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Antimicrobial Potential of Zinc Oxide Nanoparticles from Marine Macroalgae

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Abstract: ***The current study evaluates the free antimicrobial and radical scavenging properties of zinc oxide nanoparticles(ZnO-NP's) synthesized from an aqueous extract of Sargassum polycystum brown algae. The free radical scavenging potential of ZnO nanoparticles was evaluated through DPPH, Nitric oxide, Hydroxyl radical and Hydrogen peroxide scavenging assays. In addition to that, the antibacterial and antifungal activity of synthesized ZnO nanoparticles was examined against different species. The free radical scavenging and antimicrobial potential of these Sargassum polycystum ZnO nanoparticles were associated with their phenolic compounds. ZnO- NP's DPPH radical reducing potential was found to be 12.45 ± 0.19µg/mL, Nitric oxide at 0.68±0.04 µg/ml, Hydrogen peroxide to be 0.39±0.01 µg/ml and Hydroxyl radical scavenging potential with 0.63±0.04 µg/ml. Furthermore, ZnO- NP's antimicrobial efficacy showed significant results against Staphylococcus aureus, Escherichia coli, Salmonella typhi, Klebsiella pneumonia and fungal strains of A. niger Candida albicans. Further research is on purifying the selected phenolic compounds and developing their acuteness for specificity for ultimate pharmaceutical use.***
Keywords: Marine algae, ZnO nanoparticles, antioxidant and antimicrobial activity, Gulf of Mannar.

I. INTRODUCTION

Seaweeds are extensively used in nutrition and biomedicine, particularly in East Asian countries. There is evidence at these locations that certain types of cancer and breast and prostate cancer are low due to the constant use of seaweed, the main reason being their wealth of bioactive compounds that can protect against such diseases[1][2]. Several research studies in the healthcare and pharmaceutical industries have been conducted to determine the role of marine algae in human welfare[3].

Intracellular chemicals and endogenous metabolism in the human body produce hazardous reactive oxygen species (ROS) that interact with specific cellular components.

In recent years, the role of free radicals and antioxidants has increased in recent developments in disease prevention. Hence, the critical role of free radicals in disease prevention and antioxidants will need to be examined. Marine algae can remove the metabolic defects caused by various substances linked to free radicals[4].

Marine species are typically targeted to recognize antimicrobials, specifically active compounds against pathogenic bacteria[5] . In the secondary metabolites of algal groups such as Phaeophyceae (brown), Rhodophyceae (red), Chlorophyceae (green), Chrysophyceae (golden) and Bacillariophyceae (diatoms), modern screening methods defined antibacterial compounds[6].

The use of algal extracts in nanoparticles preparation has been suggested as possible ecologically friendly alternatives to chemical and physical methods.

A comprehensive literature survey shows that nanoparticle phytosynthesis is a simplistic, appropriate and feasible approach with practical applicability[7]. The study of these biosynthesized nanoparticles free radical scavenging activity gives an idea of the activity and connection of these nanoparticles with living systems biomolecules.

Herein, we targeted to estimate the free radical scavenging and antimicrobial activity of zinc oxide nanoparticles of seaweeds.

II. MATERIALS AND METHODS

A. Collection of Seaweeds

Samples from shallow waters of the Gulf of Mannar region were collected. The collected seaweeds were washed with salt water to remove all the epiphytes, and the seaweeds were subsequently air-dried. Finally, the seaweeds were cut into small pieces, powdered, and used for further tests.

B. Preparation of algal extract

The crude algal extract (CAE) was prepared using 10 g algal powder in 100 ml of water and held for 24 hours in the revolving shaker, filtering and collecting the solvent used for further research.

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C. Bio synthesis of ZnO-NP's from Sargassum polycystum aqueous extract

10 ml of aqueous extract was added to 300 ml of 4 mM zinc sulphate heptahydrate solution and stirred at room temperature for 5 minutes to achieve the pale yellow solution. Firstly, 1M sodium hydroxide solution was added to the mixture dropwise, stirring continuously at room temperature. The suspended particles were purified by dispersing them in sterile distilled water. The white particles were then washed with ethanol to remove the impurities for the end product. After drying at 60ºC in the vacuum oven for 6 hours, a white powder was then collected, and the sample was processed for further studies[8].

