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Study on Phytochemical Composition, Biosynthesis and Characterization of Zinc Oxide Nanoparticle Using *Sargassum ilicifolium*

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Abstract

Nanotechnology involves producing nanoscale materials with specific properties. Zinc oxide nanoparticles have potential applications in various fields. Due to toxic chemicals and environmental concerns, green methods using plants, fungi, bacteria, and algae have been adopted. An emerging area of nanotechnology is the green synthesis of nanoparticles using biological systems, particularly seaweed extracts. The green synthesis method has synthesized the zinc oxide nanoparticles using the aqueous extract of the brown seaweed *Sargassum ilicifolium*. The algal extract has greatly reduced the zinc acetate dihydrate salt solution to form zinc oxide nanoparticles. The synthesized zinc oxide (Zn-O) nanoparticles have been confirmed through Particle Size Analyzer (PSA), Raman spectroscopy, UltraViolet (UV)- Visible (Vis) spectroscopy, and Scanning Electron Microscope (SEM). The study used Scanning Electron Microscope (SEM) and Particle Size Analyzer (PSA) to examine the size and shape of the Zn-O nanoparticles. Raman spectroscopy and UltraViolet-Vis spectroscopy confirmed the formation of Zn-O nanoparticles. The SEM results exhibited a range of 24.4 nm to 83.4 nm. The occurrence of Zn-O nanoparticles was confirmed by Raman spectroscopy with peaks at 276.98 cm-1, 414.67 cm-1, 462.03 cm-1, 514.99 cm-1, and 998.28cm-1 and UltraViolet-Vis spectroscopy with peak at 370 nm. The present study also deals with the qualitative phytochemical constituent analysis using the aqueous extracts of *Sargassum ilicifolium*. Alkaloids, flavonoids, cardiac glycoside, tannins, amino acids, carbohydrates, and saponins were analyzed. Anthraquinone, anthocyanin, vitamin C, quinone, and phlobatannins were considered absent in the aqueous extract.

Keywords: Zinc oxide nanoparticles, *Sargassum ilicifolium*, Green synthesis, metal oxide nanoparticles, Nanotechnology, Brown macroalgae, Seaweed.

1. INTRODUCTION

The first records of Nanoscience and nanotechnology date back to 600 BCE, and their applications have been around for centuries. Norio Taniguchi coined the term “Nano” in the year 1959 [1]. Today, research and development carried out at the nanoscale between 1 to 100nm in at least one dimension is referred to as Nanotechnology [2]. By providing novel ideas regarding particle size, grain size, and the boundary of nanomaterials, Richard Feynman is recognized for having revolutionized the field of nanotechnology [3].

The study of nanotechnology is expanding quickly to produce nanomaterials that have possible attributes which include enormous volume, surface area, macro-tunneling effects, and quantum size [4]. Since they can destroy 650 cells [5], nanomaterials are regarded as a marvel of modern medicine. Aside from its antibacterial and wound-healing capabilities, metal nanoparticles also serve optical, electrical, magnetic, and catalytic purposes [6-7].

Zinc oxide (ZnO) nanoparticles are a common n-type semiconducting metal oxide because of their diverse and adjustable characteristics [8]. With its large excitation energy of 60 meV and wide bandgap direct of 3.37 eV, it can be used in various devices, such as optoelectronic, gas sensors, and surface acoustic wave devices [9]. Zinc oxide nanoparticles show great promise

in biological applications such as sensing, labeling, drug transport, gene editing, and nanomedicine [10].

Because of its large surface area, biocompatibility, and anti-bacterial qualities zinc oxide nanoparticles are widely used in textile, medical device, and electrochemistry sectors [11]. However, there are drawbacks to old technologies, such as excessive energy consumption, low purity, and pollution of the environment [12]. Green synthesis is the substitute for conventional techniques since it is inexpensive, environmentally friendly, and offers environmental sustainability when utilizing microbes, enzymes, and plant extracts [13, 14]. Algal extracts are easily obtained and require only a zinc salt solution.

Aquatic plants, such as seaweeds and microalgae, have high biomass output, photosynthetic efficiency, and pollution resistance. They can develop on ground that isn't suitable for other uses and contain bioactive substances [15]. Algae are being used more and more in green synthesis because of their abundant secondary metabolites, quick growth rate, ease of harvesting, and affordable scale-up [16]. The most pre-historic and common photosynthetic organisms are algae, which can hyper-accumulate metals and turn them into nanoparticles, which makes them perfect for green synthesis [17].

The microalgae *Sargassum ilicifolium* competes with other aquatic organisms for light and space while providing food and habitat for species linked with reefs, by offering shelter, nutrition, oxygen, and vital functions in nitrogen cycling, metabolism, and fixation, microbial interactions with algae enhance host fitness [18-20].

