

**BIOSYNTHESIS AND CHARACTERIZATION OF ZINC OXIDE
NANOPARTICLES FROM MARINE BROWN ALGAE *SARGASSUM
WIGHTII* AND ITS ANTIMICROBIAL ACTIVITY**

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ABSTRACT

In modern science, Nanotechnology is a mature and advanced field for researchers. Zinc Nanoparticles (ZnO NPs) are known to be one of the most multifunctional inorganic nanoparticles with its application in treatment of various diseases. The present study focuses on the production of ZnO nanoparticles from Zinc Nitrate Hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) using the methanol extracts of a *Sargassum wightii*. Nanoparticles were synthesized using *Sargassum wightii* extract and were characterized by UV-visible spectroscopy (UV-vis), Fourier transform infrared spectroscopy (FT-IR) and Scanning electron microscopy (SEM). Therefore, the study reveals an efficient and simple method for the green synthesis of multifunctional ZnO NPs using *Sargassum wightii*. The results showed that the extract is

optimum for the synthesis of ZnO NPs and it is also known to have the ability to inhibit the growth of various pathogenic microorganisms. The synthesized ZnO NPs can be used for various applications due to its eco-friendly, non-toxic and compatibility for pharmaceuticals. It indicates the biomedical capability of ZnO NPs.

KEYWORDS: Green synthesis, Zinc nanoparticles, Antimicrobial activity, *Sargassum wightii*.

INTRODUCTION

In recent years, Nano materials are being used in a wide variety of applications due to its varying properties on scaling down from bulk size to nanometre size (10^{-9}m). The surface

area to volume ratio plays an important role in Nanoparticles, due to which they become more reactive. Nanotechnology and Nanoparticles based product and application are increased now a days due to various fields like biotechnology, physics, chemistry, material sciences, engineering, and medicine. ZnO NPs are being widely under use in a variety of fields due to its uniqueness and attractiveness in their properties like electrical, optical, dermatological and antibacterial.^{[4][5]} This makes them to be a promising element the widely distributed fields like automobiles, electronics, optoelectronics, textiles, medicine, drug delivery and cosmetics.

Most commonly, ZnO NPs are produced through chemical methods, like sol-gel processing, precipitation and electro deposition method.^{[6][7]} Zinc has been found highly attractive because of its remarkable application potential in solar cells, piezoelectric devices, UV absorbers, pharmaceutical and cosmetic industries. Potentially, Zinc removed all the dyes and water pollutants from textile effluent under UV light have been proved. Nanoparticles exhibit completely new or improved properties with larger particles of the bulk materials and these novel properties are derived due to the variation in specific characteristics such as size, distribution and morphology of the particle.^[8] 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.^[9] The growing need of environmental friendly nanoparticles, researchers are using green methods for the synthesis of various metal nanoparticles for pharmaceutical applications.^[10]

Although different biological based synthetic methods are known for Zn are sought by researchers. Biological process has led to the development of an eco-friendly approach for the synthesis of nanoparticles. The use of non-toxic materials like plant extract & bacteria for synthesis of ZnO NPs offers numerous benefits of pharmaceutical application.^[11] Biological methods of nanoparticles synthesis using microorganisms, enzymes, fungus and plants have already shown to be possible.^[12]

Sargassum wightii is among the widely found marine brown algal species in India. It is dark brown to blackish in colour when dry. The colour of the phaeophyceae results from the dominance of the pigment fucoxanthin which masks the other pigments (including chlorophyll a and c, betacarotene and other xanthophylls). It belongs to the family Sargassaceae and order Fucales. It is found to be the most diverse genus among Phaeophyta in India and is represented by 38 species.^[1] It has tremendous biological applications and

known to be rich in sulphated polysaccharide content. It has been used traditionally for treating scrofula, goiter, tumor, edema, testicular pain and swelling.^[2] It has been reported that pharmacological activities of *Sargassum* include anticancer, anti-inflammatory, antibacterial and antiviral activities.^[3] Considering the chemical and immense pharmacological properties of brown algae, the present study was aimed to explore the nanoparticle synthesis of Zinc and its antimicrobial activity in methanolic extract of *Sargassum wightii*.

MATERIALS AND METHODS

Collection of Algae Materials

The fresh sample of *Sargassum wightii* was collected from the Rameshwaram sea shore, Tamil Nadu. Collected sample was washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles in refrigerator.

