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Green biosynthesis, characterization, and anticancer activity of *Sargassum cinctum* zinc nanoparticles

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This work aims at exploring the green synthesis of ZnO NPs from *Sargassum cinctum*; a brown marine macro algae. In the green synthesis of ZnO NPs, the bioactives present in *Sargassum cinctum* extract are capable of reducing and stabilising agents in the development of the said particles. In contrast to normal approaches, which usually involve the use of harmful chemicals to make nanoparticles, this technique is considered ecologically beneficial.

X-ray diffraction, scanning electron microscopy, and fourier transform infrared spectroscopy studies were employed to characterise the synthesised Sc-ZnO NPs. The crystalline structure, size distribution, and functional groups revealed in the seaweed extract were all supported by these studies and may be the reason for the nanoparticles stability and biological activity. In order to determine the anticancer property of the Sc-ZnO NPs, the particles were tested on breast cancer cell lines. The findings shown in the outcomes showed that Sc-ZnO NPs had the ability to suppress the growth of breast cancer cells.

In general, this research indicates that Sc-ZnO NPs prepared with the help of green techniques based on *Sargassum cinctum* might be considered as a rather effective and environmentally friendly anticancer agent for breast cancer. The observations made allow for the possibility of the further use of these nanoparticles for the creation of new, environmentally-friendly anticancer agents that have less adverse effects on the environment and high selectivity towards cancer cells.

Keywords: Nanoparticles, *Sargassum*, SEM, XRD

The field of nanomedicine emerged in the late 1990 as a result of the merging and integrating of nanotechnology with areas that are medically, biological, and pharmacological, related[1].

Globally, the discipline of nanotechnology is becoming more and more popular and is growing rapidly. The main reason for this is the distinct biological and physical properties that nanomaterials display. It greatly enhances the techniques for creating unique goods with precise form and size at the nanoscale[2].

Three basic methods are often used to synthesise nanoparticles: chemical, biological/green, and physical. Each one of these techniques has unique characteristics. Physical or chemical techniques are often regarded as preferable ways to produce stable nanostructures with identical dimensions, although they cannot lead to long-term sustainability. Green approaches must be developed utilising safe, environmentally acceptable, and emission-free procedures in light of the limitations of current techniques for producing nanomaterials[3].

The global consciousness towards green chemistry and environmentally friendly technologies has inspired a lot of concern on aspects of green chemistry especially in the synthesis of nanomaterials. Within the large family of metal oxide NPs ZnO NPs have been used in various cutting-edge applications like electronics, communication, sensors, cosmetics, environmental protection, biology, and the medicinal industry[4].

Zn is also an essential trace element that is present in the human body. Because Zn^{+2} ions are soluble, it has low toxicity and great biodegradability, which contribute to its biocompatibility.

Researchers in the field of nanotechnology, which has shown to be one of the most active, have focused on biological approaches because they can produce nanoparticles efficiently using natural reduction, capping, and stabilising agents without the use of dangerous materials or high energy consumption[5]. To develop a technique that is safe for biological systems, easily accessible, and environmentally friendly, a variety of plants and microorganisms are being studied[6].

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Coastal algae are a major source of essential biomolecules used in a range of sectors, including as biomedicine, agriculture, food, medicines, and nutraceuticals[7].

One of the most complicated genera of Phaeophyceae is *Sargassum* (*F. Sargassaceae*), a genus of brown algae that is part of the Fucales order and class Phaeophyceae. It is also referred to as sea holly or gulfweed[8,9].

The literature review indicates that *Sargassum* species have potential as sources of a number of classes of secondary metabolites, including chromanols, and chromenes, plastoquinones, glycerides, which have anti-herpes, anti-inflammatory, hepatoprotective, anti-histamine, antiallergic, and anti-cancer properties[9,10].

Several marine species have been looked at as prospective agents for the biosynthesis of ZnO NPs: one of which is *Sargassum cinctum*, a brown marine alga. This species contains high proportions of polysaccharides, polyphenols and other phytochemicals which enable the reduction of zinc salts to zinc oxide, as well as capping and stabilizing the nanoparticles. The ZnO NPs obtained in the present work are free from toxicity and have improved biocompatibility to be used in biomedical fields[11,12].

