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Green manganese nanoparticles with *Amphiroa fragilissima*

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Abstract
Green synthesis is now recognised as a sustainable alternative to traditional nanoparticle fabrication by lowering the reliance on harmful chemicals and utilizing naturally existing reducing and stabilizing biomolecules from biological sources. Within this framework, algae-mediated routes are particularly attractive due to the rich phytochemical profiles and metal-binding capabilities of macro and microalgae, yet the use of marine red seaweeds such as *Amphiroa fragilissima* for manganese nanoparticle production remains scarcely explored. The aim of the present study is to prepare aqueous extract of *A. fragilissima*, synthesise and characterize manganese nanoparticles. Manganese nanoparticles have been synthesised using aqueous seaweed extract reacted with potassium permanganate and characterized by using various instruments, such as UV–Visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and scanning electron microscopy (SEM). The visual colour change of the reaction mixture and time dependent UV-Visible spectral variation supported nanoparticle formation, while FTIR exhibited functional groups potentially involved in reduction and capping processes. XRD analysis suggested a crystalline material with broad reflections and SEM observations indicated moderately dispersed, slightly agglomerated and predominantly spherical to elliptical particles. These findings suggest that *A. fragilissima* is an effective biological agent for the sustainable synthesis of manganese nanoparticles. Additionally, the physiochemical data provided by this study serves as a critical foundation for future research exploring their potential in catalytic, antimicrobial and various biological applications.

Keywords: Nanoparticle, green synthesis, manganese nanoparticle, red seaweed

Introduction
Nanobiotechnology has emerged as a transformative platform for engineering metal-based nanomaterials with specific physiochemical properties for biomedical, environmental and sensing applications [1, 2]. Manganese based nanoparticles (Mn NPs) are especially appealing among transition metals owing to manganese's status as a physiologically essential trace element, it's multiple accessible oxidation states, pronounced redox reactivity and favorable magnetic and catalytic characteristics properties that collectively enable diverse applications in theranostics, drug delivery, imaging and biosensing [1, 3, 4]. A growing body of literature underscores the expanding role of Mn-based nanomaterials in oncological detection and therapy, where they are being integrated with chemotherapy, radiotherapy and emerging modalities such as chemodynamic and photothermal treatment [5, 6]. Nevertheless, the broader deployment of Mn NPs remains constrained by the sustainability and biosafety concerns inherent to conventional synthesis protocols, which frequently rely on hazardous reducing agents, generate toxic byproducts and demand considerable energy input [7].

Green techniques address these limitations by utilizing biological systems or naturally derived extracts as simultaneous reducing and stabilizing agent, thereby enhancing biocompatibility while substantially reducing the ecological burden associated with nanomaterial production [8, 9]. Green routes involve microorganisms and plant or algal extracts which can influence the shape, size and surface properties of biomolecules [1, 10]. Within this context, algae have emerged as particularly promising nano-biofactories due to their rich repertoire of secondary metabolites, pigments, and functional groups capable of simultaneously reducing metal ions and capping the resulting nanoparticles [11]. Beyond their biosynthetic versatility, both macro and microalgae are well established for their capacity to sequester and bio transform heavy metals in aqueous environments, reflecting strong metal ligand coordination chemistry and inherent scalability in water- based systems [12].

Algae-mediated "phyco-nanotechnology" has been thoroughly investigated for the fabrication of metallic and metal oxide nanoparticles across a broad spectrum of biological systems [13]. Among red algal species, *Amphiroa fragilissima* have sparked interest as adaptable bio-resources because their cell walls and extracellular matrix are enriched with sulfated polysaccharides, structural proteins, and phenolic compounds capable of functioning as natural reducing and capping agents. The demonstrated ability of *A. fragilissima* to produce crystalline, colloidally stable silver nanoparticles exhibiting antibacterial activity against both gram positive and gram negative pathogens underscores its considerable potential as a nanofabrication [14]. Despite encouraging results with silver, the exploitation of *A. fragilissima* for the green synthesis of manganese-based nanomaterials remains substatially unexplored with no comprehensive physicochemical characterization reported in the peer-reviewed literature.