Preparation of Extract

Crude Sample extract was prepared by Soxhlet extraction method. About 20gm of air dried sample was extracted with 250ml of methanol. The process of extraction has to be continued for 24 hours or till the solvent in siphon tube of extractor become colourless. Then the extracts were collected separately and stored at the refrigerator for further studies. Dried extract was kept in refrigerator at 4°C till future use.

Bio synthesis of ZnO Nanoparticles

To the methanol extract, Zinc Sulfate, Sodium Hydroxide solution was added slowly drop wise in a molar ratio of 1:2 under vigorous stirring, and the stirring was continued for 12 hrs. The precipitate obtained was filtered and washed thoroughly with deionized water. The precipitate was dried in an oven at 100°C and ground to fine powder using agate mortar (Vazquez *et al.*, 2013). The powder obtained from the above method was calcined at different temperatures.

Characterization of nanoparticles

The pure sample was analyzed for UV–vis absorption and optical band gap (Eg) using UV–Vis spectrophotometer (a Lambda 25-Perkin Elmer). The functional group of Nanoparticles were examined by using FTIR spectrometer (Perkin-Elmer 1725X). The shape and size of the sample were characterized by using field emission scanning electron microscope (FESEM)

(JSM-6360LA). Size distribution and the average size of the nanoparticles were estimated on the basis of FESEM image.

Determination of Antibacterial Activity

The disc diffusion method (Bauer *et al.*, 1966) was used to screen the antimicrobial activity. *In vitro* antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained from Hi-media (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The concentration of extracts is 40 mg/disc was loaded on 6 mm sterile disc. Standard antibiotic (Chloramphenicol) was used as positive control and bacterial plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Determination of Antifungal Activity

The agar well diffusion method (Perez, 1993) was modified. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar. Standard antibiotic (Fucanazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37°C for 72 hrs. The diameters of zone of inhibition observed were measured.

RESULTS AND DISCUSSION

Visual Observation

ZnO NPs have attracted great attention because of their superior optical properties. Visual colour change is the preliminary test for nanoparticle synthesis. The synthesis of ZnO NPs synthesized using freshly prepared *Sargassum wightii* fresh extract. Colour change from half white to pale yellow represents the synthesis of ZnO NPs.

UV-Visible Analysis

UV-visible spectroscopy is usually conducted to confirm the synthesis of ZnO NPs. Conducting electrons start oscillating at a certain wavelength range due to surface Plasmon resonance (SPR) effect. Fig.1 represents the UV-visible spectra of freshly prepared ZnO NPs. Peak obtained at 296.47 nm clearly demonstrates the presence of ZnO NPs in the reaction mixture.

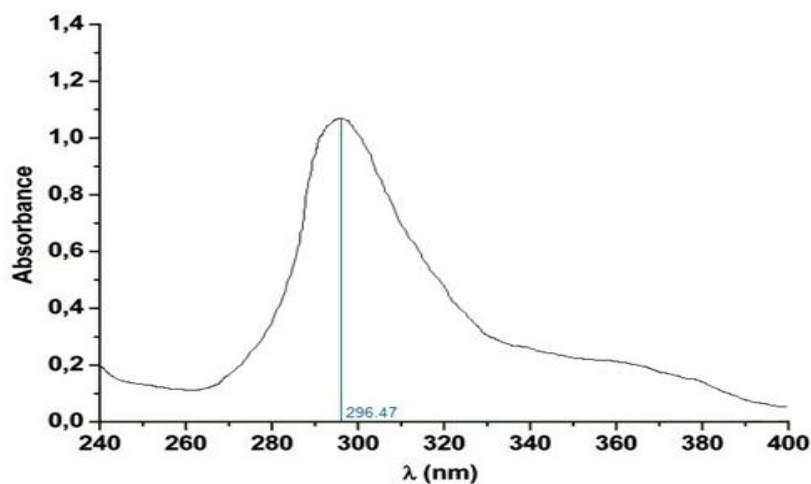


Fig 1: UV-Visible analysis of *Sargassum wightii*.

FTIR Analysis

Substance-specific vibrations of the molecules lead to the specific signals obtained by IR spectroscopy. FT-IR spectra and functional group involved in ZnO NP synthesis illustrated peak in the range of 500–3500 cm^{-1} (Fig.2). Broad peak obtained at 3496.34 cm^{-1} corresponds to primary amines (medium), 2864.17 cm^{-1} corresponds to alkanes (strong), 1738.69 cm^{-1} corresponds to aliphatic esters (very strong), 1039.05 cm^{-1} corresponds to primary amines (weak to medium) and 648.29 cm^{-1} corresponds to aromatic methane (strong).