The prevalence of cancer is rising worldwide in the modern era, necessitating the establishment of innovative and effective cancer treatment strategies. It is essential to look for novel treatments because breast cancer remains one of one of the most prevalent cancers in women. The incorporation of ZnO NPs in cancer treatment has been due to the option it has to generate oxidative stress that kills cancer cells without affecting healthy ones. The biosynthesized ZnO NPs from *Sargassum cinctum* are thought to have a high anticancer potential mostly in breast cancer cells because of the acquired surface morphology and the improved interactions[13].

This work set its objectives towards the green synthesis of ZnO NPs from *Sargassum cinctum* and analysis of physicochemical and anti-breast cancer properties. The purpose of this research is to obtain an understanding of green chemistry combined with nanotechnology in the hope of applying the knowledge to enhancing ways of abolishing cancer in the future.

Materials and Methods

Collection and preparation of extract

The collection of *Sargassum cinctum* from Ramanathapuram in Tamil Nadu, India[11]. To remove any remaining detritus and epiphytes, the gathered algae were extensively cleaned in saltwater. Shade-dried and preserved, the algae underwent a final washing with purified water to eliminate any remaining residues of salts or impurities[14,15].

100 g of dry powder and 1000 mL of distilled water were mixed, heated to boiling, and then reduced to half of their original volume in order to create an aqueous extract. Whattman filter paper was used to filter the extract, which was then stored cold for later study[9,11].

Green synthesis of zinc nanoparticles

Upon weighing and dissolving 2.195 g of zinc acetate [Zn(O2CCH3)2(H2O)2] in 100 mL of Milli-Q-Water, the mixture was placed in a magnetic stirrer for a 1 h to ensure full solubilisation. 20 mL of *Sargassum cinctum* aqueous extract and 80 mL of 0.1 M zinc acetate were combined, and the mixture was constantly agitated[16].

A magnetic stirrer was used to continuously mix the solution throughout the trial. Subsequently, 1 M NaOH was poured dropwise while gently stirring, and the solution was then adjusted to pH values of 7.5, 8.5, 9.5, and 10[17]. Subsequently, the solution heated to 60°C for a duration of one hr, and magnetic stirring was employed for an additional hr. In order to verify the generation of ZnO NPs, the absorbance of the reaction mixture was measured using UV spectrophotometry (Shimadzu UV-1800 Spectrophotometer) over the wave-length range of 200 to 800 nm. After obtaining a white, crystalline precipitation of ZnO NPs, it was repeatedly cleaned using distilled water and tested until a pH of 9.5 was reached[18].

Characterization of biosynthesized green ZnO NPs

The UV1800 Shimadzu UV-visible Double beam spectrophotometer was used to test the properties of the synthesis ZnO NPs. The Bruker BI-200SM is equipped with a beam splitter and detector for FTIR spectroscopy. X-ray diffractometry (XRD) Ultima IV from Rigaku Corporation in Japan was used to analyse the structural makeup of ZnO NPs. Using a Carl Zeiss supra55 SEM (Germany) to view the surface morphology of the material. In order to ascertain the surface charge of ZnO NPs in the solution, the Dynamic Light Scattering and Zeta potential analyser (Malvern Panalytical, Malvern, UK) was utilised.

Characterization of biosynthesized Sc-ZnO NPs

Optical property of Sc-ZnO NPs by UV–vis spectroscopy

Small quantity of the nanopowder was re-suspended in around 10 mL of de-ionized water, and the maximum absorbance was measured using a UV Spectrophotometer (Shimadzu UV-1800) between 200 and 800 nm in order to ascertain the optical property of the synthesised Sc-ZnO NPs[16].

FTIR analysis

The acronym for "Fourier Transform Infrared" is FTIR. FTIR spectra can be obtained using radiation absorbed within 400 and 4000 cm^{-1}. A wide range of spectral data is collected by the FTIR spectrometer. ZnO nanoparticles capacity to capture electromagnetic waves at different frequencies and intensities makes it easier to characterise specific functional groups as well as chemical structures. Due to their distinctive, typical organisation and shape, ZnO nanostructures can be distinguished from other clusters and structures[19-21].