In the current work, we used the aqueous extract of the brown marine seaweed *Sargassum ilicifolium* to establish a cheap, environmentally friendly, and sustainable procedure for creating the synthesis of Zinc oxide (Zn-O) nanoparticles and characterized utilizing SEM, UltraViolet-Visible spectroscopy, Raman spectroscopy, and Particle Size Analyzer (PSA).

2. MATERIALS AND METHODS

2.1 Collection of *Sargassum ilicifolium*

The dried form of the brown seaweed *Sargassum ilicifolium* was collected on 2-12-2023 (4.00 P.M) from the Central Marine Research Institute of Rameshwaram in Ramanathapuram district in Tamil Nadu.

2.2 Preparation of aqueous extract of *Sargassum ilicifolium*

To prepare the aqueous extract 10 grams of *Sargassum ilicifolium* was heated with 100 milliliter of distilled water for 30 minutes. The extract was passed through sterile Whatman No.1 filter paper in a sterile funnel, then filtered and stored in a brown bottle at 4◦C. The process of aqueous extract preparation is explained in Figure 1.

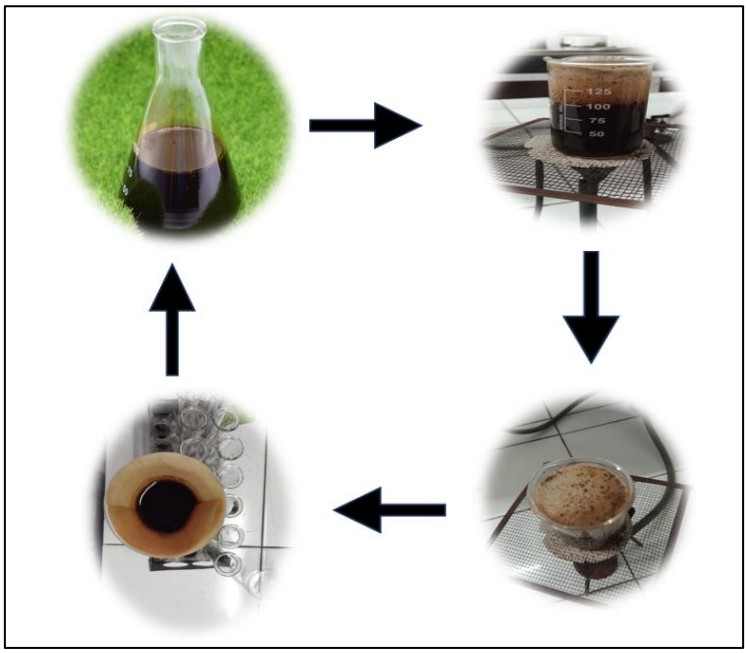

Figure 1: Preparation of aqueous extract of *Sargassum ilicifolium*

2.3 Phytochemical analysis of *Sargassum ilicifolium*

The primary types of chemicals were identified by confirmatory qualitative phytochemical screening of brown seaweed aqueous extract (Flavonoids, Tannins, Saponins, Alkaloids, Phenols, Glycosides, Terpenoids, and Steroids) existent in the aqueous extract using standard protocols [21-24].

2.3.1 Determination of alkaloids

A) Wagner's-test: 200 milligrams of *Sargassum ilicifolium* dried powder was simmered with 10 milliliters of distilled water (H_2O) and 10 milliliters of hydrochloric acid (HCl). Then ammonia (NH_4) was added to adjust the pH to 6-7, followed by 1 milliliter of Wagner's reagent which is an iodide solution in potassium iodide. Precipitation or turbidity is a sign of alkaloids [21].

B) Hager's-Test: Take 2milliliter of algal extract and add few drops of Hager's reagent which is a saturated picric acid was added. When alkaloids are present, a bright yellow colour is formed [21].

2.3.2 Determination of Cardiac glycosides

A) Baljet's test: Several droplets of Baljet's reagent which is a mixture of picric acid, ethanol, and sodium hydroxide are added to 2-3 milligrams of dried powder of brown seaweed. Orange to deep red color indicates a positive test [21].

2.3.3 Determination of Pseudotannins

A) Ferric chloride test: 1 gram of sample was heated in a water bath with 20 milliliters of distilled water for 5 minutes and filtered. Next, distilled water was used to dilute 1 milliliter of the cold filtrate to 5 milliliters, and add few drops of 10% ferric chloride ($FeCl_3$) were added. A positive test indicates the presence of a bluish-black or a brownish-green precipitate [23].

2.3.4 Determination of Tannins

A) Alkaline-reagent test: A few drops of 2 milliliters of 1N sodium hydroxide (NaOH) were added to 2 milliliters of brown seaweed extract. The presence of yellow to red color suggests the existence of tannins [23].

