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Biosynthesis of NiO_2 Nanoparticles from Marine Seaweed *HORMOPHYSA SPS* and its Antimicrobial Activity

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Abstract: ***Marine Seaweed extracts of* Hormophysa sps *was used as a reducing agent in the eco - friendly extracellular synthesis of NiO_2 nanoparticles from an aqueous solution of Nickel chloride. Biosynthesized nanoparticles were characterized using UV–visible diffuse reflectance spectroscopy (UV–vis - DRS), Fourier transform infrared spectroscopy (FTIR), X - Ray diffraction (XRD), Scanning electron microscopy (SEM), energy dispersive X - ray Spectroscopy (EDS) techniques. XRD results revealed that the average crystallite size ranges from 45 to 76 nm. The present investigation states that the marine seaweed extracts synthesized NiO_2 nanoparticles have potent antimicrobial activity even in minimum concentration against pathogens.***

Keywords: NiO_2 nanoparticles, *Hormophysa sps*, average crystallite size, antimicrobial activity

1. Introduction

The widespread practical application of metal oxide nanoparticles (particles less than 100 nm) is attributable to a number of their unique properties [1, 2]. Different physical and chemical processes are currently widely used to synthesize metal nanoparticles, which allow one to obtain particles with the desired characteristics. However, these production methods are usually expensive, labor - intensive, and are potentially hazardous to the environment and living organisms. Thus, there is an obvious need for an alternative, cost - effective and at the same time safe and environmentally sound method of nanoparticle production. During the past decade, it has been demonstrated that many biological systems, including plants and algae, diatoms, bacteria, yeast, fungi, and human cells [3] can transform inorganic metal ions into metal oxide nanoparticles via the reductive capacities of the proteins [4].

Marine algae are one of the largest producers of biomass in the marine environmental. Seaweeds are the eukaryotic organisms that live in salty water and recognized as a potential source of bioactive natural products. The prosperous and diverse marine vegetation consists of many bioactive compounds such as polyunsaturated fatty acids, proteins, polysaccharides, and pigments that form the basis of novel medicinal products [5 - 7]. Among the seaweed - born macromolecules, the sulfated polysaccharides (SPS) from marine macroalgae are reported to possess antioxidant, anti - inflammatory, anti - allergic, anticancer, antiviral, and anticoagulant activities despite their structural and nutritional roles [8 - 10]. Heterofucans from brown seaweed *Dictyopteris delicatula* was reported to have anticoagulant, antioxidant, and antitumor activities [11]. The dependence of sulfate content on the bioactivity of SPS has been well - established.

Figure 1: Dry powder of *HORMOPHYSA SPS*

Among transition metal oxides, nickel oxide (NiO) bulk and nano size have received considerable attention due to their wide range of applications in different fields, such as: catalysis, fuel cell electrodes and gas sensors, electrochromic films, battery cathodes magnetic materials, and photovoltaic devices. Furthermore, NiO is being studied for applications in smart windows, electrochemical supercapacitors and dye - sensitized photocathodes. Because of the quantum size and surface effects, NiO nanoparticles exhibit catalytic, optical, electronic, and magnetic properties that are significantly different than those of bulk - sized NiO particles [12 - 14].

In this study, a novel green synthesis of NiO_2 using *HORMOPHYSA SPS* seaweeds via co - precipitation method. The prepared nanopraticles were characterized using UV–vis - DRS, FTIR, XRD, SEM and EDS techniques. The present study aimed on the *in vitro* determination of antioxidant potential of green synthesis of NiO_2 and *HORMOPHYSA SPS*.

2. Materials and Methods

2.1. Materials

HORMOPHYSA SPS seaweeds were collected from Mantapam Kanyakumari District, Tamilnadu. All the chemicals used in this experiment were of analytical grade. Double distilled water was used throughout the experiments.