Concurrently, increasing evidence shows that biogenically synthesized manganese nanoparticles possess strong antimicrobial, antibiofilm and antioxidant properties along with acceptable cytocompatibility, making them promising candidates for addressing multidrug resistant infections [13, 16]. Notably, MnO_2 nanoparticles synthesized using plant leaf extracts demonstrate improved antibacterial, antifungal and biofilm inhibition activities as well as enhanced antioxidant capacity compared to chemically synthesized nanoparticles. These effects are largely attributed to the presence of bioactive phytochemicals on the nanoparticle surface [17]. Recent studies also highlight that phytosynthesied Mn nanoparticles can provide therapeutic benefits in controlling microbial infections while maintaining good hemocompatibility and low cytotoxicity [18, 19, 20]. Together these finding suggest that green synthesized Mn nanoparticles derived from marine biomass may combine favorable physiochemical properties with bioactive surface coatings, thereby improving their functional performance and safety. Therefore, the present study aims to develop a green synthesis protocol for manganese nanoparticles using aqueous extract of *A. Fragilissima* as a dual reducing and capping agent, and to comprehensively characterize the resulting nanomaterials in terms of optical properties, functional groups, crystalline structure, particle size, morphology, and surface charge. This work is expected to provide a foundational understanding of *A. fragilissima*-mediated Mn NP formation and to underpin future investigations into their antimicrobial and other biofunctional applications.

Experimental Methods and Characterizations

Biosynthesis of Manganese nanoparticles by means of the aqueous extract of *Amphiroa fragilissima*

Collection and preparation of seaweed aqueous extract

The seaweed (*Amphiroa fragilissima*) was collected from Rameshwarsam, Tamilnadu, India. Collected sample was brought to the laboratory in polythene bags containing natural seawater to avoid rotting or drying. The red seaweed *A. fragilissima* was first washed in tap water thoroughly to remove all debris, salt and adhering epiphytes. Then it was washed in sterile distilled water and shade dried for 4-5 days in room temperature. The sample was then ground into fine powder. The pure algal extract was prepared by adding 2gms of algal powder in 30ml of sterile distilled water and boiled for 2mins. The boiled extract was cooled and filtered through Whatmann No.1 filter paper and was stored in a refrigerated temperature for further analysis.

Biosynthesis of Manganese nanoparticle

Protocol for synthesis of manganese nanoparticles was followed according to John Peter Paul *et al.,* (2017) [21]. 1mM of potassium permanganate solution was prepared. 5ml of seaweed aqueous extract was mixed with 25ml of 1mM $KMnO_4$ solution. The reaction mixture was left for 24hrs in room temperature. After 24 hours, the nanoparticles settled at the bottom and the supernatant was discarded. The reaction mixture was then subjected to centrifugation and the supernatant was discarded. Resulting pellets were washed with 1ml of distilled water by centrifugation. Acetone was used to collect the pellets. The pellets were left for drying in the watch glass to collect the nanoparticles. Dried nanoparticles were stored in a container and used for further characterization techniques.

Characterization

The reduction of manganese ions was monitored using UV-Visible Spectroscopy at different time intervals. For each measurement, 2ml of the reaction mixture was analyzed and compared against 2ml of 1mM potassium permanganate solution which served as the blank reference [21]. Wavelength (λ max) at 0min, 15mins, and 30mins, 60mins, and 24hrs time intervals was recorded using UV-Visible spectroscopy.

FTIR

Fourier Transform Infrared (FTIR) spectroscopy was used to analyze the various functional groups associated with manganese nanoparticle formation. Spectral measurements were recorded in the range of 4000-500^{cm-1} with a resolution of 4 $^{cm-1}$, providing insights into the biomolecules responsible for reduction and stabilization during synthesis.