2.3.5 Determination of Flavonoids

A) Flavonoids-test: 1 gram of powdered seaweed sample was heated in a water bath with 10 distilled water for 5 minutes and filtered. Then 1 milliliter of 20% sodium hydroxide (NaOH) was added to the cooled filtrate. When an acid is added, the colorless solution turns to yellow, signifying a successful test [22].

B) Alkaline-reagent test: 1 milliliter of 2N sodium hydroxide (NaOH) was added to 1 milliliter of the brown seaweed extract. The existence of a yellow color denotes the presence of flavonoids [23].

C) Ferric-chloride ($FeCl_3$) test: one milliliter of the algal extract was added with a few milliliters of ferric chloride ($FeCl_3$) solution. The occurrence of blackish-red precipitate denotes the existence of flavonoids [23].

D) Zinc hydrochloride-reduction test: To algal extract a pinch of zinc (Zn) dust was added and hydrochloric acid (HCl) was added along the corners of the test tube. After a few minutes, the hue turns magenta, which is an indication that flavonoids are present [24].

2.3.6 Determination of saponin

A) Saponin-test: 2 milliliter of distilled water was combined with 2 milliliter of algal extract and the mixture was rapidly agitated lengthwise for 15 minutes in a graduated cylinder. Saponin was detected in the test sample by a layer of foam [23].

2.3.7 Determination of anthocyanin

A) Test for anthocyanin: To 1milliliter of 2N sodium hydroxide (NaOH) was added to 2 milliliter of the algal extract and subjected to heat at 100◦C for 5 minutes. The appearance of a bluish-green color denotes the existence of anthocyanin [23].

2.3.8 Determination of anthraquinone

A) Test for anthraquinone: To 2milliliter of algal extract, a few droplets of 2% hydrochloric acid were added. The formation of a red-colored precipitate denotes the existence of anthraquinone [23].

2.3.9 Determination of coumarins

A) Coumarins-test: 1 milliliter of 10% sodium hydroxide (NaOH) was added to 1 milliliter of the seaweed aqueous extract. The occurrence of a yellow color denotes the presence of coumarins [23].

2.3.10 Determination of glycoside

A) Keller-Killani test: 1 milliliter of glacial acetic acid was mixed with 1 milliliter of the seaweed aqueous extract and it was cooled. Following the cooling process, concentrated sulfuric acid (H_2SO_4) was added along the corners of the test tube, and then two drops of ferric chloride solution. The occurrence of glycosides was denoted by a ring of reddish-brown color at the junction of two layers [23].

2.3.11 Determination of phlorotannins

A) Phlobatannins-test: To 1 milliliter of algal extract, a few drops of 10% ammonia solution were added. The occurrence of a pink-colored precipitate indicates the existence of phlobatannins [23].

2.3.12 Determination of quinones

A) Test for quinones: 1 milliliter of concentrated H_2SO_4 was added to 1 milliliter algal extract. The appearance of red color denotes the existence of quinones [23].

2.3.13 Determination of acid

A) Acid-test: 1 milliliter of the brown seaweed aqueous extract was reacted with sodium bicarbonate ($NaHCO_3$). The formation of brisk effervescence denotes the existence of acid [23].

2.3.14 Determination of Vitamin C

A) DNPH-test: 1 milliliter of the brown seaweed aqueous extract was reacted with Dinitrophenyl hydrazine (DNPH) (dissolved in concentrated sulphuric acid). The development of yellow precipitate denotes the occurrence of vitamin C [23].

2.3.15 Determination of amino acids

A) Ninhydrin-test: 1 milliliter of the brown seaweed aqueous extract was boiled with 1 milliliter of the ninhydrin reagent solution (10mg ninhydrin in 200milliliter acetone). The development of a purple hue denotes the existence of amino acids [24].

B) Xanthoproteic test: To 1milliliter of algal extract 1milliliter concentrated nitric acid was introduced and the mixture was brought to a boil. After cooling, 2 milliliters of 40% sodium hydroxide (NaOH) was added. The occurrence of a yellow color indicates the existence of amino acids (Tyrosine and Tryptophan) [23].

2.3.16 Determination of carbohydrates

A) Molisch test: To 2milliliter algal extract Molisch reagent (α-naphthol in alcohol) was added and concentrated sulphuric acid (H_2SO_4) was added along the corners of the sterile test tube. A violet-colored ring was developed near the intersection of the two layers demonstrating the presence of carbohydrates [24].

B) Benedict's test: To 2milliliter algal extract few drops of Benedict's reagent which is a combination of sodium carbonate, sodium citrate, and copper (II) sulfate pentahydrate were added and heated. The occurrence of a reddish-brown precipitate denotes the presence of reducing sugar [23].