2.2. Preparation of NiO_2 using seaweed extract

Dried powdered *HORMOPHYSA SPS*, 5g was mixed with 50ml distilled water in the Erlenmeyer flask. The mixture was boiled for 30 minutes at 60ºC. Finally, the extract was collected and stored at 4º C for further uses. The precursor is prepared by dissolving nickel chloride in 50 ml of double distilled water. This solution is stirred for 30 minutes on magnetic stirrer with the addition of 5 ml of seaweed extract. Light green precipitate of nickel oxalate dehydrate so obtained is filtrated and washed with distilled water. The above obtained precipitate is calcinated at 300ºC for 2 h to remove the excess moisture and impurities. Finally seaweeds extract modified pure nickel oxide nanoparticles were obtained [15]. Fig.2. shows the systematic procedure for the preparation of NiO_2 using seaweeds extract.

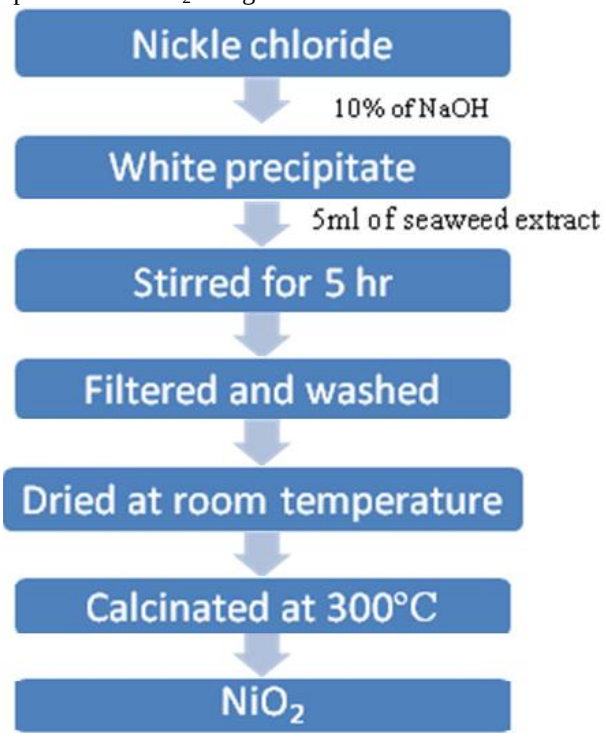


Figure 2: Systematic procedure for the preparation of seaweeds extract modified NiO_2.

2.3 Photochemical screening (Qualitative Analysis [16])

(a) Test for Reducing sugars (Fehling's test)
The aqueous ethanol extract (0.5ml in 5ml of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a color reaction.

(b) Test for cadiac glycosides (Keller - Killiant test)
To 0.5ml of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

(c) Test for Anthraquinines
0.5 ml of the extract was boiled with boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipette into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for color changes.

(d) Test for proteins
To 2 ml of extract 1ml of 40% NaOH solution and 2 drops of 1% $CuSO_4$ solution was added. A violet color indicates presence of peptide linkage molecule.

(e) Test for flavonoids
Three methods were used to test for flavonoids. First, dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow coloration that disappears on standing indicates the presence of flavonoids.

(f) Test for saponins
To 0.5 ml of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

(g) Test for tannins
About 0.5 ml of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue black coloration.

(h) Test for lignins (Maule's test)
0.5 ml of extract was added with 1% of aqueous potassium permanganate solution and few drops of ammonium chloride solution and a drop of ammonium hydroxide. Red rose color is the indicator of the presence of lignins.

(i) Test for phenol
A portion of the methanolic extract of the sample was treated with neutral ferric chloride solution and observed for the result.

2.4 Characterization

The nanoparticle was characterized by the following methods. The UV - visible diffuse reflectance spectra were obtained for dry - pressed disk sample using JASCO V - 530 double beam spectrophotometer with PMT detector equipped with an integrating sphere assembly, using $BaSO_4$ as a reference sample. The spectra were recorded at room temperature ranging from 200 nm to 800 nm. Fourier Transformation Infrared Spectrum (FT - IR) of synthesized sample was obtained using Shimadzu Fourier Transform Infrared Spectrometer (IR Affinity) with KBr – nanoparticles mixture in the form of pellets. The structure

and phase of the samples were determined by X - Ray and powder diffraction with Cu Kα radiation at 25° C using XPERT PRO X – RAY and the structural assignments were made with reference to the standard JCPDS powder diffraction files. Scanning Electron Microscope (SEM) of the nanoparticles were taken by a JM – 6701F – 6701 instrument in both secondary and backscattered electron modes. The elemental analysis was detected by an energy dispersive X - Ray spectroscopy (EDX) attachment to the SEM.

