated in acetone. The amount of total sulfate (Dodgson *et al., 1962*) and total sugar (Dubois *et al.*,1956) present in the crude fucoidan was estimated and recorded.

BIOSYNTHESIS OF ZnO NANOPARTICLES

For the biosynthesis of ZnONPs 5 ml of fucoidan extract and 95 ml of 0.01 M zinc acetate dihydrate solution ($Zn(C_2H_3O_2)_2.2H_2O$) were mixed together. The mixtures were continuously stirred for one hour at 70°C during the incubation period. NaOH was used to bring the pH to an alkaline (pH = 10) range. Following the incubation period, the mixtures produced powdered precipitates, which were subsequently centrifuged for 30 minutes at 3000 rpm. After decanting the supernatant, distilled water was used to completely wash the precipitate. Pellets were then moved to Petri dishes and left overnight at 60 °C to ensure thorough drying (Naseer M *et al.*, 2020).

OPTICAL CHARACTERISATION OF ZINC OXIDE NANOPARTICLES

The obtained Zn-NPs were first characterized by UV-vis absorption spectroscopy (Halo DB-20 UV-vis double beam). The reaction mixtures were scanned in the wavelength (*λ*) range between 200 and 800 nm.To determine the functional groups of the ZnO NPs, FT-IR analysis was conducted (SHIMADZU IR TRACER-100).

ANTIBACTERIAL ACTIVITY ASSAY BY DISC DIFFUSION METHOD (Kirby & amp; Bauer1966)

Using the agar disc diffusion method, antibacterial susceptibility tests were performed against five drug-resistant bacterial species. The five drug-resistant pathogenic microorganisms utilized in the experiment were *Pseudomonas aeruginosa, Bacillus subtilis, Bacillus cereus, E. coli,* and *Bacillus subtilis*. The test bacteria was inoculated in peptone water and incubated for 3 – 4 hours at 35 ºC. Mueller hinton agar plates were prepared and poured in sterile petri plates. 0.1 ml of bacterial culture was inoculated on the surface of Mueller hinton agar plates and spread by using L-rod. The inoculated plates were allowed to dry for five minutes. The disk loaded with samples concentration 1000 µg/ml was placed on the surface of inoculated petri plates using sterile technique. The plate was incubated at 37 ºC for 18-24 hours. The plate was examined for inhibitory zone and the zone of inhibition was measured in mm.

RESULTS AND DISCUSSIONS

UV-visible spectroscopic analysis of ZnO NPs

The biogenic production of ZnO NPs was verified by UV-visible spectroscopic spectroscopy. The sample was dissolved in deionized water for this standard analysis. The wavelength range of UV-visible light was 200–800 nm. The peak measured at 370 nm (Fig. 1) indicated that ZnO NPs were present in the mixture. The wide band of absorption that reaches beyond wavelengths could be attributed to the mobility of the electronic cloud on the overall skeleton of ZnO NPs.

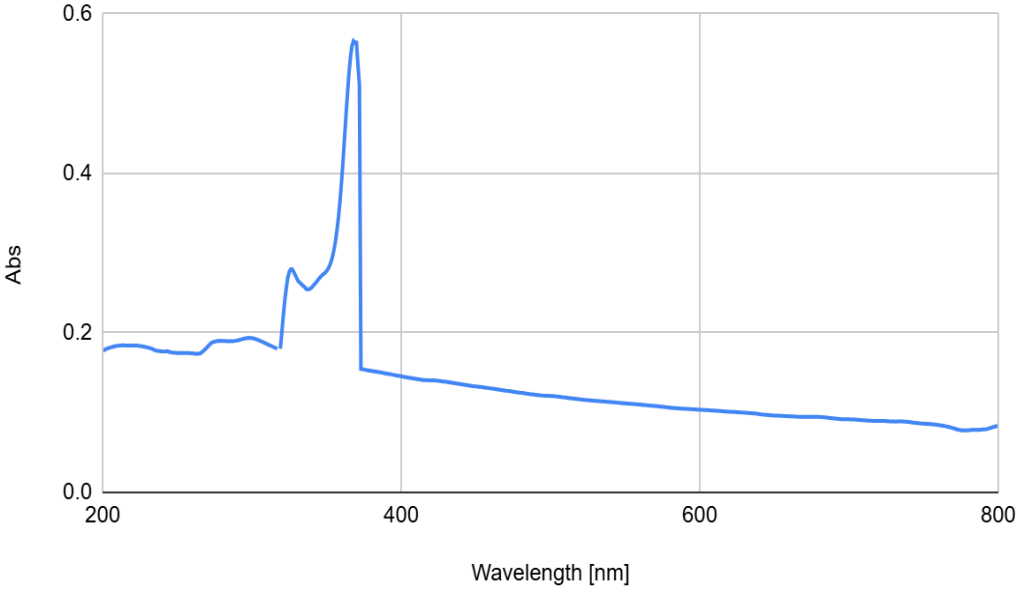

Fig.1 UV-Vis Spectra graph of ZnONps obtained from Fucoidan

FT-IR ANALYSIS

FT-IR spectroscopy is used to identify where the Zn coordinates with the structure of fucoidan. The spectrum of fucoidan exhibited a broad band at 3452 cm−1 and a band at 1638 cm−1 assigned to the –OH stretching and bending vibrations. The bands within 2924cm−1 and 1407 cm−1 are assigned to the C–H stretching in the pyranoid ring. The band at 1777 cm−1 is associated with C=O stretching. The bands at 1189, 1048 and 1104 cm−1 are responsible for C–O stretching and a band at 1255 cm−1 indicates S=O asymmetric stretching of sulfate groups. The signal at 815 cm-1 characterizes the sulfation at the equatorial position where the sulfate ester binds to the of fucose to form sulfate fucose. The spectra show the peak around 785 cm-1 due to the stretching mode of the zinc and oxygen bond (ZnO). The significant changes might be due to complexes of zinc with the fucoidan sites.The FT-IR spectrum of phyto-fabricated ZnO NPs in the wavenumber range from 500 to 4500 cm−1 is shown in Fig.2.