SEM

The morphology and average particle size of the synthesized Mn NPs were examined using Scanning Electron Microscope (SEM). This analysis provides detailed insights into the structural characteristics and surface features of the nanoparticles.

XRD

X- ray diffraction (XRD) analysis was employed to investigate the crystallographic structure of the synthesized manganese nanoparticles. The diffraction patterns provided information on the crystalline phases present, lattice parameters and degree of crystallinity, thereby confirming the structural organization of the sample.

Results & Discussion

Formation of Manganese nanoparticles

Exposure of the reaction mixture to the plant extract was accompanied by a distinct colour change. Manganese nanoparticles in aqueous solution are known to display vivid hues, shifting from violet to red, as a result of surface plasmon resonance phenomena. The emergence of the red colouration confirmed nanoparticle formation [21].

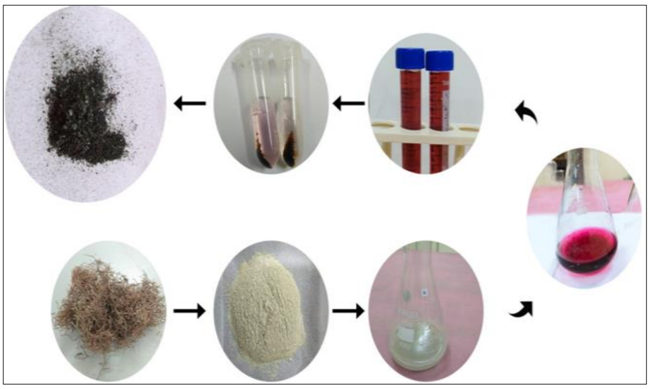

Fig 1: Process for synthesis of Manganese nanoparticle using aqueous extract of *A. fragilissima*

UV–Vis spectral analysis

The appearances of the peaks show the characteristics of surface plasmon resonance of nanoparticles. With increase in time interval the absorbance is decreasing. The absorption spectra exhibit a gradual decrease of the absorbance, accompanied by a shift in the wavelength from 500-580nm. The increase in the intensity of the plasmon bands indicate the decrease in the bandwidth (Picardi *et al.,* 2016). Decrease in peak intensity might be because of the formation of larger nanoparticles as a result of aggregation of small nanoparticles. (Mason *et al.,* 2012).

FTIR

FTIR measurements were carried out to identify the possible biomolecules in the manganese nanoparticle. Various functional groups were found. Mn peaks confirmed that the nanoparticle was of manganese chemical composition. The spectrum was recorded in the range of 4000-500cm-^{1}. The

FT-IR spectrum shows characteristic peaks. From the data, broad peak is observed at 3198.47 cm-1, which indicates the occurrence of alcoholic and phenolic groups. The slight vibration at 2922.77 cm-^{1} denotes the antisymmetric stretching vibrations of methylene. Then the peak shift to a higher level and forms a peak at 1619 cm-^{1} which confirms the presence of amide I protein and the peak shifted to 1623cm-^{1}. The vibrations at 1411cm-^{1} indicates the presence of alkane groups. And sharp peak at 1027.68cm-^{1} is contributed by (C-O) groups of polysaccharides.

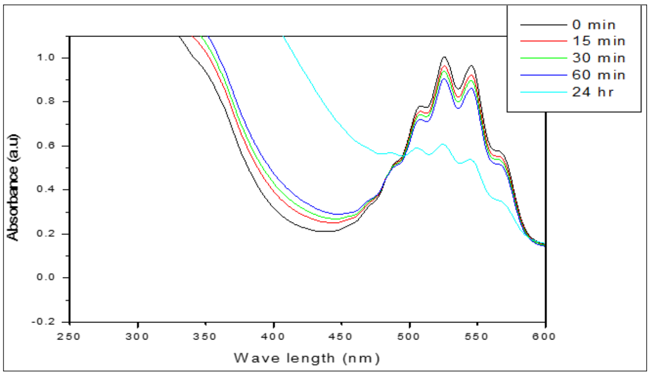

Fig 2: UV-vis spectrum of manganese nanoparticle recorded during the reduction reaction from 0 to 24 hours (nm: wavelength in nanometers)

