

Structural characterization of marine macroalgae derived silver nanoparticles and their colorimetric sensing of hydrogen peroxide

Ramesh Vinayagam^a, Vasundra Nagendran^a, Louella Concepta Goveas^b,
Manoj Kumar Narasimhan^c, Thivaharan Varadavenkatesan^d, Narendhar Chandrasekar^e,
Raja Selvaraj^{a,*}

^a Department of Chemical Engineering, Manipal Institute of Technology, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India

^b Nitte (Deemed to be University), Department of Biotechnology Engineering, NMAM Institute of Technology (NMAMIT), Nitte, India

^c Department of Genetic Engineering, School of Bioengineering, College of Engineering and Technology, SRM Institute of Science and Technology, Potheri, Kattankulathur, Tamil Nadu, Chengalpattu District, 603203, India

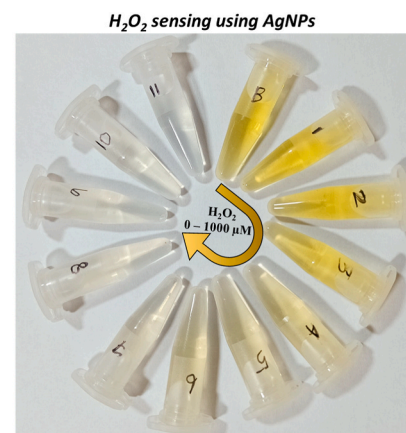
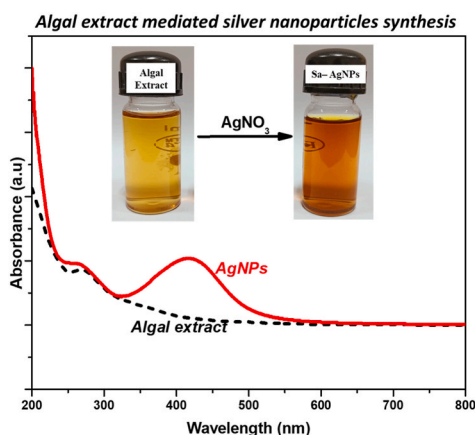
^d Department of Biotechnology, Manipal Institute of Technology, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India

^e Department of BioNano Technology, Gachon University, 1342, Seongnam-Daero, Sujeong-Gu, Seongnam-si, 13120, Gyeonggi-do, Republic of Korea

HIGHLIGHTS

- AgNPs were synthesized using *Sargassum* spp. extract.
- The AgNPs were observed to be highly stable zeta potential value (−22.6 mV).
- Developed colorimetric method to detect H₂O₂ in aqueous samples.

GRAPHICAL ABSTRACT



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ABSTRACT

The present study investigates the algae-mediated synthesis, characterization, and H₂O₂ sensing ability of silver nanoparticles using *Sargassum* spp. extract. The synthesized nanoparticles were spherical agglomerates and exhibited an absorbance peak at 418 nm, as analyzed by FE-SEM and UV-vis spectrophotometric analysis. TEM-SAED analysis interpreted the size between 2 and 35 nm and crystallinity corresponding to face-centered cubic (FCC) silver structure. This was further supported from XRD findings, which ascertained the presence of (111), (200), (220), and (311) lattice planes. FT-IR analysis confirmed the capping of algal phenolics as corresponding

* Corresponding author. Department of Chemical Engineering, Manipal Institute of Technology, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India.

E-mail address: raja.s@manipal.edu (R. Selvaraj).

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functional groups, which was further confirmed by XPS studies. The nanoparticles were observed to be highly stable in aqueous suspensions, due to the zeta potential value (-22.6 mV). On incubation of synthesized nanoparticles, with H_2O_2 solutions, decolorization of nanoparticle solution was observed along with the reduction of absorbance peak at 418 nm. This color change could be utilized for H_2O_2 detection in contaminated samples. Therefore, this study reveals the potential of sustainable *Sargassum* spp. biomass in synthesizing beneficial silver nanoparticles which could be exploited for environmental detection applications.

1. Introduction

Nanotechnology is a fast-evolving subject that works with materials at the nanoscale with dimensions $1\text{--}100$ nm. It has attained lot of focus in the recent decade because of its enormous potential and diversified uses in material engineering, nanomedicine, microfluidics, electronics, energy, and environment [1]. Nanomaterials, as opposed to bulk materials, have superior physicochemical characteristics owing to minute size and distinct properties. This results in their reactivity as well as their exceptional chemical, electrical, optical, magnetic, and mechanical capabilities [2]. Nanotechnology paves the way to design, manipulate and tailor various well known attributes of nanoparticles (NPs) like stability, high surface-area-to-volume ratio, charge and shape [3]. NPs are divided into many categories such as organic, inorganic, metals, metal oxides, ceramic, and carbon-based nanoparticles [4]. NPs produced from metals such as gold, silver and platinum have been extensively investigated upon, as they offer various benefits and hence, have been exploited for variety of purposes. Metal nanoparticles are widely used in healthcare, electronics, catalysis, and environmental applications. Metal nanotechnology is a developing field that is advancing through research [5].