Dynamic light scattering

By measuring the dynamic variations in light scattering intensity caused by the Brownian motion of the particles, the size distribution of the particles was determined by the Zetasizer Nano ZS. The experiment showed significant peaks for the hydrodynamic diameter distribution, average hydrodynamic diameter,and particle size distribution width. The polydispersity index (PdI) was found to be an extra measure. On the PdI scale, 0 represents monodispersity and 1 represents polydispersity.

Each measurement was carried out three times at 25°C for 1 min each to allow for temperature equilibration. The data processing method utilised was high multi-modal resolutio[22,23].

Zeta potential

Zeta potential was examined in order to ascertain the charge on the formed zinc nanoparticle. With the Zetasizer Nano Series, we assessed the nanosynthesis materials size and zeta potential throughout a 0.1-1000 mm range[24]. The electrokinetic potential is another term for the zeta potential. It entails determining the charge stability of the colloidal NPs and computing the "effective" charge that exists on the NP surface.

An NP with a net surface charge has a concentration of oppositely charged ions around it that acts as a "screen" for any charges present. The combination of the external charge layer and the oppositely charged ion layer is known as an electrical dual layer because the ion layer is opposite charged and moves with the NP. The fluid surface that has oppositely charged ions bonded to its surface and the bulk fluid that contains the nanoparticle are measured for difference in electric potential, which is known as the zeta potential. Positively charged surfaces are attracted to particles having a positive Zeta potential, and vice versa. The Zeta potential indicates particle stability; higher magnitude potentials suggest greater stability and electrostatic repulsion[25].

X-Ray diffraction analysis

A lattice constant (d-spacing) can be found and the validity of this structure confirmed using X-ray diffraction (XRD). Zinc nanoparticle crystallite average size can be ascertained *via* XRD. By relating the crystallite size and peak width in the XRD pattern, the Scherrer equation was able to determine the average size of the nanoparticles. During an XRD research, X-rays are guided onto the material using an X-ray diffractometer.

By examining and gathering these diffracted X-rays at various angles (2θ), one can generate a diffraction pattern. The data was collected within a range of 2θ. The D. Scherrer equation was used to calculate the crystalline domain size[26].

$$D = \frac{\delta\lambda}{\beta \cos\theta}$$

where D = average crystallographic domain size, λ = X-ray wavelength (1.54 A0), β = Full Width Half Maximum (FWHM), K = 0.94, and θ is the Brags angle.

Scanning electron microscopy (SEM)

The sample surface morphology is seen by SEM. The electron reflecting off the substance creates a picture. We may learn useful information about the nanoparticle's electrical conductivity, size, shape, composition, topography, and other properties from the high-resolution surface image[27]. Sc-ZnO NPs were examined under a 10 kV scanning electron microscope to learn more about the morphological characteristics of the particles. To determine the averaged particle size of ZnO NPs, the sizes of randomly selected nanoparticles in the sample were examined[24].

***In vitro* anticancer activity (MTT Assay)**

Using the MTT technique, the anticancer effects of Sc-ZnO NPs were evaluated against MCF-7 cells. 5 flurouracil, a positive control, were employed[9,11]. The cells were grown in the culture medium at a density of 1×10^4 cells/mL for a duration of 24 h at 37°C and 5% CO2. In control wells, 0.2 percent DMSO was added to the cell line and cultured. Incubated each sample three times. To determine the percentage of live cells in the control cell after culture, controls were maintained.

Cells were grown for twenty-four hrs at 37°C and 5% CO2 in a CO2 incubator. Once the incubation period was over and the media had been fully withdrawn, 20 µL of MTT solution (5 mg/min PBS) was added. Incubation of the cells in CO2 for four hrs followed the addition of MTT. Formazan crystal development was observed by looking at the wells under a microscope. A dark formazan could only be produced by living cells from yellowish MTT.

After removing all of the medium, 200 µL of DMSO was added, and it was then incubated (covered with aluminium foil) at 550 nm for 10 min at 37°C. Using a microplate reader (Benesphera E21), the absorbance of the sample was taken in triple at a wavelength of 550 nm[9,11].