3. Result and Discussion

3.1 Characterization

3.1.1. UV - vis spectral studies

The diffuse reflectance spectra of chemical NO and *HORMOPHYSA SPS* extract modified NiO_2 (HNO) are displaced in Fig.3. Hence, wavelength determined for excitation of NO and HNO are 350 and 500 nm respectively. This reveals that the absorption of edge of NO is extended to visible region by loading of seaweed extract this makes HNO more visible light sensitive. The band gap energies of NO and HNO are found to be 2.25 eV and 1.89 eV, respectively using the tauc plot using the following equation [17] as shownin Fig.4 and 5.

$$\alpha = \frac{C(h\nu - Eg^{bulk})^2}{h\nu}$$

where α is the absorption co - efficient, hγ is the photon energy, E_g the optical band gap energy and C is the constant depending on the electron – hole mobility.

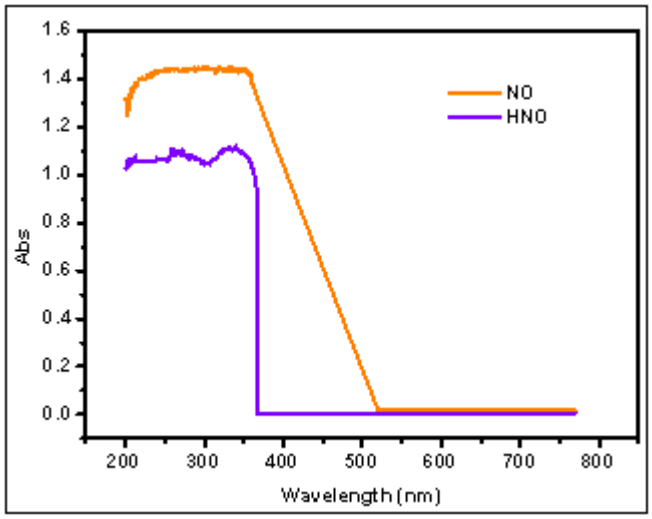


Figure 3: UV - vis - DRS of NO and HNO

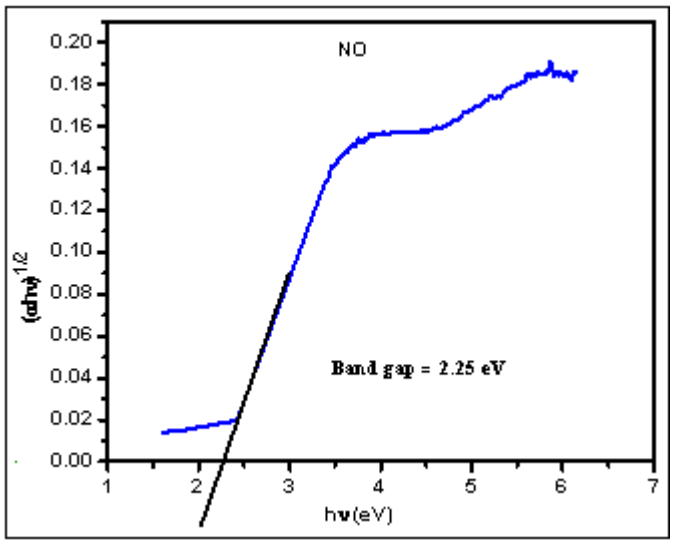


Figure 4: Tauc plot of NO

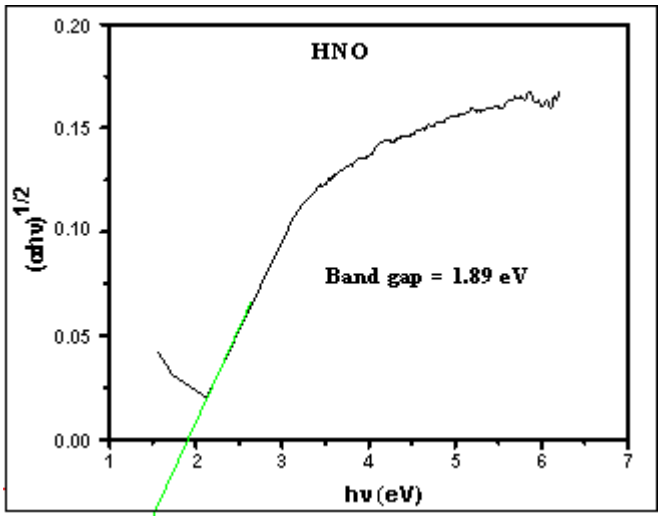


Figure 5: Tauc plot of HNO

3.1.2. FT - IR

The absorption bands at 459 cm^{-1} and 522 cm^{-1} is due the presence of NiO band. The broad band at 1035 $cm^{-1}$and 1114 cm^{-1} is associated with the glycosidic linkages present in the seaweeds extract [18] in Fig.6. The band between 2860 - 2929 cm^{-1} is due to the C - H stretching vibration of alkane groups sharp peak observed in 2374 cm^{-1} represents the presence of nitrogen, hydrogen atoms (N - H) band. The band at 1620 cm^{-1} and 3485 cm^{-1} indicates the presence of stretching of hydroxyl group [19] present in the seaweed extract. The absorption bands at 459 cm^{-1} is associated to Ni - O vibration band, and band at 688 cm^{-1} is assigned to Ni - O - H stretching band [20] as in the Fig.7