Silver nanoparticles (AgNPs) are predominantly investigated, as they are highly stable and minimally chemically reactive, in comparison with other metallic NPs [6]. AgNPs have long been recognized to have antimicrobial characteristics, and fortunately, they appear to be more effective and efficient antibacterial agents than other noble metal nanomaterials [7]. AgNPs have been researched for their potential applications in dentistry [8], wound healing [9], food packaging [10], cancer treatment [11], dye degradation [12], and various other environmental applications.

Most of the physico-chemical and biological methodologies of NP synthesis are built on the principles of top-down and bottom-up methods. The top-down method emphasizes on condensation of bulk material to fine NPs by monomer formation, while in bottom-up, they are constructed from the atom. Physical methods include aerosols, arc discharge, and thermal decomposition [13], requiring extreme pressure and temperature. Chemical methods include chemical vapor deposition, micro-emulsion, sol-gel and precipitation, micro-emulsion [14]. However, these solutions come at a cost in terms of the environment and energy consumption. Some of the downsides of this technology include the use of toxic materials, difficulties in scaling up, and lack of control over size and shape. Owing to these disadvantages, green synthesis is steadily substituting the physico-chemical synthesis of NPs [15].

The risks associated with physico-chemical synthesis methods are substantially reduced by green nanotechnology, an effective approach that suitably simplifies NP manufacturing and applications [16]. Plant parts such as rhizome, seed, stem, fruit, flower, peel, bark and callus, as well as microbes like algae, fungi and bacteria are routinely employed. These biological sources' organic molecules can be employed to reduce metal salts to nanoparticles [17]. Algae are popular for their abilities in accumulation of heavy metal ions and subsequent modification to simpler forms [18]. Algae have been used in the field of nanotechnology owing to their significant potential in bio-nanomaterial production as they are cost-effective, versatile, and eco-friendly [19].

Brown algae, also known as *Phaeophyceae*, familiar its applications in various fields including industries. Food and nutrition, pharmaceuticals, bioremediation, biofuel generation, and many others are among them.

Owing to its eco-friendly approach, *Sargassum* species are utilized for the preparation of several NPs such as gold [20], silver [21], and zinc [22]. Recently, our research team has reported the synthesis of magnetite NPs using *Sargassum* species and explored the methylene blue dye degradation potential [23].

Hydrogen peroxide (H_2O_2) is known for its peculiar properties such as both oxidizing agent and as a disinfectant make it useful in various applications [24]. Nevertheless, proper handling and safety precautions are required for guaranteed safe use [25]. As a result, developing precise and rapid ways to detect H_2O_2 is critical. Many techniques have been proposed to detect H_2O_2 , including spectroscopic, electrochemical, fluorescence, and colorimetry-based methods. Among these methods, colorimetric detection stands out as the most preferred option due to its simplicity, quick response time, high detection limit, cost-effectiveness, and practicality [26]. Of particular interest in H_2O_2 detection are AgNPs due to their intriguing optical properties. The use of green chemistry principles in synthesizing AgNPs has yielded specific properties, such as enhanced stability through the capping action of phenolic compounds and a consistent UV-vis absorption spectrum of the solution [27]. Recent research has explored various green resources for AgNPs synthesis, including *Capsicum baccatum* extract [28], *Benincasa hispida* fruit extract [29], cellulose nanowhiskers [30], and lignin [31]. These environmentally friendly approaches have shown promise in producing stable AgNPs for H_2O_2 detection. Our team has also contributed to this area by publishing a research paper on rapid detection of H_2O_2 using *Calliandra haematocephala* leaf extract [27]. This complements the rising body of knowledge and underscores the significance of green resources in advancing H_2O_2 detection methods.

According to our earlier study [23], *Sargassum* species-mediated magnetite nanoparticles could be employed to build a low-cost, long-term solution for wastewater treatment. Likewise, in this case, there is a need for more specific and direct methods of sensing H_2O_2 such as the one sought in this study. Literature reveals that there have been no studies on employing AgNPs from *Sargassum* species extract for H_2O_2 sensing. Hence, in this study, *Sargassum* spp. extract-mediated AgNPs were synthesized, and characterized and their potential in H_2O_2 detection was investigated.

2. Materials and methods

2.1. Chemicals

Silver nitrate and hydrogen peroxide utilized in this study were purchased from Merck, India. Analytical grade reagents were employed here. Distilled water was employed to make all the working solutions.