Results and Discussion

Characterization of *Sargassum cinctum* ZnO - NPs

UV–vis spectral analysis

Quantitative and qualitative research are simultaneously integrated using the UV-vis approach. This is a fast, preliminary, and crucial step to find out if a plant can produce the NPs. Plant extracts contain a variety of phytochemicals that lead to the formation of nanoparticles and the decrease of metal ions.

NPs stability, capping, and synthesis are all aided by these phytochemicals. Reducers bring the metal ions down to metal zero, while capping agents cling to the surface of NPs through covalent bonds or contact. To confirm the generation of Sc-ZnO NPs and characterise the optical property, the UV–vis spectroscopy approach was used. The crucial sign of the creation of Sc-ZnO NPs is revealed by the white precipitate that results from adding zinc acetate to the extract, as seen in (Fig. 1).

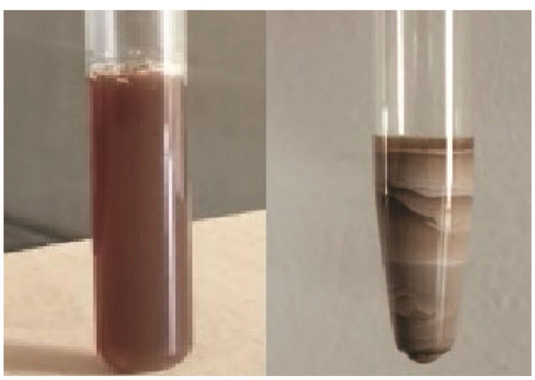

Fig. 1 — Change in color of the solution. (A) Aqueous extract of algal; and (B) Zinc Oxide

Nanoparticle formation in aqueous crude extract after the addition of zinc acetate dihydrate

Zinc ions in the fluid are reduced to zinc oxide by secondary metabolites found in plants. SPR It was brought on by the interaction of the light with the freely circulating Sc-ZnO NPs surface electrons. Previous research findings have identified the SPR of Sc-ZnO NPs as the peaks at wavelengths between 289 and 385 nm. Additionally, SPR bands may vary based on the reaction other variables and the quantity of plant extracts[4].

The creation of ZnO-NPs was linked to an SPR signal at 385 nm in (Fig. 2), which displayed the UV–vis spectra of the algal extract and ZnO-NPs[28]. At pH 9.5, a distinctive peak absorption was seen at 385 nm, which suggested that metal oxide had been reduced and that zinc acetate had been converted into ZnO NPs. There were free electrons in the ZnO NPs, which suggests that they have an active surface that is rich in electrons, as indicated by the Surface Plasmon resonance (SPR) absorption band at about 385 nm. Intrinsic band-gap absorption is seen at 385 nm for ZnO as a result of electron transitions from the valence band to the conduction band (O2p–Zn3d). The UV analysis study produced is also consistent with previous results[16].

Fourier transform infrared (FTIR)

According to Figures 3 & 4, the FT-IR spectra of the extract and Sc-ZnO NPs from *Sargassum cinctum* reveal the chemical composition of many functional groups. A comparison between the two peaks indicated that the increased or decreased transmission peaks were the result of metal nanoparticles adhering to bioorganic molecules. The presence of phenols,