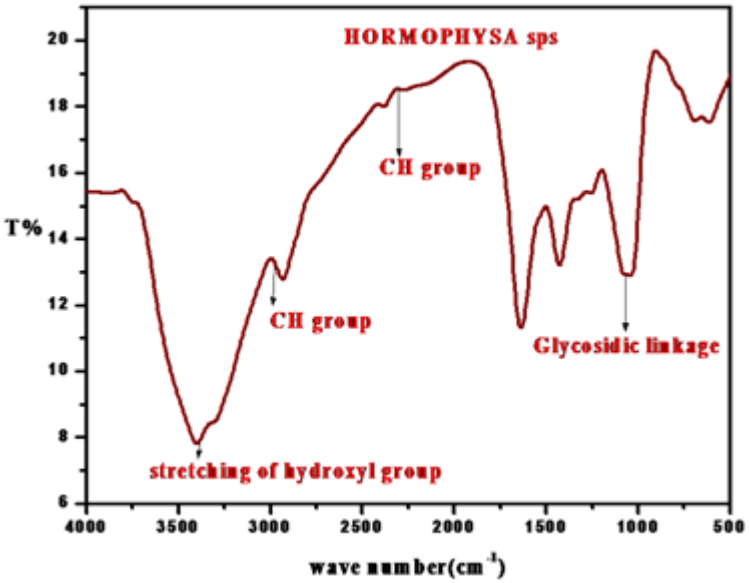


Figure 6: FTIR spectrum of HO

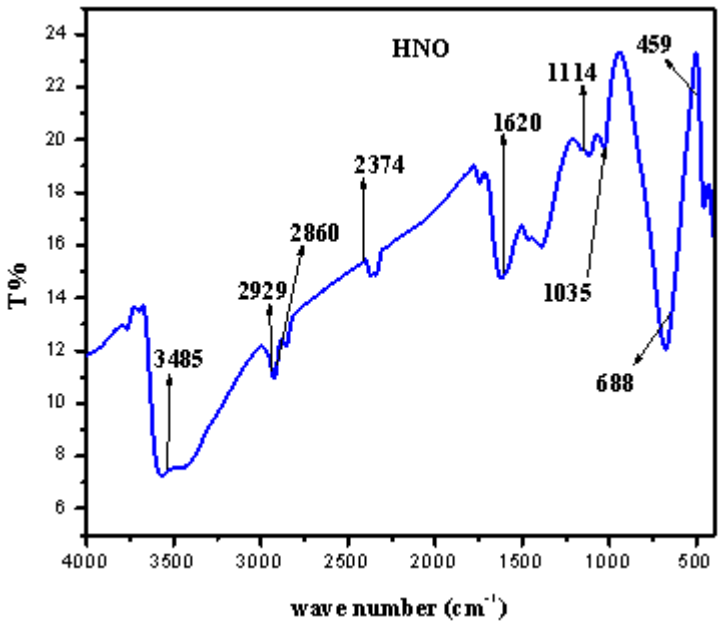


Figure 7: FTIR spectrum of HNO

3.1.3 XRD

Fig.8 Shows the XRD pattern for NO and seaweeds modified Nickel oxide nanoparticles under (HNO) optimum condition. Relatively strong intensive and sharp diffraction peaks are obtained for the HNO nanoparticles. When compared to chemical method. The diffraction peaks of NO can be indexed to tetragonal primitive lattice. While HNO belongs to JCPDS NO 47 - 1049 of thin nanorods with face centre cubic lattice (Fig.9) average crystalline size is calculated using Debye's Scherer's equation.

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

Where, β is the full width at half height maximum of the most intense 2θ peak, K is the shape factor (0.89). θ, λ are the incident angle and wavelength of X – rays respectively [21]. The average particle crystalline size obtained for NO and HNO nanoparticle are found to be 15 nm, and 10 nm respectively.