2.2. Algal extract and AgNPs preparation

The gathered brown algae [23] were cleaned, dried, and pulverized before being stored as a powder. This powdered algal biomass was blended with distilled water (100 mL) in a ratio of $1:100$. The resultant solution was heated at 80°C for 60 min before cooling to room temperature. Post filtration of cooled solution on Whatman No. 1 filter paper, filtrate was collected and stored at 4°C until use [23]. Algal extract (25 mL) and silver nitrate solution (1 mM, 25 mL) were combined and held in a thermostat at 80°C for 1 h. As the AgNPs took shape via a bottom-up approach, it led to a bright golden-brown colloidal

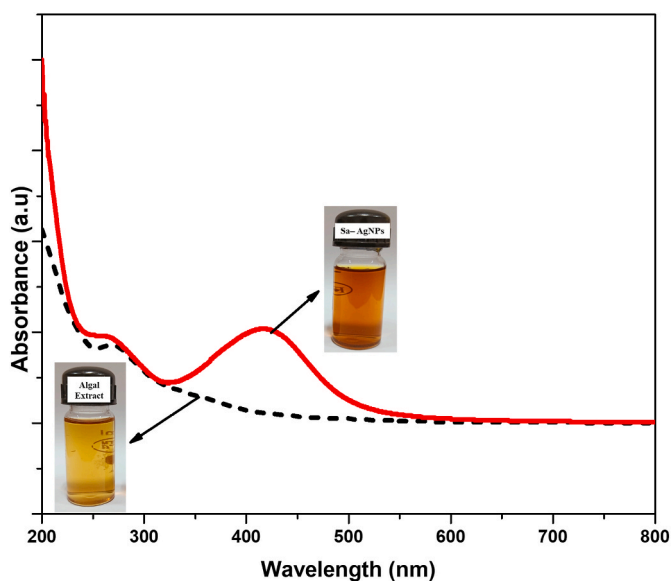


Fig. 1. UV-vis spectra of Sa-AgNPs and algal extract.

solution. Characterization and other applications were carried out with colloidal algal-silver nanoparticles that were referred to as Sa-AgNPs. The nanoparticles were termed so as they were created by the reducing effect of the phenolic components found in *Sargassum* spp. extract [32].

2.3. Characterization

Neon 40-Carl Zeiss, X-act was used to perform Field emission scanning electron microscopy (FESEM) and capture high-quality pictures, whereas, the corresponding elemental compositions were acquired using Oxford Instruments, UK correspondingly. High-Resolution Transmission Electron Microscopy (HRTEM) and Selected Area Electron Diffraction (SAED) pictures were attained using JEM-2100 Plus, JEOL Japan. The D8 Advance - Bruker, Germany X-ray diffractometer documented XRD patterns as well as the data on their crystalline states. Functional groups found on NPs surface were analyzed by a FTIR Spectrophotometer (Cary 660 – Agilent Technologies, USA). Spectral information on the elemental valency states of the samples were recorded by X-ray Photoelectron Spectrometer, XPS (K-Alpha, Thermo Fisher Scientific, UK). Zeta analysis and hydrodynamic size were investigated by Malvern/Nano Zeta Sizer (ZS-90 m), United Kingdom.

2.4. H_2O_2 sensing experiments

Various concentrations of hydrogen peroxide (0–1000 μ M) were prepared to assess the colorimetric assay sensitivity. Subsequently, a 1

mL solution of H_2O_2 at the specified concentration was mixed with 3 mL of Sa-AgNPs and left to incubate for 3 h [30]. After the incubation period, the spectra and the reduction in absorbance were monitored by UV-vis spectrophotometer, at the respective concentration of H_2O_2 .

3. Results and discussions

3.1. Visual and UV-vis spectral studies

Post-visual inspection, UV-vis spectral examination was used to establish the synthesis of Sa-AgNPs. The steady color shift to golden brown that occurs after heating the mixture of algal extract and silver nitrate solution, acts as a preliminary confirmation for the formation of AgNPs [33]. Additionally, it reveals that the phenolic compounds amend the valency of silver from 1 to 0 in the synthesis process [34]. The spectrum of both algal extract and the Sa-AgNPs between 200 and 800 nm is depicted in Fig. 1. Both spectra depicted absorption peaks at 276 nm, which were due to ($n \rightarrow \pi^*$) or ($\pi \rightarrow \pi^*$) electronic transition of the phenolic moieties inherent to the extract [35]. The Sa-AgNPs spectrum portrays the realization of NPs, authenticated by the surface plasmon resonance (SPR) peak (418 nm), a distinguishing attribute of AgNPs [32]. This substantiates that reducing agents of algal extract are involved in Sa-AgNPs formation. A similar peak was noted for AgNPs using the *Galaxaura rugosa* extract [36]. Additionally, SPR peaks such as 420 nm using *Teucrium polium* extract [37] and 424 nm using *Callistemon lanceolatus* leaf extract [38] were also documented.

