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Green Synthesis, Characterization and Application of Ferric Nanoparticles from *Sargassum wightii* in the Gulf of Mannar Biosphere Reserve, Tamil Nadu.

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Abstract
In recent years, there has been an increased interest in easily manufacturing nanoparticles. Conventionally, physical and chemical techniques are employed in the synthesis of nanoparticles, however due to limitations of these methods, the focus of research has been recently shifted towards the development of clean and eco-friendly synthesis protocols. The green synthesis of ferric nanoparticles FeNPs has been achieved using environmentally acceptable aqueous extract. The aim of this study is to synthesize ferric nanoparticles using *Sargassum wightii* extract in an environmental and sustainable way. Phytoconstituents individually or in combination determine the therapeutic value of an aqueous extract. Flavonoids, Terpenoids, saponins are some important phytochemical activities with biological activities. The synthesized Iron nanoparticles were characterized using Scanning Electron Microscope (SEM) demonstrated that the synthesized nanoparticles were filamentous like structure and Fourier Transform Infrared (FTIR) analysis of *Sargassum wightii* aqueous extract revealed various study indicated that the hydroxyl(-OH), carbonyl(>C=0), tertiary Amino(R3N) functional compounds. UV-visible spectrophotometer of *Sargassum wightii* aqueous extract of FeNPs showed a 267nm is considered a peak. Energy Dispersive X-Ray Analysis EDX showed a 1.59keV. This study shows that the Ferric Nanoparticles can be synthesized using *Sargassum wightii* extract. In the future, they might be created for use in medicine creation and to decrease environmental contamination.

Keywords: Nanoparticles, Characterization, Ferric, Environmental, Medicines, Aqueous.

INTRODUCTION

Marine macroalgae, commonly known as seaweeds, are plant-like organisms classified into three major groups based on their pigmentation: red (Rhodophyta), brown (Phaeophyta), and green (Chlorophyta). Owing to their rich content of lipids, minerals, vitamins, and a variety of bioactive compounds such as proteins, polysaccharides, and polyphenols, seaweeds are widely recognized as functional foods. These compounds have demonstrated therapeutic potential against cancer, oxidative stress, and other diseases. The hydroxyl, carboxyl, and amino functional groups present in these phytochemicals can act as boseth capping and metal-reducing agents, enabling the green synthesis of metal nanoparticles in a single step (Bhuyar *et al.,* 2020).

Nanotechnology refers to the manipulation of matter at the atomic or molecular level, typically between 1–100 nm, to produce materials with specific properties for diverse applications. The high surface area to volume ratio is a key characteristic contributing to the broad applicability of nanomaterials. Although conventional synthesis of nanoparticles often involves toxic reducing agents with adverse environmental impacts, biological synthesis particularly green synthesis is emerging as a safer alternative. This bottom-up approach relies on redox reactions and eliminates the need for hazardous chemicals, high temperatures, and pressure. It is cost-effective, environmentally friendly, and easily scalable, offering better control over crystal growth and stability (Prasad *et al.,* 2013).

Several brown algae (Phaeophyta) species such as *Laminaria japonica*, *Hizikia fusiformis*, and *Undaria pinnatifida* are widely consumed in East Asian countries like China, Korea, and Japan due to their high nutritional content. Among these, the genus *Sargassum*, abundant in tropical marine waters, is known for its rich nutritional and bioactive compound profile (Sangeetha *et al.,* 2011).

MATERIALS AND METHODS

COLLECTION OF SEAWEED

Seaweed samples of *Sargassum wightii* Greville ex J. Agardh were collected from Uvari and the Gulf of Mannar Biosphere Reserve in Tamil Nadu. The collected seaweeds were identified based on morphological characteristics using

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the taxonomic keys described by Jha *et al.,* (2009). After identification, the samples were thoroughly washed, shade-dried, and powdered separately for further research use.

PREPARATION OF PLANT EXTRACT

Whole plants of seaweed collected from the Mandapam region of the Gulf of Mannar were thoroughly washed several times with distilled water to remove dust and debris and then shade-dried. The dried seaweed was cut into small pieces, and an aqueous extract was prepared using a Soxhlet apparatus. The resulting extract was concentrated under reduced pressure to obtain a dry powder, following the method described by Vijaya Kumar *et al.,* (2014).

PREPARATION OF EXTRACTS

A 10 g sample of powdered seaweed was dissolved in 100 mL of distilled water and allowed to macerate for 30 minutes at room temperature with intermittent shaking. The mixture was then filtered using Whatman No. 1 filter paper until no further weight change was observed in the filtrate, following the method described by Kokate (1999).