2.4 Green synthesis and the characterization of Zn-O nanoparticles utilizing the aqueous extract of *Sargassum ilicifolium*

We have produced using the green synthesis method and are employing the bottom-up strategy to prepare zinc oxide nanoparticles. The protocol was followed as per the procedure with some slight modifications following Azizi *et al.*, [25].

2.4.1 Materials

i) Zinc acetate dihydrate was used as the precursor. 109.75 mg of zinc acetate dihydrate ($(CH_3COO)_2Zn.2H_2O$) was weighed and dissolved in 100milliliter distilled water **(concentration=0.1M)**

ii) Dried powder of *Sargassum ilicifolium*

iii) Distilled water

2.4.2 Methodology

Step 1: Preparation of algal extract 2 grams of dried powder of *Sargassum ilicifolium* was weighed and heated up to 80◦C with 100 millilitres of distilled water in a magnetic stirrer for about 30 minutes. The mixture was purified from the filtrate using the Whatman 41 filter paper.

Step 2: 50milliliter of 0.1M zinc acetate dihydrate ($(CH_3COO)_2Zn.2H_2O$) solution was combined with 50milliliter of algal extract in a 250milliliter conical flask

Step 3: The mixture has been kept in a magnetic stirrer with a constant stirring at 70◦C for 3-4 hours.

Step 4: After continuous stirring, a pale white solid product is obtained which is collected using a centrifuge at 4000 rpm.

Step 5: The white solid product was carefully cleaned with distilled water and then it was dried overnight at 100◦C.

Step 6: The pure Zn-O nanoparticles can be obtained by calcinating at 450◦C for four hours.

Step 7: The powdered zinc oxide nanoparticles were weighed and stored in an Eppendorf tube for future use. The yield of Zn-O nanoparticles was found to be 0.191 g. The process of green synthesis of Zn-O nanoparticles is illustrated in Figure 2.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

The seaweed's chemical makeup varies depending on the species, location, weather, and time of harvest [26]. Because they produce a wide spectrum of molecules, seaweeds are currently considered potential species for contributing new physiologically active chemicals for the development of innovative nutritional, cosmetic, and medicinal substances. It is believed that there are many different types of bioactive molecules, such as carotenoids, peptides, tocotrienols, proteins, carbohydrates, phlobatannins, minerals, and vitamins [27].

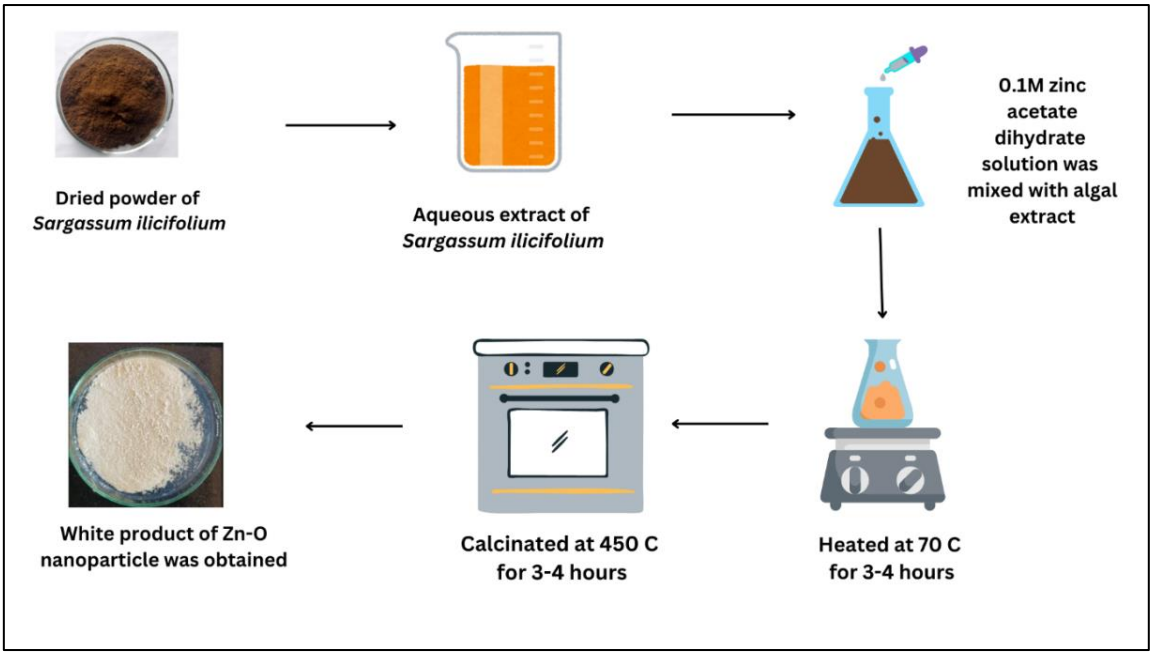

Figure 2: The process of green synthesis of Zn-O nanoparticles.