3.2. FE-SEM and EDS studies

The topography and surface morphology of Sa-AgNPs were visualized using an FESEM image. Spherical-shaped Sa-AgNPs were witnessed (Fig. 2a) with agglomeration, forming spherical clusters which might have been owing to capping of phenolics in the algal extract [32]. Energy Dispersive X-Ray Spectroscopy (EDS) was exploited to catalog the elements present in the AgNPs. As observed from Fig. 2b, the spectrum indicates signals for Ag, C, Cu, and other elements. It is noticeable that the prominent occurrence of silver signal (3 keV) is undoubtedly from the synthesized Sa-AgNPs [39]. The signal for C at 0.28 keV is due to the phenolics of the algal extract. Further, the presence of Cu is due to the copper grid that was used during the analysis. The weak signal for other elements could be due to the components present in the algal extract.

3.3. TEM studies and SAED patterns

The TEM images (Fig. 3a and b), corroborated that the produced Sa-AgNPs were certainly spherical which aligned with the FE-SEM results. The images also revealed the presence of agglomeration in the sample which was attributed to the biomolecules capping the surface of nanoparticles [23]. The nanoparticles had diameters between 2 and 35 nm, and 11.99 nm average diameter (Fig. 3c). Fig. 3d shows the SAED pattern of Sa-AgNPs, which depict concentric rings with occasional dots,

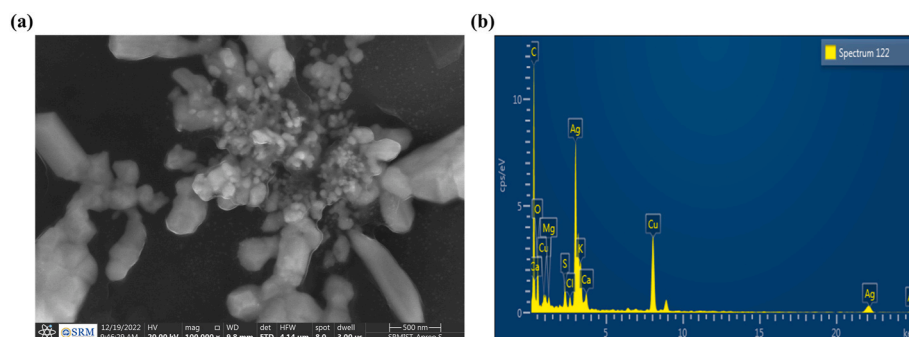


Fig. 2. FE-SEM image (a) and EDS image of Sa-AgNPs (b)