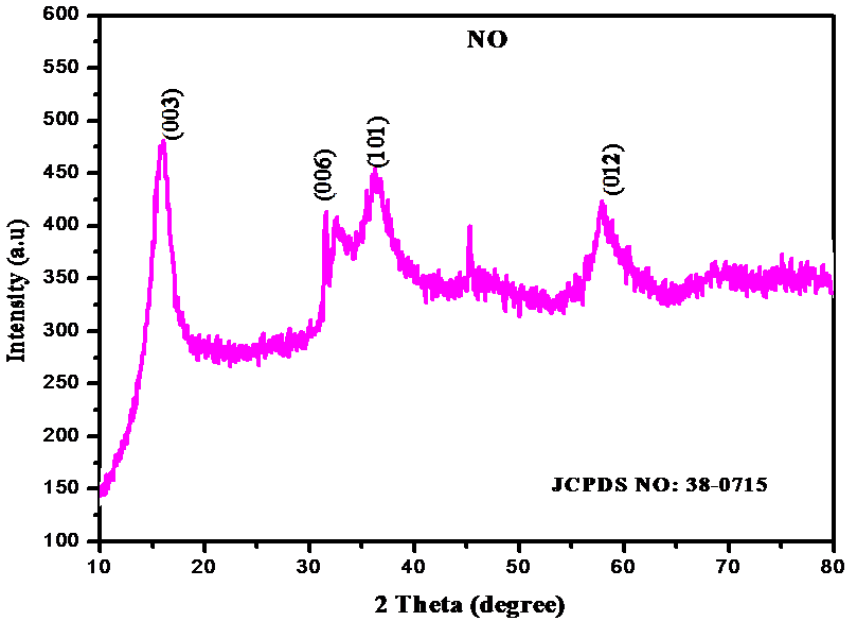


Figure 8: XRD image of NO

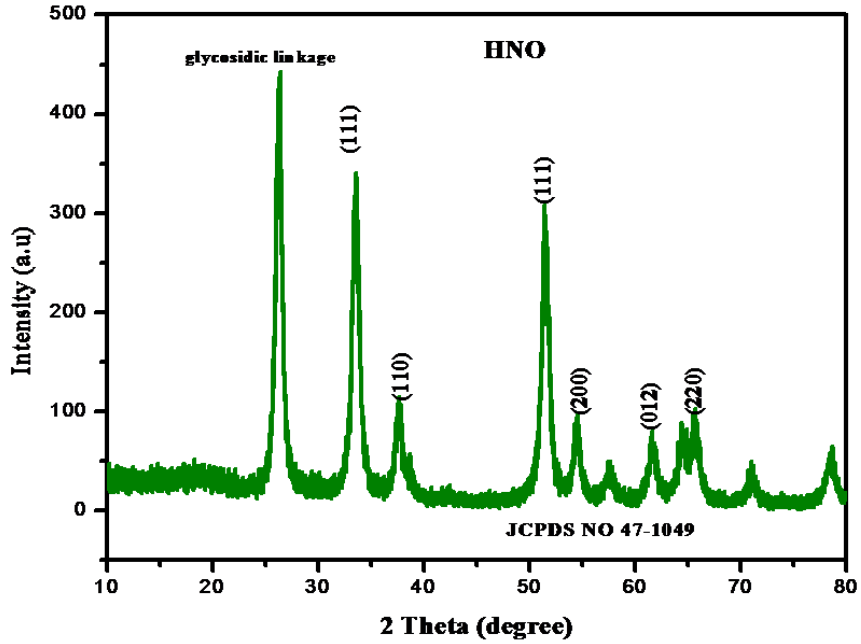
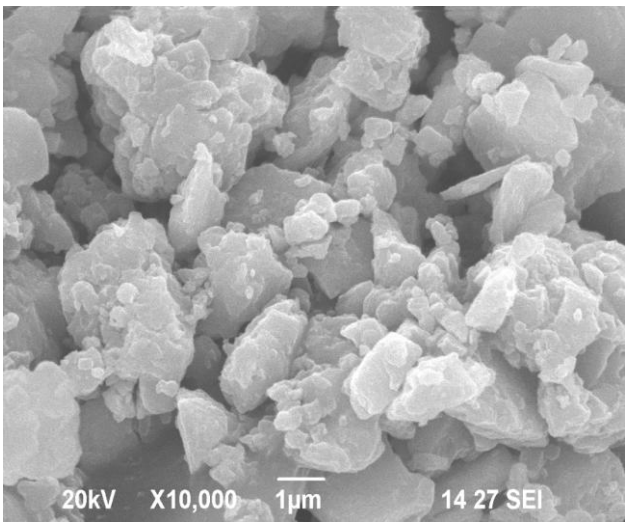


Figure 9: XRD image of HNO

3.1.4 SEM and EDX

The different morphologies of the as - prepared materials were depicted in the Fig.10 the NO sample exhibited plate structure which was consistent with XRD analysis. On