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Phytochemical analysis of methanol extracts of *Sargassum wightii* Greville ex J. Agardh was conducted following the procedure of (Harborne, J. B.*et al.,* 1998).

REAGENTS AND CHEMICALS

A 0.001 M solution of ferric chloride ($FeCl_3$) was obtained from Sigma-Aldrich. Freshly prepared triple-distilled water was used throughout the experiment to avoid contamination. Fresh specimens of *Sargassum wightii* were collected from the local coastal region. The seaweed was washed thoroughly with triple-distilled water to remove debris and epiphytes, then blotted dry using water-absorbent paper. The cleaned material was cut into small pieces using an ethanol-sterilized knife and crushed using a sterile mortar and pestle. Approximately 10 mL of sterile distilled water was added to the crushed material, and the mixture was heated at 70–80 °C for 2–3 minutes. The extract was then filtered through Whatman No. 1 filter paper using standard aseptic techniques, and the filtrate was collected in a clean, sterile conical flask and stored at 4 °C for further use, following the method described by Kathiraven *et al.,* (2015).

PREPARATION OF IRON OXIDE NANOPARTICLES

Iron oxide nanoparticles were synthesized using a green approach. A 1% iron oxide nanoparticle solution was prepared using tannic acid as a reducing and stabilizing agent in an alkaline medium. In this method, 0.5 g of iron was dissolved in 50 mL of double-distilled water. To this, 5 mL of 0.004 M tannic acid solution was added, followed by 2 mL of 0.5 M sodium hydroxide (NaOH). The entire reaction mixture was stirred continuously for 30 minutes. A visible change in color of the solution indicated the formation of iron oxide nanoparticles, as described by Sivaraj *et al.,* (2014).

CHARACTERIZATION OF IRON OXIDE NANOPARTICLES

UV-VISIBLE SPECTRA ANALYSIS

The reduction of pure Fe+3 ions to Fe0 was monitored by measuring the UV-Vis spectrum by sampling of aliquots (0.3 mL) of Fe nanoparticle solution diluting the sample in 3 mL distilled water. UV-Vis spectral analysis was done by using UV-Visible spectrophotometer Systronics 118 at the range of 200–600 nm and observed the absorption peaks at 216–268 nm regions due to the excitation of surface plasmon vibrations in the FeNPs solution, which are identical to the characteristic UV visible spectrum of metallic iron, and it was recorded.

FOURIER TRANSFORM INFRARED SPECTROSCOPY

For FTIR spectrum analysis the silver nano bioconjugates were centrifuged at 15,000 rpm for 15 min to remove free proteins or other compounds present in the solution. The filtration after complete reduction of FeCl3 ions was subjected to repeated centrifugation at 15,000 rpm for 15 min, and the supernatant was replaced by distilled water each time to concentrate FeNPs. The process was repeated three times and finally the centrifuged part containing silver nano bio conjugates were redispersed in double distilled water and subjected to FTIR spectroscopy. The presence of unreacted silver ions leads to white precipitation in addition to sodium chloride. However, no precipitate was formed after the addition of sodium chloride indicating the absence of unreacted silver in the nanoparticle solution. FTIR measurements were carried out to identify the possible biomolecules responsible for the reduction of the FeCl3 and capping of the bioreduced silver nanoparticles synthesized by the leaf broth. The Fourier transform infrared spectroscopy (FTIR) spectrum of the sample was recorded on a Perkin-Elmer FTIR spectrum in the range 450 to 4000 cm-1 at resolution of 4 cm-1.

SEM ANALYSIS

Scanning electron microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a FeCl3 grid by just dropping a very small amount of the sample on the grid, extra solution was

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removed using a blotting paper and then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 min.

ENERGY-DISPERSIVE X-RAY (EDX) MICROANALYSIS

Energy-dispersive X-ray (EDX) spectrum analysis employing an X-ray micro-analyzer (Module Oxford 6587INCA X sight) connected to JEOL JSM 5500 LV was used to study the structure of ferric using a scanning electron microscope to identify the other basic compositions of the particles and to verify that iron is present in them.

RESULTS AND DISCUSSION

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Phytochemical analysis of methanol extracts of *Sargassum wightii* reveals the presence of Flavonoids, Terpenoids, Saponins, and Alkaloids.