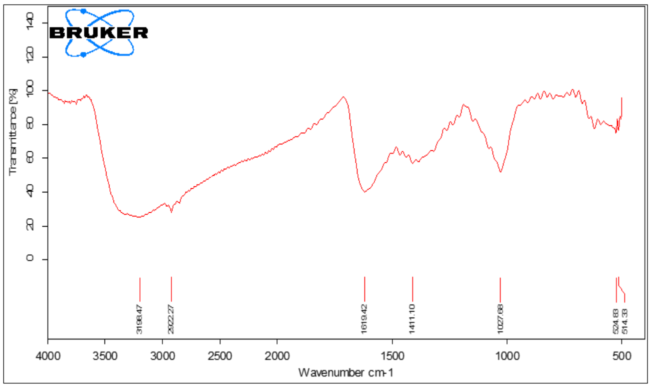

Fig 3: FTIR absorption spectra of biosynthesised manganese nanoparticle

XRD

XRD pattern was examined using powder X-ray diffractometer instrument (XRDML) in the angle range of 10˚C-80˚C of the manganese nanoparticles at 2θ, scan axis. The amorphous nature of biosurfactant moieties surrounding the manganese nanoparticle and the small size of the nanoparticle results in the collectively broad peak. The crystalline structure of manganese nanoparticle was confirmed by X-ray diffction pattern along with impurities. Broad crystalline reflections are at 24.62º, 27.02º and 32.4º. The lattices are depicted as 201º, 211º and 301º.

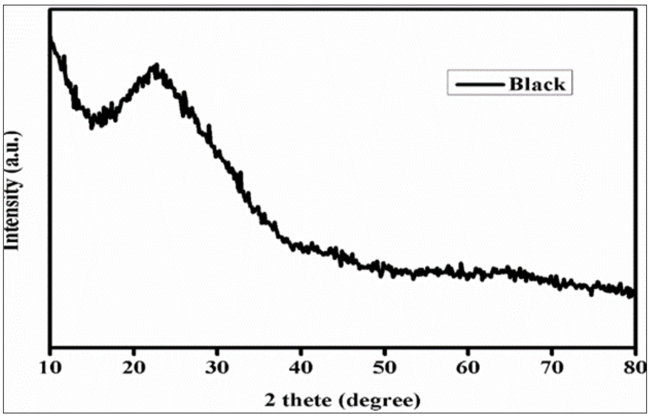

Fig 4: XRD pattern of the biosynthesised Manganese Nanoparticle

SEM

SEM micrograph was observed at magnification of 10.0 kx, 5.0. Kx 2.5 kx, 1.0 kx. The formed manganese nanoparticle shows moderate dispersion and slight agglomeration. SEM images show that most of the particles are polymorphic. SEM analysis revealed that the average size of manganese nanoparticle was 76.29 ± 147.61 nm. The micrographs also revealed that the manganese nanoparticle is spherical/ elliptical in shape.