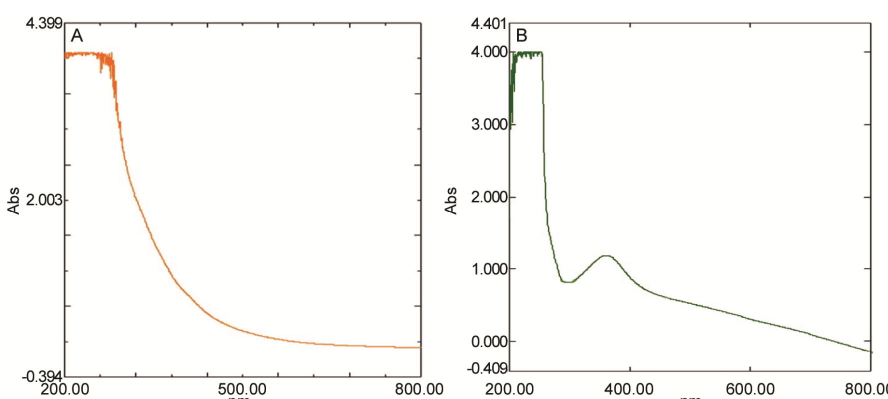

Fig. 2 — Green synthesis of zinc nanoparticles from *Sargassum cinctum* extracts

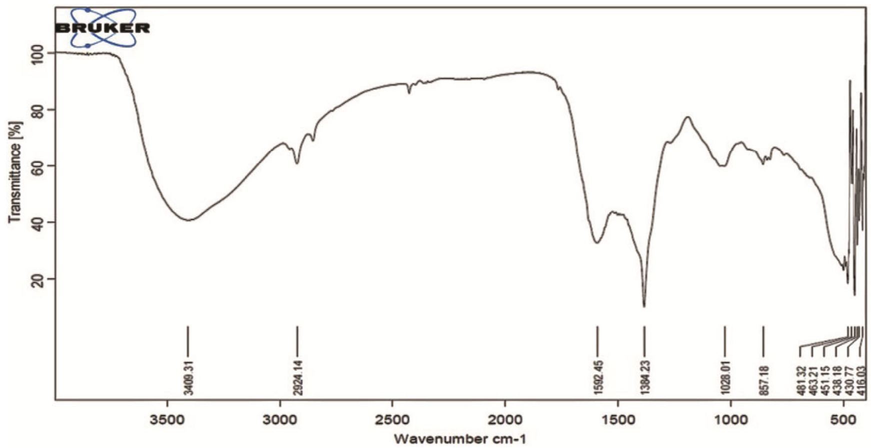

Fig. 3 — FT-IR spectra of *Sargassum cinctum* aqueous extract

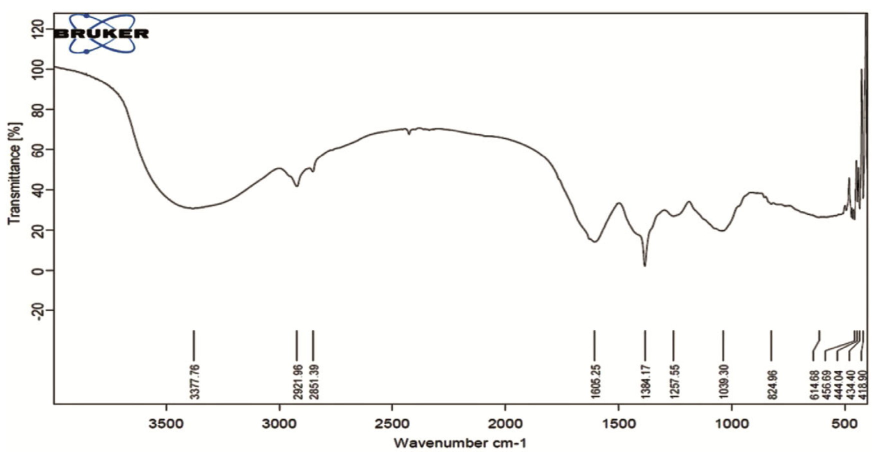

Fig. 4 — FT-IR spectra of *Sargassum cinctum* ZnO NPs

alcohols, or the NH group of amides or amines is indicated by the hydroxyl group (OH) stretching spectrum bands at 3409.31 cm^{-1} in *Sargassum cinctum* extract and 3377.76 cm^{-1} in ZnO NPs.

The extract spectra show that CH-stretching is responsible for absorption band 2921.96 cm^{-1}. This peak is an indication of the capping agents found in the plant extract. Capping agents are in charge of controlling the solubility of uncontrolled agglomerated particles in various solvents and preventing their expansion.

The C=O group of the carboxylic acid is indicated by the extract at 1592.45 cm^{-1}. This peak is identifying the reducing agents found in extracts made from *Sargassum cinctum*. Providing the electrons required for the current ions to change into zero-valent atoms is the main job of the reducing agent. These atoms will then come together to form an NP. C=C group of alkenes is responsible for the peaks of the Sc-ZnO NPs at 1384.17 cm^{-1} and the extract from *Sargassum cinctum* at 1384.23 cm^{-1}.