Table 1: Represents the results of the preliminary phytochemical screening study and shows that the aqueous extract of *Sargassum ilicifolium* has a broad range of phytochemicals which includes alkaloids, cardiac glycosides, tannins, flavonoids, saponin, amino acids and carbohydrates

S. No	Phytochemicals	Name of Test	Aqueous Extract	Results
1.	**ALKALOID**	Wagner's-Test		+++
2.		Hager's-test		+++
3.	**CARDIAC GLYCOSIDES**	Baljet's Test		++
4.	**PSEUDOTANNINS**	$FeCl_3$-test		+
5.	**TANNINS**	Alkaline- reagent test		++

6.	**FLAVONOID**	Flavonoids		++
7.		Alkaline- reagent test		+
8.		FeCl3 test		++
9.		Zinc hydrochloride test		-
10.	**SAPONINS**	Foam test		++
11.	**ANTHOCYANIN**	Sodium hydroxide test		-
12.	**ANTHRAQUINONE**	Hydrochloric acid test		-
13.	**GLYCOSIDES**	Keller Killani test		-
15.	**PHLOBATANNINS**	Ammonia test		-

16.	**QUINONES**	Sulphuric acid test		-
17.	**ACID**	Sodium bicarbonate test		-
18.	**VITAMIN C**	DNPH test		-
19.	**AMINO ACID**	Xanthoproteic test		++
20.		Ninhydrin test		+
21.	**CARBOHYDRATE**	Molisch test		+++
22.		Benedict test		-
23.		Fehling's test		+++

(+++)- indicates phytochemicals in very high concentration.
(++)- indicates phytochemicals in moderately high concentration.
(+)- indicates phytochemicals in small concentrations.
(-)- indicates phytochemicals do not detect

This phytochemical study shows the presence of secondary metabolites of the seaweed *Sargassum ilicifolium* in which the aqueous extract revealed the presence of alkaloids, carbohydrates in very high concentration, whereas, cardiac glycosides, tannins, flavonoids, saponin, and amino acid in moderately high concentration. As a comparative study with Basha *et al.*, our phytochemical results showed the presence of saponins and amino acids. Further, anthraquinone, anthocyanin, vitamin C, quinone, and phlobatannins were considered absent in *Sargassum ilicifolium* as per the recent study.

According to Marimuthu *et al.*, [28], it is frequently discovered that alkaloids possess anti-bacterial qualities against both gram-negative bacteria and gram-positive bacteria which is known as "nature's tender drug". Flavonoids have a collective of pharmacological and biological effects which consist of anti-viral, anti-oxidant, anti-fungal, anti-inflammatory, anti-allergenic, anti-thrombic, anti-carcinogenic, hepatoprotective, and cytotoxic [29-32]. Unsaponifiable, non-toxic sterols of therapeutic significance have been demonstrated to be abundant in marine algae [33]. Saponins exhibit multiple biological characteristics which include anti-bacterial, anti-inflammatory, anti-feedent, and hemolytic actions [34]. Tannins have the ability to lower the risk of neurological disorders like Alzheimer's and cardiovascular disease, as well as cancer and as a nutraceutical, these compounds could help improve a healthy gut microbiome [35]. Cardiac glycosides reduce the heart rate and limit the activity of sodium-potassium adenosine triphosphatase (Na^+/K^+ ATPase) exchanger to enhance cardiac contractility in cardiomyocytes. These substances are used as pharmacologic treatments to treat congestive heart failure and atrial fibrillation [36]. A wide range of cardiovascular and hematological conditions, from inflammation illness and anti-thrombotic therapies to wound healing, are treated with carbohydrate-based or modified medicines [37]. The existence of flavonoids, alkaloids, saponins, tannins, cardiac glycosides, and carbohydrates in the aqueous extracts of *Sargassum ilicifolium* implies that seaweeds can be utilized as anti-microbial, anti-carcinogenic, anti-thrombic, anti-oxidant, anti-allergenic, anti-feedent, cardiovascular, and haemolytic actions shortly.

3.2 Characterisation of Zinc oxide nanoparticles

3.2.1 Particle Size Analyser

The zinc oxide nanoparticles have a wide range of particle size distribution between 30 to 200 nm [38], and at low pH, zinc nanoparticles would aggregate to produce larger nanoparticles rather than nucleate [39]. A particle size analyzer (MAL-1049897) was used to measure the diameter of the Zinc oxide nanoparticles in the range between 0.1 to 10,000 nm. A peak with an intensity of 100% was detected at 1622 nm. Due to aggregation and agglomeration, the nanoparticles have been highly excited, which on sonication can range between 30 to 200 nm. The outcomes of the particle size analyzer are explained in Figure 3.