D. Screening of anti-radical Scavenging Potential

1) *DPPH radical Scavenging Activity:* Various concentrations of the ZnO-NPs were mixed with 1.0 ml of DPPH methanolic solution, where the final concentration of DPPH ended with 0.2 mM. The above solution mixed thoroughly and left to stand for 30 min, and the absorbance was measured at 517 nm. BHA (Beta Hydroxy Acid) was used as a control. The percentage of DPPH decolourization of the sample was calculated according to the equation % decolourization = [1-(ABS sample/ABS control)] x 100[9].
2) *Nitric Oxide Radical Scavenging Assay:* The reaction mixture made with 4.0 ml of sodium nitroprusside solution, 1.0 ml of phosphate buffer saline and various concentrations of ZnO-NPs (50 - 250µg) in DMSO was incubated at 250ºc for 15 minutes.). 0.5 ml of solution was removed after the incubation period, sulphanilic acid solution 1.0 ml was added, mixed well and allowed to stand for 5 mins to complete diazotization. Then 1.0ml NEDD was added and stood for 30 mins in diffused light. A pink coloured chromophore formed at 540nm against the corresponding blank solution. Ascorbic acid was used as standard[10]
3) *Hydrogen Peroxide Scavenging Activity:* The reaction mixture was prepared with various concentrations of standard and ZnO-NPs (50 - 250µg) followed by 0.6 ml of H2O2 was added to all the tubes. Finally, the samples were read at 230 nm in UV /Visible spectrophotometry. 3.4 ml of buffer and 0.6 ml of H2O2 alone served as the blank solution. Ascorbic acid was used as standard[11].
4) *Hydroxyl Radical Scavenging Activity:* The formulated reaction mixture contains 0.7 ml of 6mM Hydrogen peroxide, 0.3 ml of 20 mM sodium salicylate, 1.0 ml of 1.5 mM FeSo4 and various concentrations of the ZnO-NPs (50 – 250 µg). The remaining solution was kept it for one hour incubation with 37^{o}c. The reaction mixture was measured at 562 nm with the absence of the hydroxylated salicylate complex. The percentage of scavenging effect was calculated with standard Ascorbic acid[12].

E. Screening of Antimicrobial Properties

1) *Preparation of Culture Media:* Dehydrated media and standard antimicrobial drugs were obtained from Hi-Media Laboratories Ltd, India. The bacterial cultures used in the present study include *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia*, *A. niger and Candida albicans.* The cultures were obtained from the Department of Microbiology, P.S.G College of Arts and Science, Coimbatore. To determine the antimicrobial properties of ZnO-NP's disc diffusion method was followed. The plates were investigated for evidence of an inhibition zone, which appears as a clear area around the wells. The diameter of such inhibitory zones was measured with a meter ruler. Fluconazole and Amphotericin B were used as positive reference[13].

III. RESULTS AND DISCUSSION

A. Green synthesis of zinc oxide Nano particles (ZnO-NPs)

ZnO-NPs were synthesized from an aqueous extract of *Sargassum polycystum* by the green synthesis method, which is more reliable and less toxic. The formation of pale white colour within three hours of preparation showed the synthesis of ZnO-NPs. The colour change is attributed to the collective oscillation of free electrons induced by an interacting electromagnetic field in metallic nanoparticles

B. UV–vis analysis of ZnONPs

The formation of ZnO-NPs was confirmed by the appearance of turbid yellow solution and change in Surface Plasmon Resonance (SPR) UV–vis spectroscopic analysis. The ZnONPs formations were identified via colour change after 15 minutes. Figure 1 shows that the ZnONPs UV–vis spectra recorded at 285 nm correspond to the shape. The UV spectrum results revealed that the absorbance peak at 285 nm corresponds to the characteristics of ZnO-NPs [14].