TABLE: 1. PRELIMINARY PHYTOCHEMICAL ANALYSIS

S.No	Secondary Metabolites	Methanol Extract
1	Tannins	-
2	Flavonoids	+
3	Terpenoids	+
4	Saponins	+
5	Steroids	-
6	Phlobatannins	-
7	Glycosides	-
8	Alkaloids	+
9	Anthraquinones	-
10	Anthocyanins	-
11	Carbohydrates	-
12	Proteins	-
13	Emodins	-
14	Coumarins	-
15	Leucocyanins	-

+Presence, - Absence

UV-VISIBLE SPECTROSCOPY ANALYSIS

The Green approach for the formation of iron chloride nanoparticles using *Sargassum whitti* extract was reported. Formations of iron chloride nanoparticles were confirmed by UV-visible spectrophotometry. Fig1. Shows the UV-Visible absorption spectrum of iron chloride nanoparticle. The adsorption spectrum was recorded for the sample in the range of 200 – 800 nm. The spectrum showed the absorbance peak at 267 nm corresponding to the characteristic band of iron chloride nanoparticle (Tran *et al.*, 2010).

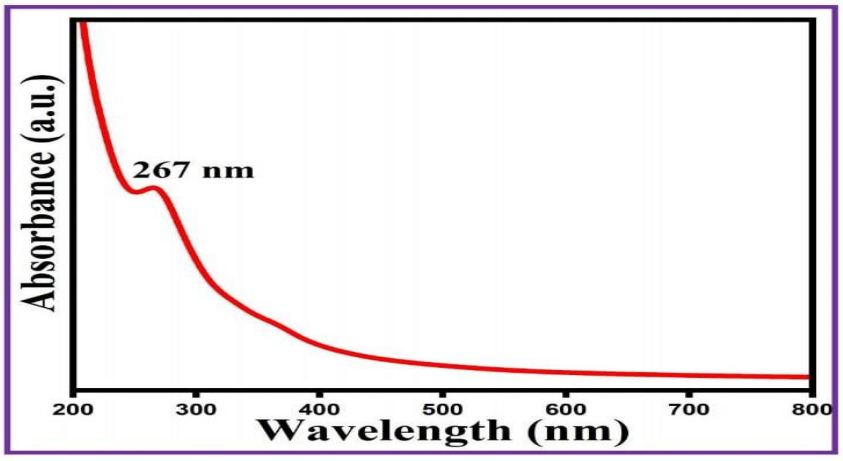

FIG1. UV-VISIBLE SPECTROSCOPY ANALYSIS

FTIR ANALYSIS

FTIR Spectroscopy analysis also revealed the possible biomolecules and functional group responsible for capping or stabilizing of synthesized ferric was expressed in (Fig – 2). Taking the Spectrum of Seaweed extract as control the

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involvement of different functional groups of *Sargassum wightii* extract in reducing and stabilizing process of nanoparticles synthesis was evaluated. Absorbance bands at 3300cm-1,1630 cm -1,1415 cm -1,1245 cm -1,1022 cm -1 were observed in the spectrum of Seaweed extract. A broad band at 3300-1 was due to the O-H Stretching of hydroxyl compounds. The peaks at 1630 cm-1 carbonyl (>C=O), 1415 cm-1 tertiary amino acids, 1245 cm-1 (R3N) Band, 1022cm-1 (C=O) stretching presence of compounds. (Kavitha .2019)

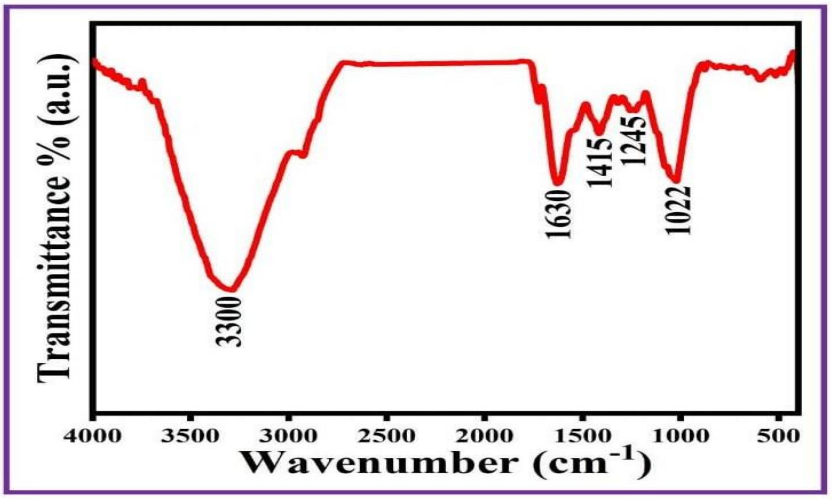

FIG2. FTIR SPECTRUM OF SEAWEEDS EXTRACT

SCANNING ELECTRON MICROSCOPY

Ferric produced from *S. wightii* cell extract was captured using a scanning electron Microscope. The nanoparticles' morphology was filamentous and had a structure similar to that of a rock. It is evident from close inspection that the ferric is encircled by a thin, pale coating of other components, which we assume are covering the organic material from the extract of seaweed. The resulting ferric particles range in size from 100 to 200 µm. (Fig. 3) (Berra *et al*., 2018).