Sc-ZnONPs appear to be associated with the weak stretch peak located at 456.69 cm^{-1}. The vibration of ZnO deformation and metal-oxygen can be attributed to the peak at 614.68 cm^{-1}, respectively. By comparing the spectra of (Figs 3 & 4), it is possible to predict that *Sargassum* uses hydroxyl and amide groups in areas 3409.31cm^{-1} and 1039.3 to be reducing and capping agents during the biosynthesis of ZnO-NPs[28].

These groups show that the Sc-ZnO NPs include metabolites with functional groups, such as aldehyde, ketone, alcohol, carboxylic acid, and amine. The implicated phenolic compounds function as reducing agents, while proteins that act as stabilising agents are linked to the C-H and N-H groups.

Because carbonyl groups have a strong affinity for zinc, they were reduced and stabilised in carboxylic acid[29]. According to the FT-IR spectra, the *Sargassum cinctum* extract contains several kinds of functional groups that could operate as Sc-ZnO NPs capping and reducing agents.

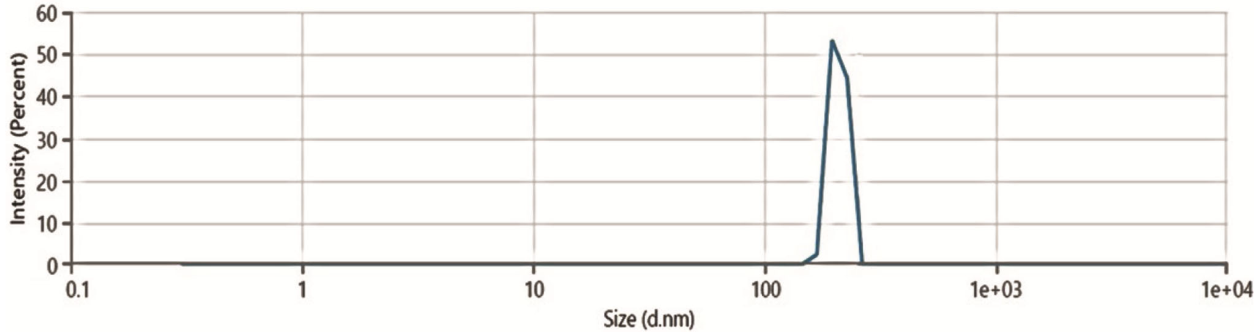

Fig. 5 — DLS histogram showing particle size distribution of synthesized Sc-ZnO NPs

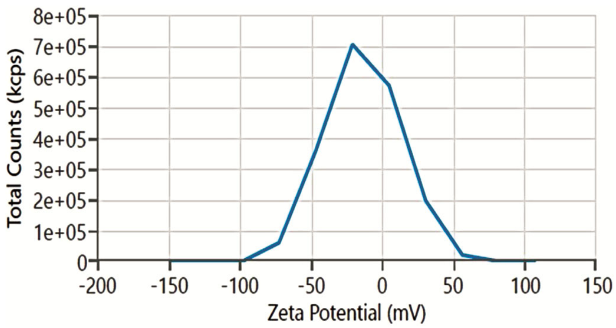

Fig. 6 — Zeta potential of synthesized Sc-ZnO NPs

Dynamic light scattering (DLS)

The experiment gave information regarding the particle population in a short amount of time. The polydispersity index (PdI) and hydrodynamic size distribution of Sc-ZnO NPs were evaluated using DLS. As shown in Figure 5, the findings indicated that Sc-ZnO NPs had an ideal cumulant diameter of 282 nm and a PdI of 0.305. The quality of the green, freshly manufactured zinc nanoparticles was demonstrated by the single peak that emerged.

Zeta potential

Higher zeta potential particles in solution will reject one another rather than grouping together. This can happen for both positive and negative particles. On the other hand, there will not be any force stopping the particles from drifting together if their zeta potential values are low.