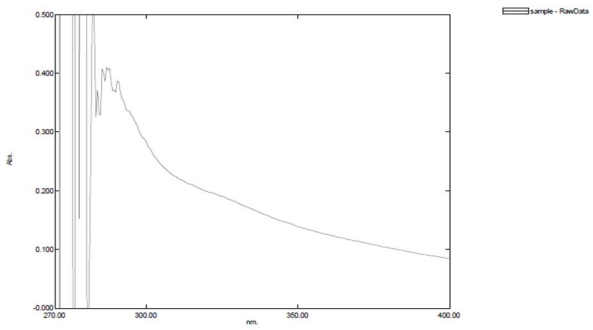


Fig. 1 UV- Vis spectrum of zinc oxide nanoparticles

C. Inhibition of DPPH radicals – Photometric assay

The most vital DPPH radical scavenging activity was exhibited by ZnONPs were IC50 = 12.45 ± 0.19µg/mL, for standard ascorbic acid, IC50 was found to be 20.26 µg/ml. The percentage of radical scavenging activity and thus the degree of discolouration of free radicals by ZnONPs of *Sargassum polycystum* were determined against DPPH. Regression equations to derive the IC50 values showed an inverse relationship between the IC50 value and the percentage scavenging potential of a sample. ZnONPs exerted a promising DPPH scavenging effect with an increase in the concentration of the pieces. Bio compounds are absorbed on the surface of the ZnO-NPs during the synthesis. Due to the higher surface area of ZnO-NPs, they appear to have a high propensity to communicate with DPPH. The DPPH contains an unusual electron responsible for the 517 nm absorption and the deep purple colour[15].

D. Inhibition of Nitric oxide (NO) Radical Generation

In vitro inhibition of NO radical is also a measure of antioxidant activity. The *S.polycystum* ZnONPs have exerted the maximum nitric oxide inhibition (66.59% inhibition). The IC 50 value was 0.68±0.04 µg/ml in nanoparticles, respectively. Standard Ascorbic acid inhibits 53.95% of Nitric oxide radical. IC50 value of normal ascorbic acid was 0.54±0.03. The radical inhibition might be due to antioxidants present in the ZnONPs, which entirely react with nitric oxide, thus inhibiting nitrite generation. The NO revolutionary development is the product of sodium nitroprusside decay in an aqueous environment. Increased growth of these nitric oxide and superoxide radicals leads to the pathogenesis of inflammatory diseases[16].

E. Inhibition of Hydrogen Peroxide Radical Generation

The maximum scavenging potentials of nanoparticles were observed at 250µg/ml concentration, and it is found to be 61.59% in *Sargassum polycystum* ZnONPs. The IC50 was 0.39±0.01 µg/ml in *S. polycystin* ZnONPs respectively. Standard Ascorbic acid had 58.36% inhibition, and the IC50 value of Ascorbic acid was 0.26±0.01. The scavenging activity of *S.polycystum* ZnONPs increased dose-dependent through the neutralization of H_2O_2. Hydrogen peroxide is a natural cellular metabolite produced and maintained continuously at low concentrations. It inactivates a few enzymes, usually by oxidation of thiol groups. It can easily cross cell membranes and react to hydroxyl radicals with Fe2 + and possibly Cu2 + ions, which might cause many of its toxic effects[17]. The results show that the *S.polycystum* ZnONPs can decompose H_2O_2, which could be due to the presence of biologically active phytoconstituents.

F. Hydroxyl Radical (OH-) Scavenging Activity

As the concentration of algal nanoparticles increased, the scavenging potential against hydroxyl ions has also increased. The maximum scavenging potentials of nanoparticles were observed at 250 µg/ml concentration, and it is found to be 70.033% in *S.polycystum* ZnONPs. The IC50 was 0.63±0.04 µg/ml in *S.polycystum* ZnONPs, and the standard ascorbic acid had 63.45% scavenging activity; IC50 value was found to be 0.59±0.03 respectively. Hydrogen peroxide and superoxide are required in the presence of a metal catalyst to form hydroxyl radical, which is the altered free radical responsible in combination with molecular oxygen for cellular damage and oxidative degradation of macromolecules[18]. Therefore, the marked ability of algal ZnO-NPs scavenges both hydrogen peroxide and superoxide radical inhibit hydroxyl radical formation and protect the macromolecules from oxidative damage.

G. Screening of Antimicrobial Properties

1) *Antibacterial activity of Sargassum polycystum:* Nanoparticles were found to produce a maximum zone of inhibition against *Escherichia coli* (11 mm) *Klebsiella pneumonia* (17 mm), and a minimum inhibitory area was located against *Bacillus substiles* (8 mm). It was found to be much effective against all the Gram harmful bacteria. Examined bacterial strains were positive response to the standard drug Amphotericin B and DMSO was used as the negative control that did not exhibit any zone of inhibition against the tested bacteria.
2) *Antifungal activity of Sargassum polycystum:* ZnO-NPs exhibited effective inhibition against all the fungal strains tested with a zone of inhibition ranging from 10 mm (*A.niger*) *to* 7 mm (*Candida albicans*), respectively. All fungal strains were found to be sensitive with standard antibiotic Fluconazole. DMSO was used as the negative control, which shows no zone of inhibition against tested fungi. Similarly, the antibacterial and antifungal activity of the ZnO-NPs against *Campylobacter jejuni*, *Salmonella enterica*, and *Escherichia coli* has been reported previously[19]. Furthermore, the antimicrobial effect of ZnO-NPs might be attributed to disruption of the bacterial cell membrane. The shape and size of the nanoparticles also play a vital role in determining antimicrobial activities[20].