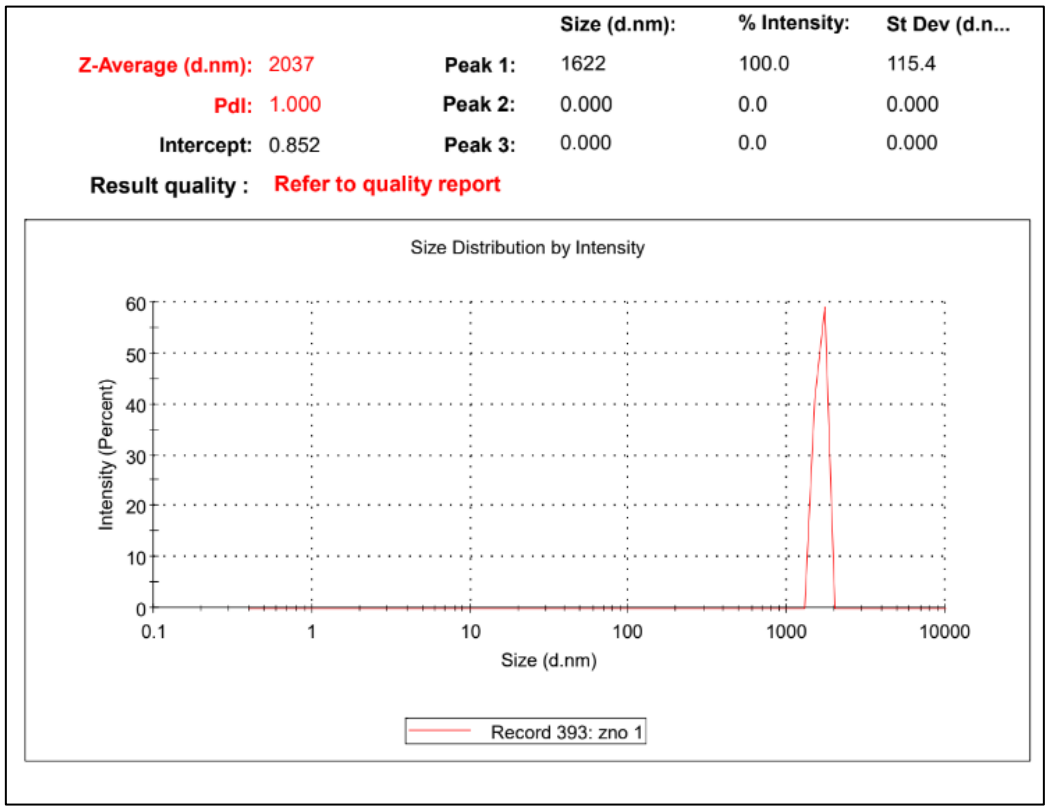

Figure 3: Results of Particle Size analyser

3.2.2 RAMEN SPECTROSCOPY

The Sargassum Zinc oxide nanoparticles Raman spectrum displays multiple unique peaks at 276.98 cm^{-1}, 414.67 cm^{-1}, 462.03 cm^{-1}, 514.99 cm^{-1}, and 998.28 cm^{-1} after sonication. Zinc oxide nanoparticles excited between 325 cm^{-1} and 532 cm^{-1} exhibit a hexagonal wurtzite structure. Stronger intensity and smaller line width are present at the highest point at 437 cm^{-1}, which is a sign of strong Raman scattering in hexagonal crystals [40]. As per Sharma *et al.*, [38], the peak at 414.67 cm^{-1} can be related to oxygen vibration, and the peak at 514.99 cm-1 can correspond to the existence of -OH bond and certain oxygen-containing groups, as COO, O_2, and H_2O connected to the zinc oxide nanoparticles. According to [40], the nanometer between 416.67cm-1 and 462.03 cm-1 has a shorter line width and a greater intensity with a hexagonal structure and good crystal quality which confirms the presence of the zinc oxide nanoparticles even though these peaks can also be influenced by impurities and contaminants. The results of Raman spectroscopy are explained in Figure 4.