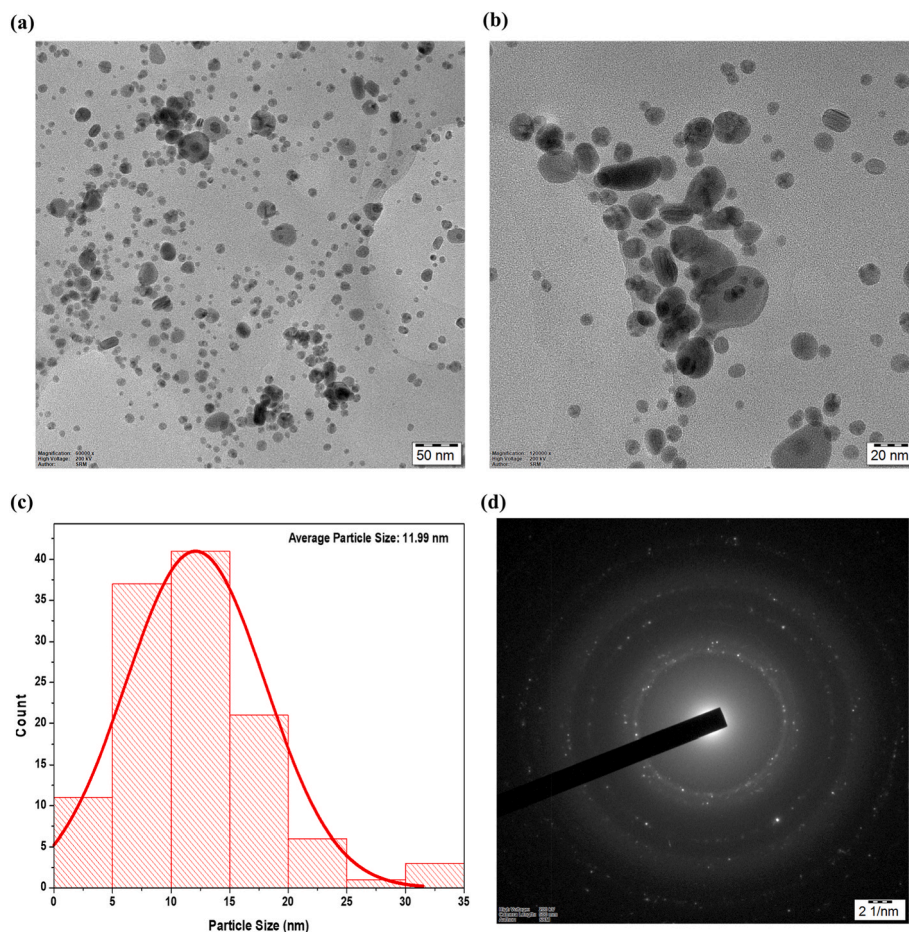


Fig. 3. TEM images at different magnification (a) & (b), size distribution (c) and SAED patterns of Sa-AgNPs (d)

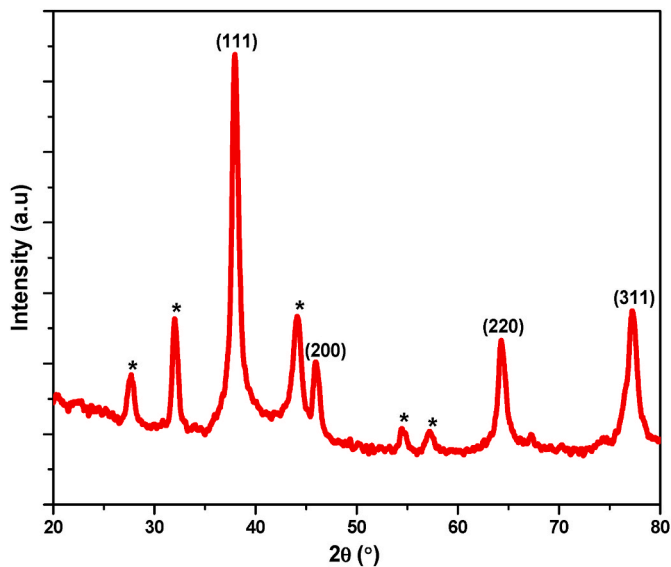


Fig. 4. XRD image of Sa-AgNPs.

indicative of crystallinity of the nanoparticles [40]. This corresponds to the elemental silver face-centered cubic (FCC) structure.

3.4. XRD results

The XRD spectrum of the Sa-AgNPs (Fig. 4) shows Bragg's reflection

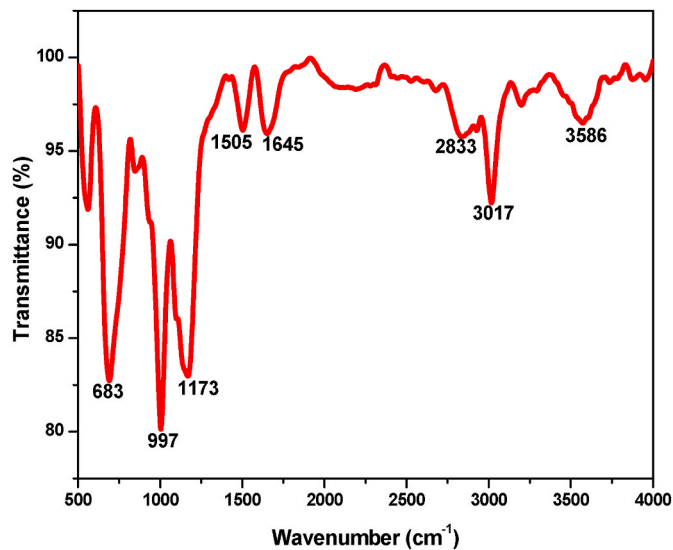


Fig. 5. FTIR image of Sa-AgNPs.

peaks at 2θ ($^\circ$) of 37.87, 45.92, 64.34 and 77.36. On comparison to reference (JCPDS Card No. 04-0783), the produced Sa-AgNPs closely match the crystallinity data. The peaks correspond to Ag crystalline planes (111), (200), (220), and (311) correspondingly. Similar results was reported by Jemal et al. [41] and indicates that the Sa-AgNPs are crystalline, cubic and face-centered, corroborating the SAED pattern. A