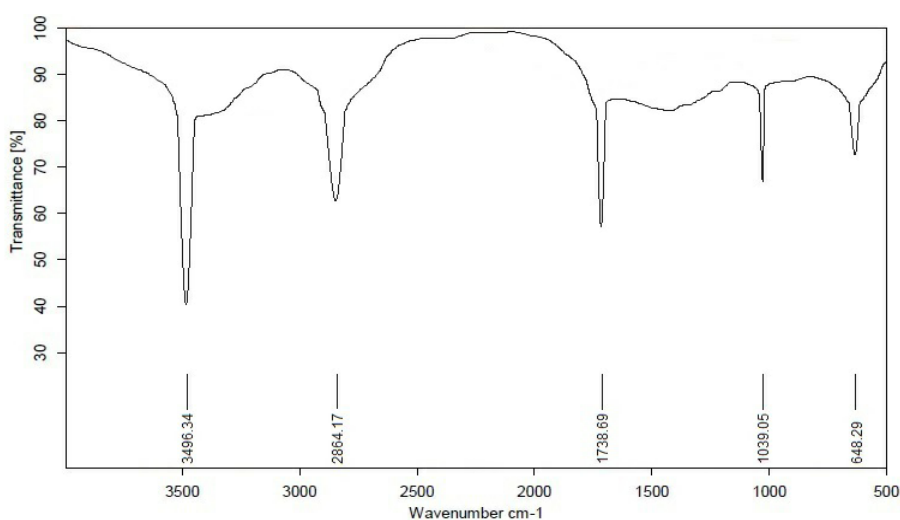


Fig 2: FTIR analysis of *Sargassum wightii*.

SEM Analysis

SEM analysis is done to visualize shape and size of nanoparticle. Scanning electron microscope was used to determine the shape of *Sargassum wightii* capped ZnO NPs in Fig.3.

SEM images were seen in different magnification ranges like 2 μm which clearly demonstrated the presence of spherical shaped nanoparticle with mean average diameter.^[13]

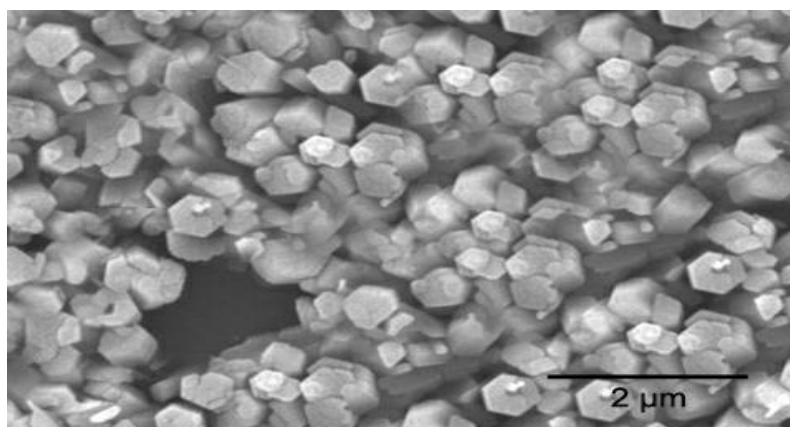


Fig 3: SEM analysis of *Sargassum wightii*.