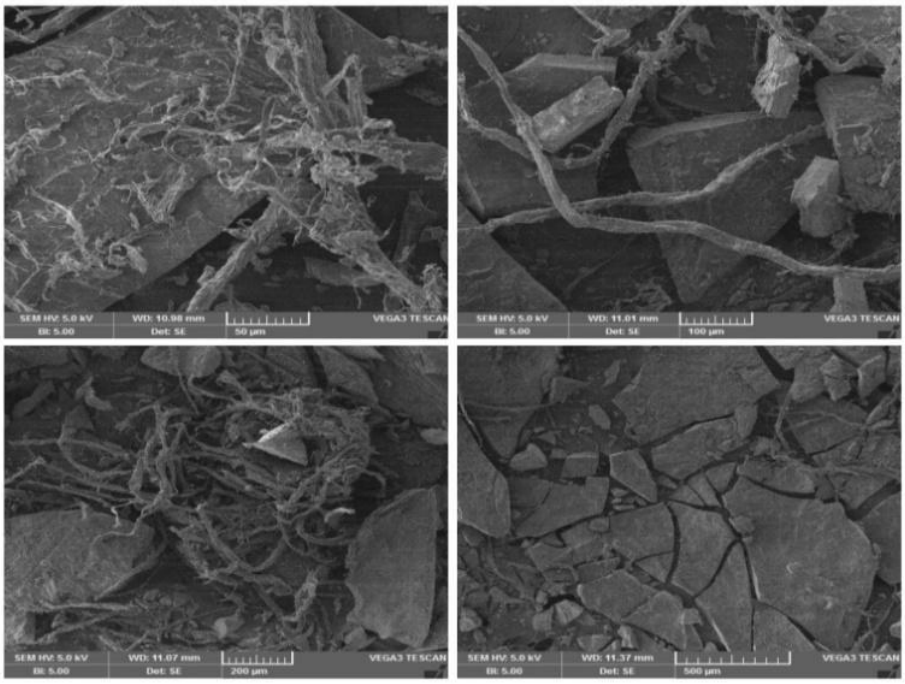

FIG 3. SCANNING ELECTRON MICROSCOPY

EDX ANALYSIS

Fig. 4 shows the elemental composition of the two ferric compounds that were biosynthesized with S. wightii. According to the peaks at 1.59 keV are associated with the Fe binding energies in the biosynthesised ferric colloids. As a result, the two biosynthesized nano colloids' existence of ferric was verified by the ferric's EDX spectra.

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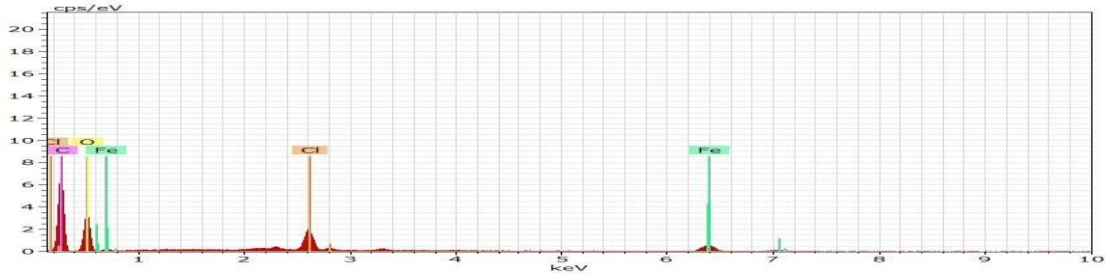

FIG4.ENERGY DISPERSIVE X-RAY DIFFRACTIVE (EDX) ANALYSIS

CONCLUSION

This work devised a biological approach to synthesis ferric nanoparticles that is easy to use, inexpensive, and environmentally benign. The FTIR study indicates that the polysaccharides found in the S.wightii aqueous extract contributes to the creation of ferric nanoparticles. With a mean size of 200µm, the ferric nanoparticles contain carbonyl, OH shapes and range in size from 200 to 297 nm. It is anticipated that the biosynthesized ferric nanoparticles made from S.wightii will find significant uses in the biomedical, pharmacological, and cosmetic sectors.

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