The zinc nanoparticles generated by the environment are rejected from one another by the biosynthesised ZnO NPs, as evidenced by their negative zeta potential, this also boosts the formulation's endurance.

As evidenced by their overall negative zeta potential, or polydisperse nature, Sc-ZnO NPs have an electrostatic repulsive force among themselves that is particularly helpful for the long-term stability of the solution since it prevents the nanoparticles from aggregating.

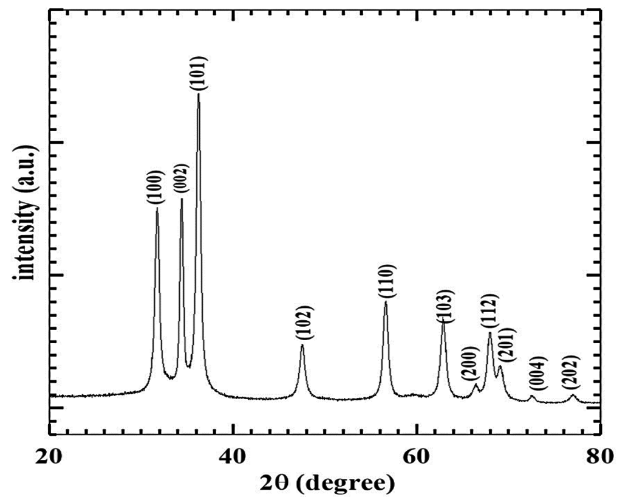

Fig. 7 — XRD pattern of Sc-ZnO NPs

Z-potential analysis was utilized to delve deeper into the surface charge and resilience of the NPs. With a peak value of -13.53 mV, it is discovered that Sc-ZnO NPs were more concentrated in the aqueous nanosuspension of *Sargassum cinctum*. Figure 6 illustrates that a single signal indicated the presence of repulsion among the synthesised nanoparticles.

X-Ray diffraction (XRD) analysis

The zinc nitrate precursor and *Sargassum cinctum* extract were used as reducing agents to create the ZnO-NPs seen in (Fig. 7). No extra peaks were seen in the XRD pattern, which supports earlier research that indicated the great purity of biosynthesised ZnO-NPs. Diffractions from plans (100), (002), (101), (102), (110), (103), (200), (201), (004), and (202) were recorded in the XRD pattern. These plans corresponded to normal angles (2θ) of 31.68, 34.40, 36.16, 47.54, 56.60, 62.88, 66.40, 67.96, 68.99, 72.58, 76.96, and 81.40 sequentially.

Scherrer formula was used to estimate the average crystallographic size of the produced nanoparticle's, which came out to be 29.61 nm. The Sc-ZnO NPs

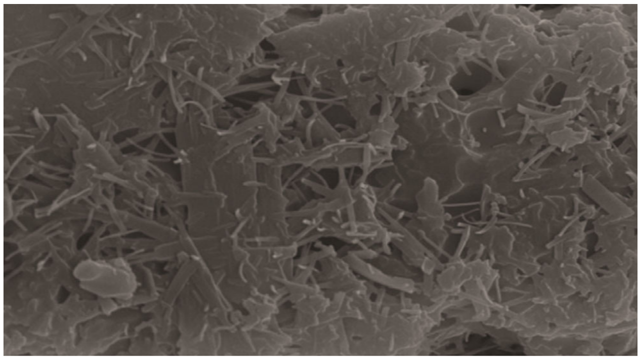

Fig. 8 — SEM images of Sc-ZnO NPs synthesized using *S. cinctum*

hexagonal crystal structure is confirmed by the results. Furthermore, the capping agent which stabilised the NPs is responsible for the observation of these Bragg sharp peaks.

The independent crystallisation of the capping agent was excluded by the centrifugation procedure and subsequent re-dispersion of the generated pellet in Millipore water as a purification step. Compared to the DLS approach, XRD examination revealed the existence of smaller particles. The DLS approach yielded an average particle size of about 282 nm, which is somewhat larger than that of the X-ray diffraction method. This difference in particle size could be the result of smaller nanoparticles aggregation[28].