IV. CONCLUSION

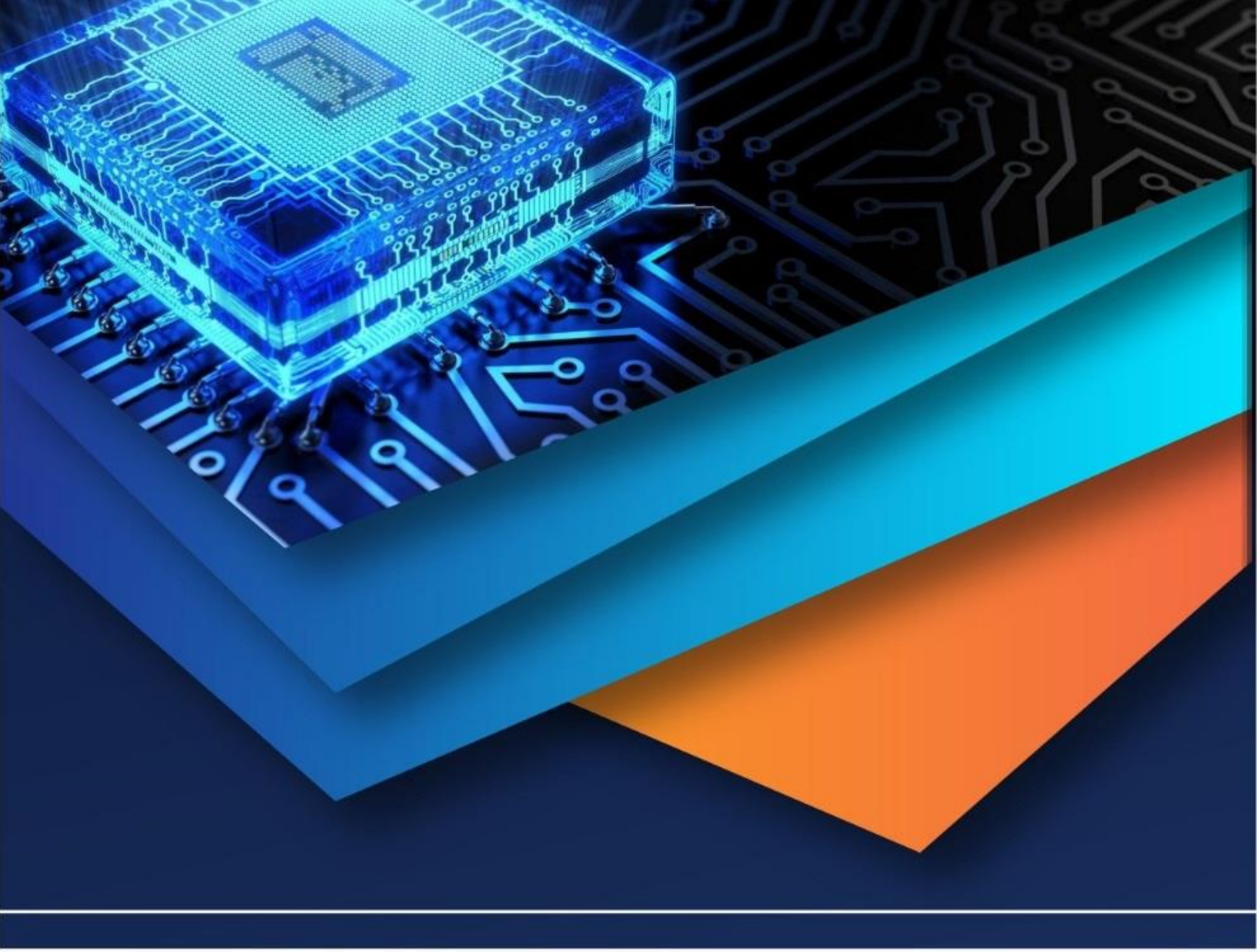
It can be ended that the *S. polycystum* can be an essential source for the biosynthesis of Zinc oxide nanoparticles which shows free radical scavenging and antimicrobial activity. The potential outcome of the study will be the development of value-added products from marine algae *S. polycystum* for biomedical and nanotechnology-based industries.

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