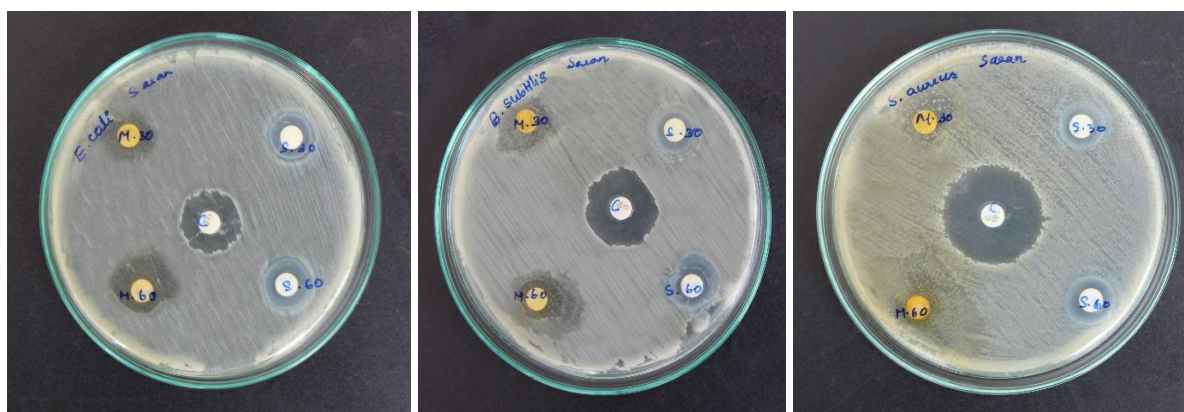
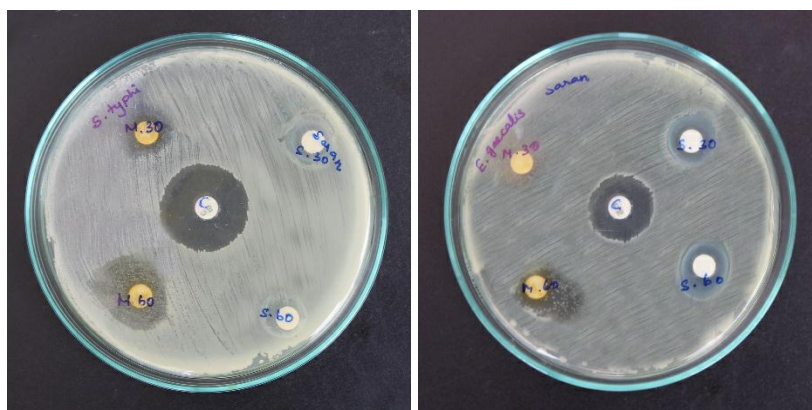
Antibacterial Activity

Agar disc diffusion technique was adopted to perform the assay. Anti-bacterial effect of ZnO NPs was visualized against pathogen like *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi* and *Enterococcus faecalis*. Chloramphenicol disc was used as a control. Results of Table.1 clearly demonstrate that the nanoparticle showed anti-bacterial effect in a dose-dependent manner.

Table 1: Comparison of Antibacterial activity using Disc Diffusion method.

Organisms	Control	Methanol extract		ZnO NPs	
		30 μl	60 μl	30 μl	60 μl
<i>Escherichia coli</i>	18 mm	16 mm	20 mm	13 mm	15 mm
<i>Bacillus subtilis</i>	21 mm	13 mm	18 mm	13 mm	15 mm
<i>Staphylococcus aureus</i>	27 mm	14 mm	22 mm	11 mm	15 mm
<i>Salmonella typhi</i>	25 mm	15 mm	18 mm	14 mm	17 mm
<i>Enterococcus faecalis</i>	19 mm	13 mm	18 mm	15 mm	16 mm

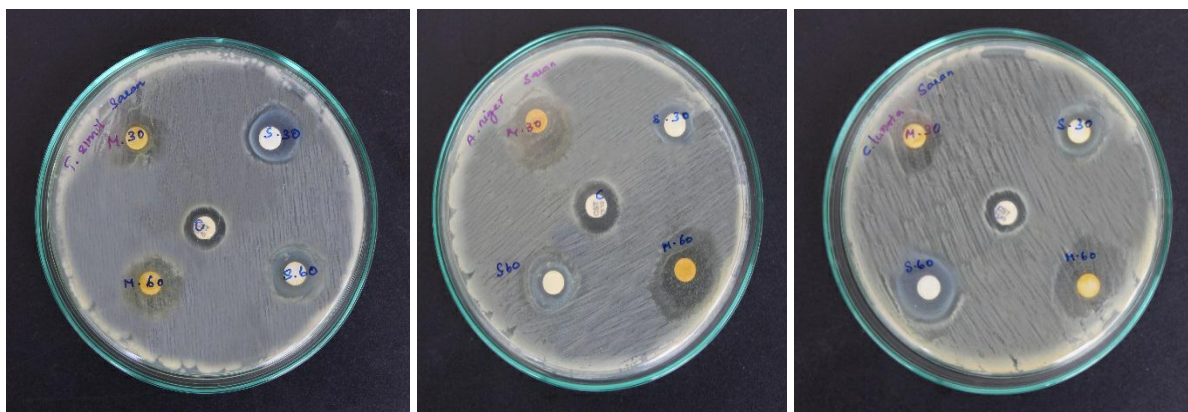
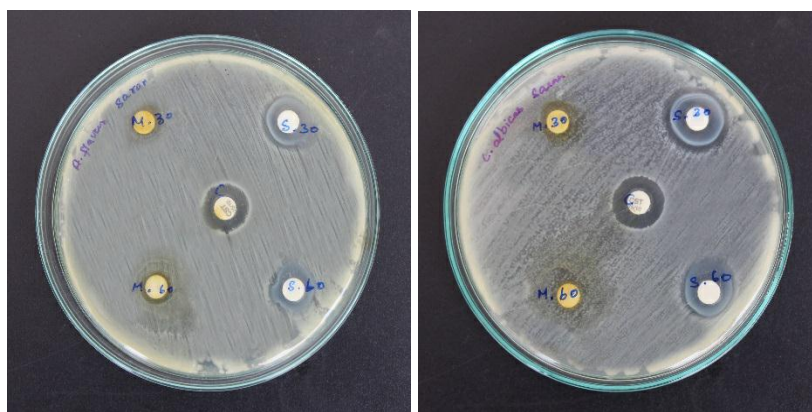
Fig. 1 visually shows zone of inhibition for nanoparticles was much lower than standard disc, which mandates the need of further engineering to achieve the desired effects from nanoparticles. The ZnO NPs are inhibit the microbial growth in *in-vitro* antimicrobial activities.^{[14][15]} Methanol extract showed better antibacterial activity when compared with ZnO NPs. The highest zone of inhibition occurs in *Staphylococcus aureus*.