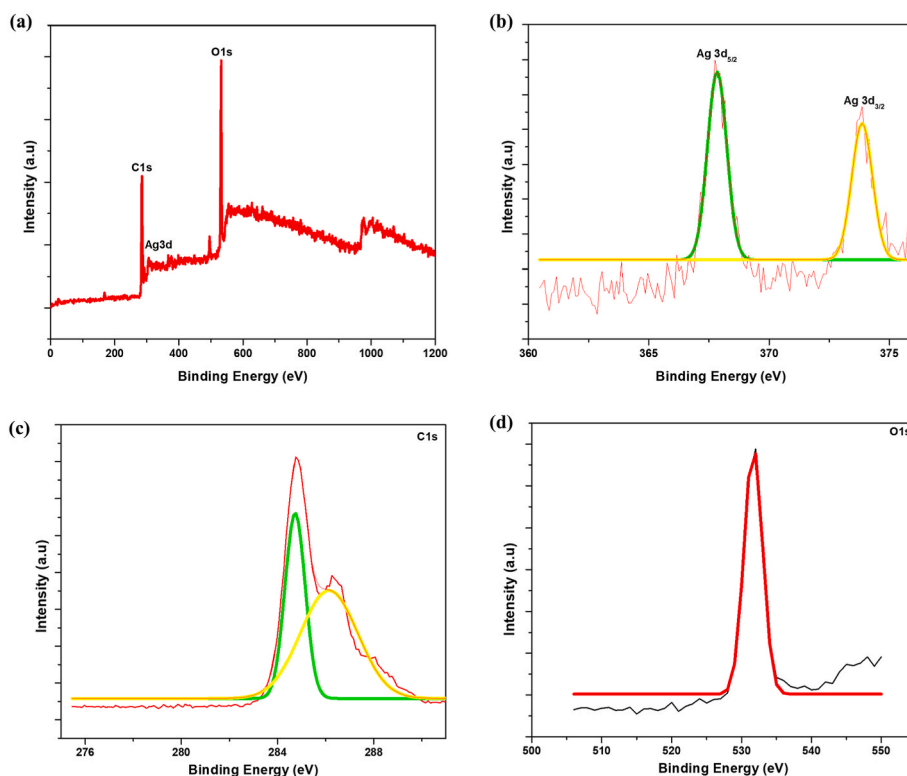


Fig. 6. XPS images: complete spectra (a), Ag 3d (b), C1s (c) and O1s (d) of Sa-AgNPs.

number of unassigned peaks (*) were noticed, which could be due to crystallization of algal extract organics on silver surface [41], acting as capping agents [35]. Crystalline nature was computed by Bragg's equation and the average crystallite size was 11.244 nm which was close to the average mean crystalline sizes from TEM studies.

3.5. FTIR studies

FTIR spectroscopy was performed for identification of functional groups in the synthesized Sa-AgNPs. The transmittance spectra for the sample can be seen in Fig. 5. Significant grooves around the regions 683, 997, 1173, 1505, 1645, 2833, 3017 and 3586 cm^{-1} were observed. Upon comparing with the literature, the band around the region 3586 cm^{-1} can be attributed to the alcoholic O–H stretching vibrations and closely matches the results reported by the researchers [32]. The band at 2833 cm^{-1} indicates C–H stretching by methylene groups as per the report [42], while that seen around 1645 cm^{-1} is accredited to C=C or C=O moieties as described in a study [43]. The 1173 cm^{-1} signal can be ascribed to the secondary amine C–N stretch [44], whereas the signals around 683 and 542 cm^{-1} could be the innate proteins that interact with the synthesized Sa-AgNPs. The significance of the band seen at 1505 cm^{-1} certainly matches the N–H stretching by nitro compounds exhibited by AgNPs synthesized from *Cannabis sativa* extract [45]. 3017 cm^{-1} signal could be possibly explained by the moderately strong C–H stretching of alkenes and the one at 997 cm^{-1} is owing to strong =C–H or C–H bending of monosubstituted alkenes [46]. Hence, the existence of the multiple functional moieties of the prepared Sa-AgNPs confirmed the role of the algal extract in reducing and stabilizing the nanoparticles.

3.6. XPS studies

XPS assessed the binding state of Ag ions contained in the AgNPs. Fig. 6a depicts the XPS spectra of the synthesized Sa-AgNPs and their matching high-resolution spectra of silver (Ag 3d), oxygen (O 1s), and carbon (C 1s). The notable signals (Fig. 6b) at binding energies of 367.75

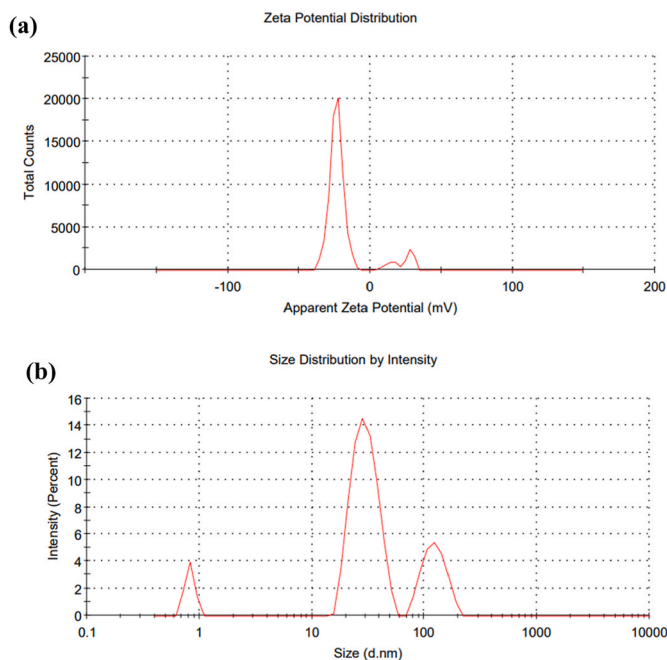


Fig. 7. Zeta potential (a) and size distributions (b) of Sa-AgNPs by DLS technique.