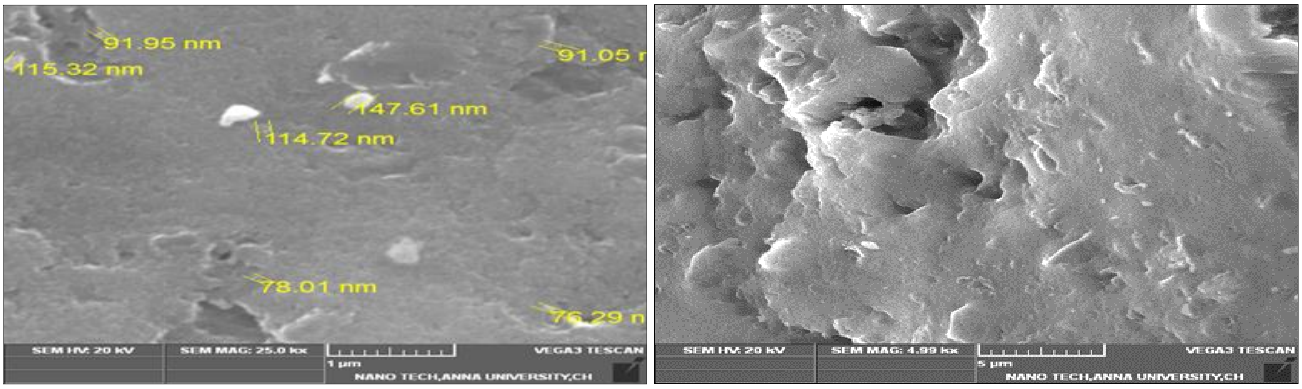

Fig 5 (a, b): SEM images of biosynthesized Manganese Nanoparticle

Discussion

This study investigated the potential of the aqueous extract of the red seaweed *A. fragilissima* to serve as a biogenic matrix for the green synthesis of manganese nanoparticles (Mn NPs) and examined the influence of its phytoconstituents on the properties of the nanoparticles. The extract effectively reduced the manganese precursor to stable Mn NPs, as evidenced by changes in UV–Vis absorption, XRD confirmation of crystallinity, FTIR detection of various organic functional groups, and SEM images depicting predominantly spherical particles with moderate agglomeration. These outcomes align with recent reports that green synthesis routes can reduce use of toxic reagents, production cost and improve colloidal stability compared with conventional chemical methods [1]. They also support evidence that marine algae act as efficient "green factories," providing polyphenols, proteins, and polysaccharides that direct nucleation, growth, and capping of metal nanoparticles [9]. The crystalline, mostly sphere-shaped Mn NPs obtained here are comparable to other biogenic MnO_2 systems synthesized using plant extracts.

The practical significance of these findings lies in the growing use of Mn-based nanomaterials as platforms for drug delivery, imaging, catalysis, sensing, and antimicrobial applications [16]. Surface functionalization by seaweed-derived biomolecules may provide additional antioxidant and antimicrobial potential, but this remains to be experimentally verified. Key limitations include the use of a single algal species and precursor, limited parameter screening, and the absence of detailed biological and stability assessments, all of which constrain immediate translational conclusions. Overall, this work identifies *A. fragilissima* as a promising marine algal platform for eco-friendly synthesis of manganese nanoparticles and provides foundational physicochemical data that can guide future optimization and application-oriented studies in biomedical and environmental nanotechnology.

Conclusion

In summary, this study shows that red seaweed *A. fragilissima* is an effective natural platform for the grenn synthesis of Mn nanoparticles. Its phytoconstituents appear to function as both reducing and stabilizing agents during nanoparticle formation. The UV–Vis profile, XRD, FTIR, and SEM analyses confirmed the successful formation of stable, mainly spherical and crystalline Mn nanoparticles with functional surface groups. These results suggest that algal biomolecules play an important role in controlling nucleation, growth and capping, in agreement with recent studies on phyconanotechnology [1, 9, 16] The eco-benign route established here is particularly relevant because Mn nanostructures are gaining importance as bioactive platforms for antimicrobial applications, imaging, drug delivery and catalysis [16, 21] *in vitro* or *in vivo* assays—may restrict immediate translational interpretation and further work is needed to evaluate dose–response, mechanistic bioactivity, and long-term safety. Taken the findings indicate that marine macroalgae can serve as sustainable nano-biofactories and support the future development of *A. fragilissima* derived Mn nanoparticle for biomedical and environmental applications.

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