A) *Escherichiae coli*B) *Bacillus subtilis*C) *Staphylococcus aureus*D) *Salmonella typhi*E) *Enterococcus faecalis***Fig. 4: Antibacterial activity of methanol extract and ZnO NPs****Antifungal Activity**

Anti-fungal effect of ZnO NPs was visualized against pathogen like *Trichophyton simii*, *Aspergillus niger*, *Cochliobolus lunata*, *Aspergillus flavus* and *Candida albicans*. Fucanazole disc was used as a control. Results of Table 2 clearly demonstrate that the nanoparticle showed anti-fungal effect in a dose-dependent manner. The methanol extract and ZnO NPs extract showed similar activity to some extent. In Fig.5, *Aspergillus niger* and *Candida albicans* shows higher zone of inhibition in methanol and synthesised extract respectively.

Table 2: Comparison of Antifungal activity using Disc Diffusion method.

Organisms	Control	Methanol Extract		ZnO NPs	
		30 µl	60 µl	30 µl	60 µl
<i>Aspergillus niger</i>	19 mm	21 mm	23 mm	12 mm	16 mm
<i>Cochliobolus lunata</i>	14 mm	13 mm	18 mm	13 mm	16 mm
<i>Aspergillus flavus</i>	12 mm	9 mm	13 mm	14 mm	16 mm
<i>Candida albicans</i>	16 mm	11 mm	17 mm	19 mm	21 mm
<i>Trichophyton simii</i>	12 mm	8 mm	16 mm	12 mm	20 mm

A) *Trichophyton simii*B) *Aspergillus niger*C) *Cochliobolus lunatus*D) *Aspergillus flavus*E) *Candida albicans***Fig. 5: Antibacterial activity of methanol extract and ZnO NPs****CONCLUSION**

In conclusion, the field of nanoscience and nanotechnology is the development of eco-friendly processes for synthesis of ZnO NPs. Green synthesis of nanoparticles used in this experiment is found to be eco-friendly, non-toxic and less usage of chemicals compared to physical and chemical method. The presence of phytochemicals in the extract itself helps in the synthesis of metal nanoparticle by inducing oxidation and reduction reaction. Here we have reported the ZnO NPs were successfully synthesized by using *Sargassum wightii* extract for the antimicrobial activity. The structural characteristics and morphology of the obtained ZnO NPs were studied using the SEM techniques.

The results confirmed the presence of ZnO NPs without any impurities and in stable state. The optical characteristics of ZnO NPs were studied using the UV analysis. The peak in the absorption spectrum is confirmed the formation of ZnO NPs. The functional group present in the extract was confirmed by FTIR analysis. Further antimicrobial activity of *Sargassum wightii* extract and synthesized ZnO NPs were investigated in the disc diffusion method.

From the results it is clear to know that the ZnO NPs from *Sargassum wightii* extract also have the ability to inhibit the growth of various pathogenic microorganisms like *E.coli*, *B.subtilis*, *S.aureus*, *S.typhi*, *E.facelis*, *T.simii*, *A.niger*, *C.lunata*, *A.flavus* and *C.albicans*.

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