addition of seaweeds extract the surface morphology of NiO change to Nano rods [22]. The EDX spectra of NO, and HNO are presented in Fig.11. The peaks corresponding to Ni and O are clearly observed at their normal energy and their corresponding keV values are shown in the Table.1.



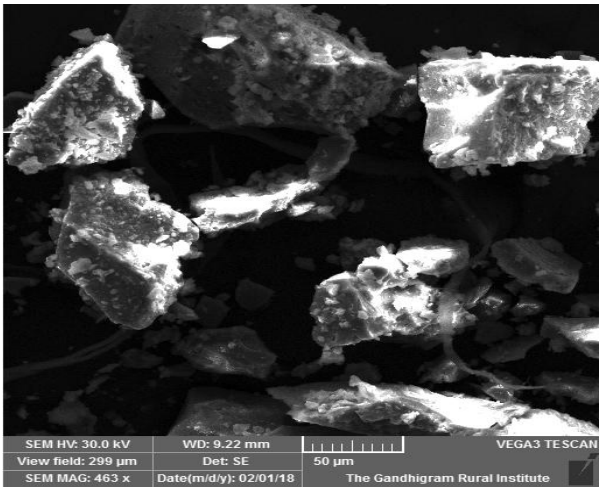
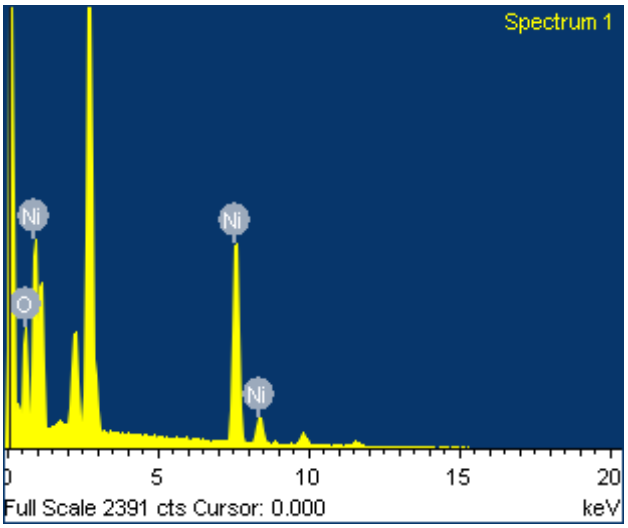