Morphological analysis by SEM

An overview of the dimensions, form, and surface characteristics of the biosynthesised Sc-ZnO NPs was provided by the SEM image. ZnO nanoparticles (NPs) were seen to be rod irregular. They ranged in diameter between 143.6 nm to 385.6 nm, having an average size of 262.23 nm (see Fig. 8). This result offered strong support for the theory that *Sargassum cinctum* extract could act as reducing and capping agents while Sc-ZnO NP is being synthesised.

***In vitro* anticancer activity (MTT Assay)**

MTT reagent and other tetrazolium dyes undergo a colour shift during reduction, which is dependent on cellular metabolic activity and is caused by NAD(P)H-dependent cellular oxidoreductase enzymes. The extent of cytotoxicity towards MCF-7 cell lines increases with zinc nanoparticle concentrations. To evaluate a cytotoxicity of the Sc-ZnO NPs, the half-life concentration (IC_{50}) at which half of the treated cells were killed was also calculated.

Following the introduction of ZnO NPs, significant percentage declines in cell viability were noted, and the IC_{50} was determined as 98.41 µg/mL.

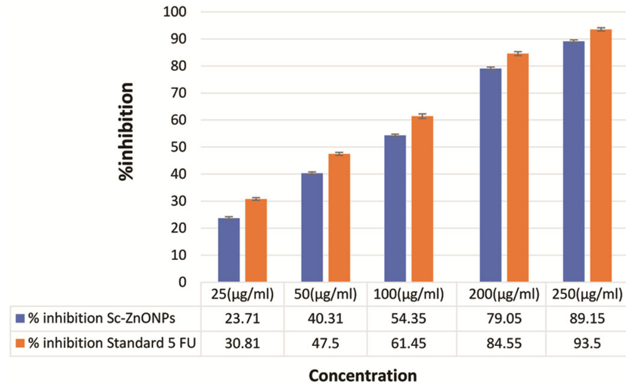

Fig. 9 — Sc-ZnO NPs anticancer activity against the MCF-7 cell line

Once biosynthesised Sc-ZnO NPs were added to the MCF7 cell line, dead cells were seen in large quantities along with morphological changes that defined the cytotoxic increased effect. Some tiny particles may have a greater cytotoxic effect because of their greater surface area to volume ratio. Furthermore, it is important to note that the aqueous extracts phytoconstituents, including as polysaccharides and polyphenols, have been shown to have significant anticancer effects, mostly through targeting different cancer-related proteins.

Possible mechanisms include DNA damage pathways; paraptosis; autophagy; radio sensitization; overcoming chemoresistance; inhibition of carcinogen-activation of $P4_{50}$ enzymes (phase I metabolism); induction of carcinogen-detoxifying enzymes (phase II metabolism); modulation of oxidative stress; induction of apoptosis; or arrest of the cancer cell cycle[16]. Research is still being done to assess any possible mechanisms underlying its anticancer efficacy. The anticancer capacity of green synthesised Sc-ZnO NPs is demonstrated by the MTT experiment in (Fig. 9). The information is presented as mean ± SD (n = 3).

Varying raw material quality, uncertainty in the mechanism of anticancer activity, cytotoxicity, safety concerns, stability, and scalability challenges, and are some of the limitations.

Conclusion

The biogenic synthesis of zinc oxide nanoparticles (ZnONPs) using *Sargassum cinctum* seaweed is a novel process which combines the eco-friendly synthesis with the possibility of using it for the cure of cancer. This method utilizes the reducing and stabilizing agents which are naturally imbibed in the brown marine algae and cause the formation of ZnONPs. The biosynthesized nanoparticles reveal some favourable characteristics such as stability, well defined particle size which are significant when applied in biological systems.

These Sc-ZnONPs when assessed for their anticancer activity exhibited marked cytotoxicity against breast cancer cell lines. These findings highlight the dual benefits of using marine algae as a bio-resource. The applications of green synthesis route for nanoparticles and the natural therapeutic intervention for cancer are presented below.

This paper not only adds to the body of knowledge in green nanotechnology but it also points to the possibility of marine algae-derived nanoparticles in the development of improved cancer treatments, hence calling for a more profound investigation for the improvement of its efficiency in the treatment field.

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Conflict of interest

All authors declare no conflict of interest.

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