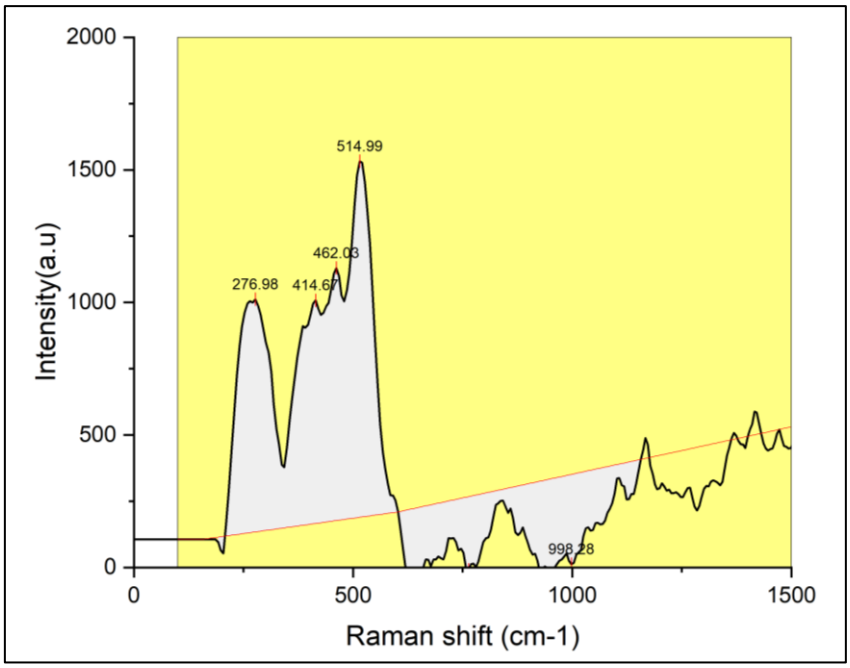

Figure 4: Peaks of Ramen Spectroscopy

3.2.3 UV-VISIBLE SPECTROSCOPY

Utilizing UltraViolet-Vis spectroscopy the structural characteristics of the Zinc Oxide (Zn-O) nanoparticles (NPs) synthesis from aqueous extract of *Sargassum ilicifolium*. The absorption spectra displayed the characteristic peaks of Zinc oxide nanoparticles, which were explained by the electron transfer from the valence to the conduction band. Zinc oxide nanoparticles exhibit an absorbance peak between 200 and 600 nm [41].

The UV-visible absorption of Zinc oxide nanoparticles was measured between 200 to 450 nm. The absorbance at its highest was noted at 370 nm, and as per Singh *et al.*, [42] the bandgap energy was about 3.38eV and the average bandgap for a normal-sized zinc oxide nanoparticle is 3.3eV. Figure 5 depicts the UV-visible absorption spectrum for the zinc oxide nanoparticles. According to Gurgur *et al.*, [43], the energy gap widens as quantum size effects cause the particles to shrink semiconductors' electrical energy bands. This is especially apparent when nano-crystallites are smaller than that of Bohr radius. The exchange of coulomb forces between the electrons and holes plays a significant role in nano-scale materials. The valence and conduction bands of semiconductors are altered by the quantum confinement of charge carriers. The exceptionally clear zinc oxide absorption peaks verify that the distribution of the nanoparticle is monodispersed.