and 373.85 eV correspond to the unperturbed metallic silver orbits Ag 3d_{5/2} and Ag 3d_{3/2} respectively [42]. The carbon peak (Fig. 6c) is between 284.95 eV and 286.36 eV, corresponding to C–C and C–O bonds [47]. Furthermore, as can be noticed, the high-resolution spectra reveal the presence of oxygen at 533.85 eV (Fig. 6d), which corresponds to the O–C=O bond of O1s [48]. Notably, the absence of additional peaks in the XPS spectra confirms the formation of pure AgNPs, implying that no

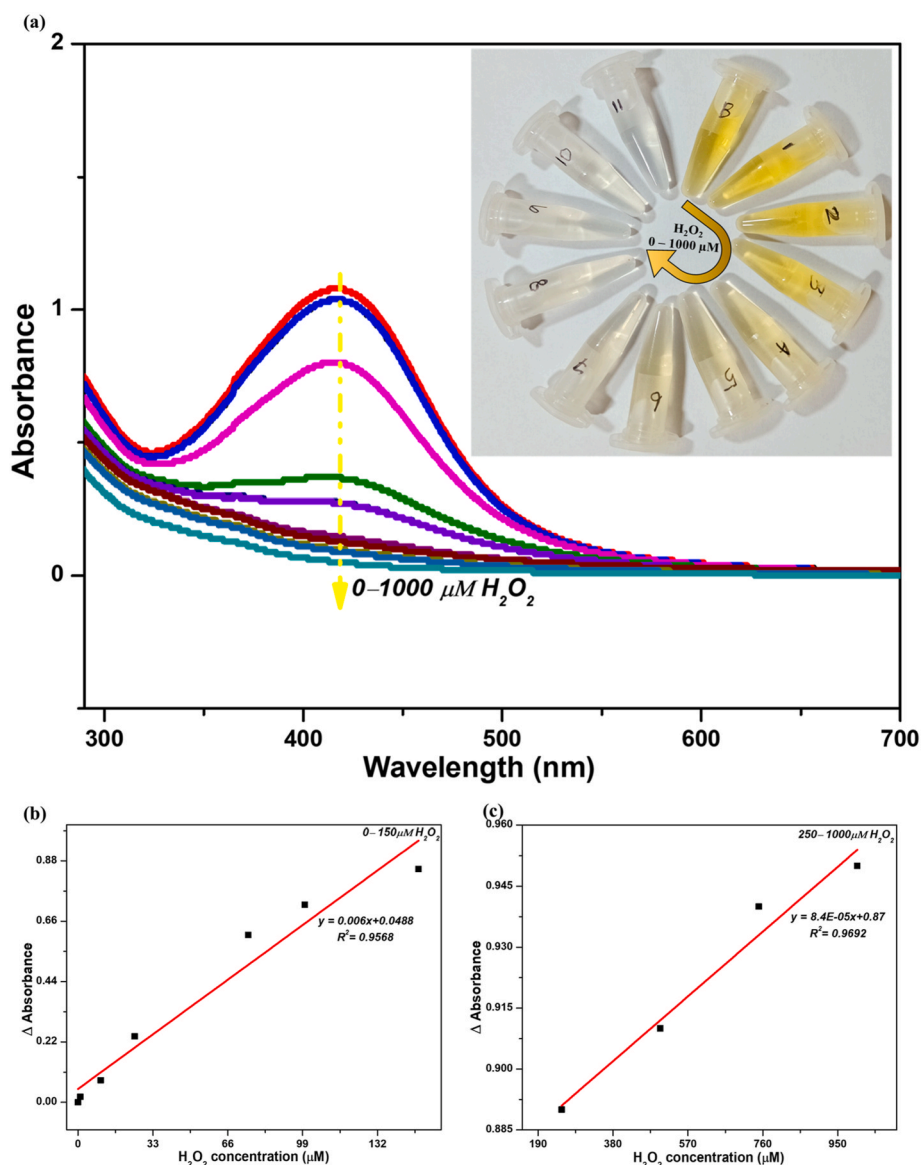


Fig. 8. Colorimetric sensing of H_2O_2 using Sa-AgNPs: UV-vis spectra of Sa-AgNPs at various H_2O_2 concentrations (0–1000 μM) with their respective photographs (a), linear response (Δ Absorbance at Ex_{418}) of the colorimetric assay against increasing H_2O_2 concentrations (b & c).

unreduced Ag (I) was present. Also, the obtained findings are consistent with the EDS pattern. Altogether, the presence of the unassigned diffraction planes in XRD, the various functional groups in FTIR, XPS analysis, and UV-vis studies provides evidence to support the part of the polyphenolic components as capping agents in Sa-AgNPs synthesis. The findings are coherent with the *Acacia ehrenbergiana* mediated AgNPs [35].