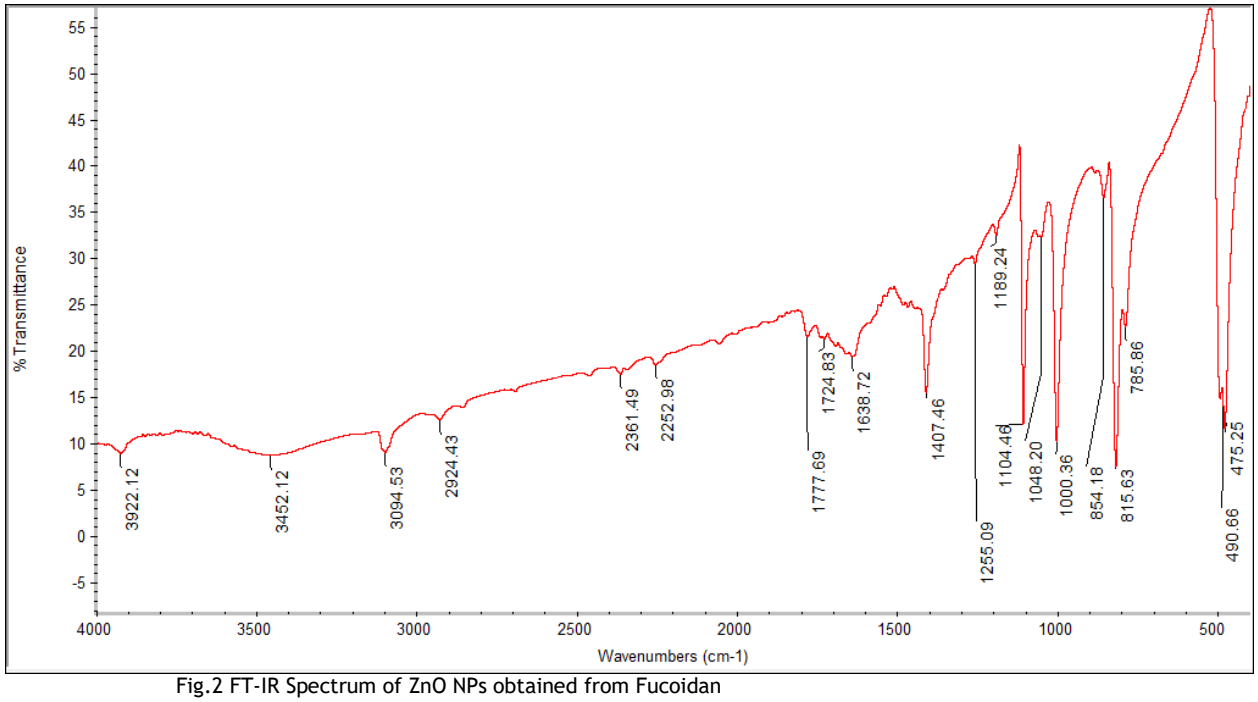

Fig.2 FT-IR Spectrum of ZnO NPs obtained from Fucoidan

ANTIBACTERIAL ACTIVITY

The antibacterial activity of the disc diffusion method was employed in this investigation. This approach is uncomplicated and economically viable. The disc diffusion method is also employed to determine which particular antibiotic is vulnerable to resistance in an organism. The National Committee of Clinical Laboratory Standards (NCCLS) has approved this standardized process for assessing antibiotic susceptibility, according to Salvador *et al.*, (2007). The findings showed that ZnO NPs have strong bactericidal effects on both Gram-positive and Gram-negative microscopic organisms. In comparison to Gram-negative bacteria (*Pseudomonas aeruginosa* and *E. coli*), Gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis,* and *Bacillus cereus*) showed larger inhibitory zones (Table 1). This could be because of the differences in their cell walls' composition (S. Shrivastava *et al.*,2007; Shah *et al.*,2014).

Table -1 ANTIBACTERIAL ACTIVITY OF BIOSYNTHESIZED ZnONPs

Bacteria	INHIBITION ZONE IN mm	
	Ab Ampicillin	ZnO NP
E. coli	8	9
Staphylococcus aureus	8	11
Bacillus subtilis	7	10
Bacillus cereus	8	9
Pseudomonas aeruginosa	9	8

CONCLUSION

This study has identified a simple, economical, and environmentally friendly way to synthesize zinc oxide nanoparticles (ZnO NPs) using fucoidan extract from *Sargassum wightii*, marine brown algae at room temperature as a useful green stabilizing and reducing agent. The synthesis of stable, amorphous ZnO NPs is demonstrated by the spectroscopic characterisation techniques. ZnO NPs that have been produced have strong antibacterial efficacy against the five pathogenic microorganisms that have been chosen. Conventional chemical methods of synthesising nanoparticles are expensive, time-consuming, and dangerous to dispose of in the environment. Major ailments like lung and skin cancer can arise from interaction with these chemically manufactured nanoparticles. As a result, this green synthesis strategy seems to be a more environmentally friendly option than traditional chemical procedures and serves as a useful substitute medication that may be employed in the future in biomedical applications.

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