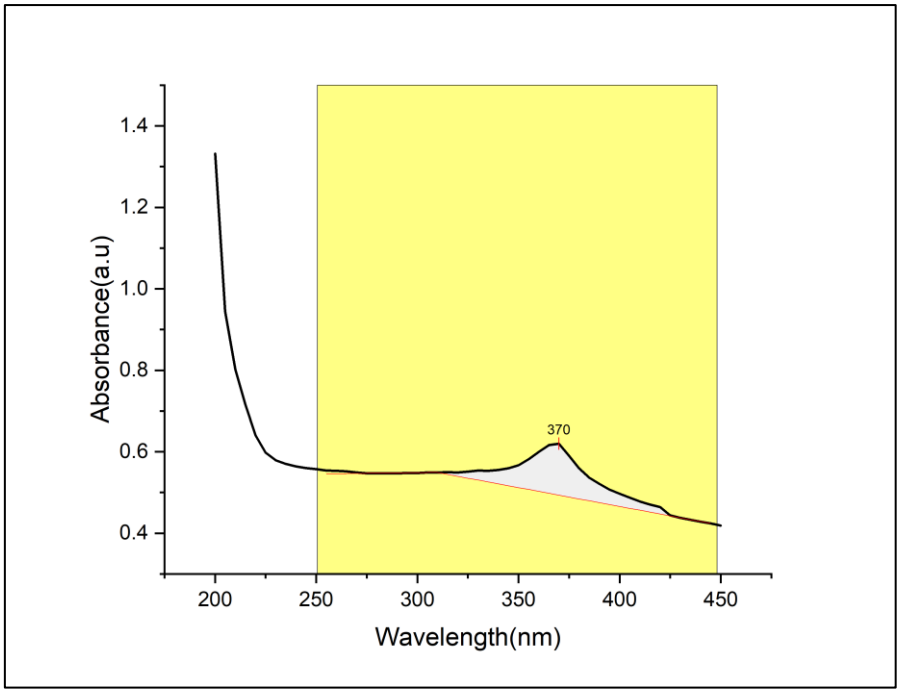

Figure 5: Results of UV-Visible Spectroscopy

3.2.4 SCANNING ELECTRON MICROSCOPE

After sonication, the Zn-O nanoparticles were examined morphologically using a Scanning Electron Microscope (SEM). The dimensions of the nanoparticles were discovered to be 24.4 nm, 41.4 nm, 43.9 nm, 53.7 nm, 65.5 nm, and 83.4 nm which is shown in Figure 6. The zinc oxide nanoparticle with a nanometer size of 24.4 corresponds to the hexagonal shape [44] which is the smallest nanoparticle size. It has also an average size of 83.4 nm which corresponds to the rod shape of the nanoparticle [45]. The nanoparticle size of 24.4 nm confirms the presence of zinc oxide nanoparticles using the precursor zinc acetate recorded by Fakhari *et al*., [46]. After sonication, previously agglomerated nanoparticles according to the Particle size analyser shrank in size. The large surface area and elevated surface energy of the generated nanoparticles could be the cause of the accumulation [47]. The agglomeration can be due to the electrostatic attraction and polarity of the zinc oxide nanoparticles [46]. Our Particle size analysis results clearly show that this may have boosted the attractive forces among the nanoparticles. The SEM analysis results we have gotten, fall below the nanoparticle size range. We can therefore verify that they are zinc oxide nanoparticles.

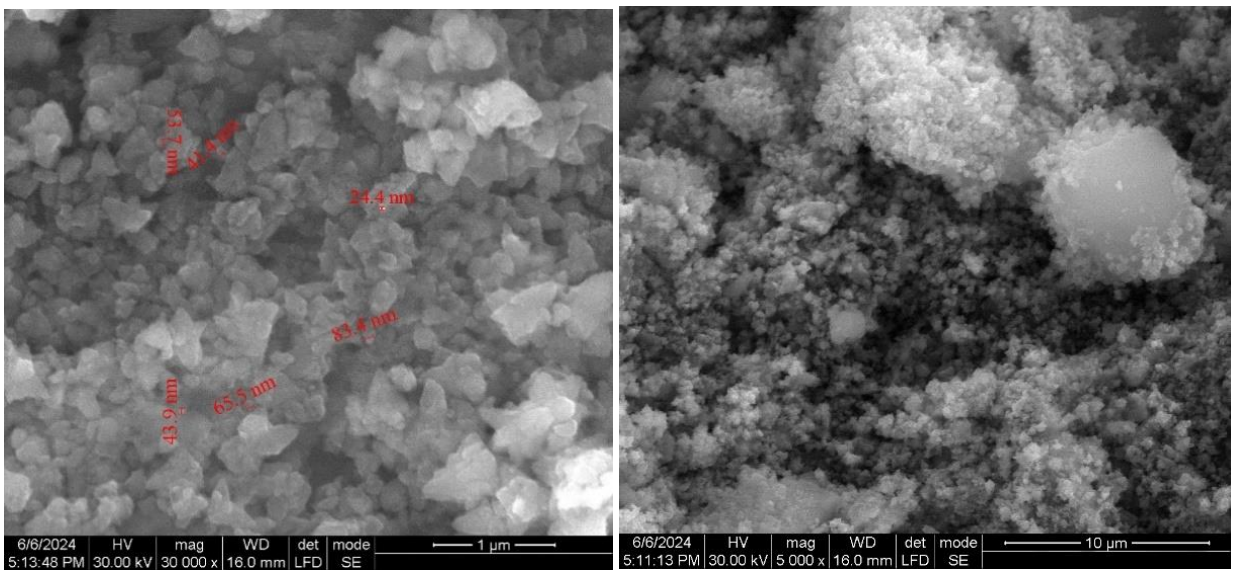

Figure 6: SEM analysis of Zn-O nanoparticles

CONCLUSION

The green synthesis approach has been accomplished and used for the first time to prepare zinc oxide (Zn-O) nanoparticles, using an aqueous extract of the brown seaweed *Sargassum ilicifolium* as the reducing agent. It has been discovered that the procedure is very easy to use and may be done in an aqueous solution at a moderate temperature. Using methods from scanning electron microscopy, Raman spectroscopy, particle size analyzer, and ultraviolet-visible spectroscopy, the bio-reduced zinc oxide nanoparticles were described. According to the findings of phytochemical analysis, these obtained zinc oxide nanoparticles have potential uses in the biochemical field from a technological standpoint. This straightforward process has various benefits, including affordability, and suitability for use in pharmaceutical and medical applications.

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