Figure 10: (a) SEM image of NO (b) SEM image of HNO



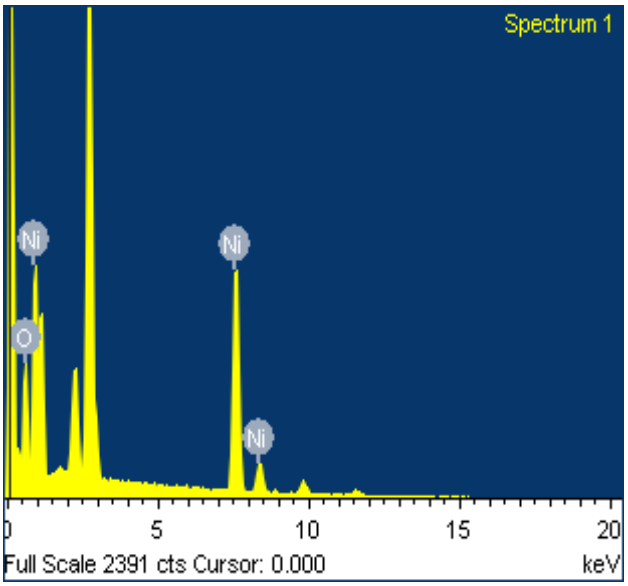


Figure 11: (a) EDX spectrum of NO (b) EDX spectrum of HNO

Table 1: EDX elemental analysis of NO and HNO

S. No	Sample	Atomic %	keV
1	NO	Ni =27.08 O = 72.92	Ni = 6.404, 7.478, 8.26 O = 0.5
2	HNO	Ni =27.08 O = 72.92	Ni = 6.404, 7.478, 8.26 O = 0.5

3.2 Qualitative analysis of seaweeds extracts [*Hormophysa sps*]

The result of the qualitative photochemical screening carried out on aqueous extract [*Horomophysa sps*] show the followed by Table.2.

Table 2: Photochemical screening test for *Hormophysa sps*

S. No	*Qualitative Test*	*Observation*	*Indication*
1	Reducing sugars (Fehling's test)	No Color	Absence
2	Cadiac glycosides (Keller - Killiant test)	Brown ring	**Presence**
3	Anthraquinines	Color change	**Presence**
4	Proteins	Violet Color	**Presence**
5	Flavonoids	No Color	Absence
6	Saponins (frothing test)	Emulsion Formation	**Presence**
7	Tannins	Blue - Black Color	**Presence**
8	Lignins (Maule's test)	No Color	Absence
9	Phenol (ferric chloride test)	Color reaction	**Presence**

The various phytochemicals detected from the seaweeds are known to have beneficial importance in industrial and medicinal sciences [23]. Seaweeds with antimicrobial compounds have enormous therapeutical potential and from these results it can be concluded that the selected seaweed extracts may be used as broad - spectrum antimicrobial, bioactive agent after extensive investigation. In the present study saponins and tannins were present in the extracts. Tannins were used therapeutically as antiviral, antibacterial, antiulcer and antioxidant agents. Many tannin containing drugs are used in the treatment of piles, inflammation, burns and as astringent [24]. Proteins, Phenol, Anthraquinines, Cadiac glycosides were noticed in seaweed extracts. Further work will emphasize the isolation and characterization of active principles responsible for bio - efficacy and bioactivity.

4. Conclusion

*HORMOPHYSA SPS*extract was used to synthesis NiO_2 nanoparticles through green method. The structure, morphology and optical properties of the nanoparticles were confirmed by XRD, SEM, EDX, FT - IR, and Uv - Vis DRS measurement. The result of the qualitative phytochemical screening carried out on aqueous extract of *HORMOPHYSA SPS* show the following by result (Reducing sugar Fehling's test (-), cardiac glycosides (keller - killiani test) (+), Anthraquinones (+), Terpenoids (salkowski test) (+), Proteins (+), flavonoids (-), Saponins (Frothing test) (+), Tannis (+), Lignins (-), phenols (Ferric chloride test) (+)).

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