3.7. Zeta potential and DLS studies

The stabilities and surface charges of the Sa-AgNPs in aqueous suspensions, were studied using zeta potential measurements. A strong peak at -22.6 mV (Fig. 7a), was noticed, that is indicative of excellent stability since this value is within the range of $+30$ and -30 mV, a standard for stable colloidal systems [49]. Sa-AgNPs exhibited colloidal state in the solution, as confirmed from the negative value, confirming relative stability [50]. The negative value confirms the repulsive force between the particles, indicating that the nanoparticles are incredibly stable. As like charges repel each other, the constituent particles in the solution do not agglomerate, resulting in a stable colloidal solution.

Fig. 7b depicts the outcome of particle size distribution by Dynamic Light Scattering (DLS) method. The poly-dispersity index (PDI) value of the produced Sa-AgNPs was 0.246, signifying the fact of devoid of agglomeration leading to better stability and monodispersity as described elsewhere [32,51]. A similar value (PDI: 0.237) was reported in our earlier study for the AgNPs synthesis by *Arachis hypogaea* nut extract [32]. The mean hydrodynamic diameter was measured as 50.73 nm which is much higher than TEM results (11.99 nm) due to the difference in the basic principle behind the two methods. Similar observations have been reported for AgNPs synthesized using various sources such as *Mentha spicata* essential oil (Diameter TEM: 24 nm and Diameter DLS: 72 nm) [52], *Trichoderma longibrachiatum* cell filtrate (Diameter TEM: 10 nm and Diameter DLS: 24.43 nm) and *Pterodon emarginatus* leaves extract (Diameter TEM: 33.20 nm and Diameter DLS: 68.72 nm).

3.8. Colorimetric detection of hydrogen peroxide

The absorbance spectral pattern of the colloidal suspension of Sa-AgNPs and various H_2O_2 concentrations was monitored (Fig. 8a). Absorbance peak at 418 nm reduced, while the solution gradually varied

from yellow to colorless with the rise in H_2O_2 concentrations (Fig. 8a inset). This implies that the silver in the Sa-AgNPs, which was initially in its neutral state (Ag^0), is oxidized to Ag^+ by the addition of H_2O_2 which in other words causes degradation of the NPs [27] which underscores the mechanism of detection. Similar outcomes in the literature for H_2O_2 detection using AgNPs and AgCl-NPs, wherein, the reductions of 410 nm [30] and 417 nm [53] peaks were respectively monitored with increasing H_2O_2 concentrations. This further strengthens the evidence for the concentration dependency of H_2O_2 in degrading Sa-AgNPs.

Fig. 8b&c displays the calibration curves of the colorimetric assay responses. The plot of $\Delta\text{Absorbance}$ ($\text{Absorbance}_{\text{Sa-AgNPs}} - \text{Absorbance}_{\text{sample}}$) against H_2O_2 concentration within two concentration ranges (1–150 μM and 250–1000 μM) demonstrates a linear relationship. The limit of detection (LOD) calculations were performed by following the procedures described in the literature [30,53]. The obtained LOD values for H_2O_2 , using our colorimetric assay were 44.60 μM and 232.42 μM for concentration ranges of 1–150 μM and 250–1000 μM , respectively. Remarkably, these LOD values are comparable to the reported values of 112 μM (H_2O_2 : 60–600 μM) [30] and 110 μM (H_2O_2 : 200–500 μM) [26] achieved using AgNPs for H_2O_2 detection. This comparison suggests that the synthesized Sa-AgNPs can be considered a reliable choice for detecting H_2O_2 in various samples.

4. Conclusions

Sargassum spp. biomass was effectively utilized for the synthesis of small-sized crystalline silver nanoparticles. The confirmation of the involvement of algal phenolics in the nanoparticle synthesis was ascertained by FTIR and XPS analysis. Their structure, shape, and size were confirmed from FE-SEM and TEM-SAED analysis. Sophisticated techniques like zeta potential and XRD assisted in the identification of superior qualities of synthesized nanoparticles. These nanoparticles could easily detect H_2O_2 in aqueous solutions, visually, as the decolorization of nanoparticle solution occurred with the H_2O_2 concentration rise. The use of these AgNPs ensures an easy and visual detection of H_2O_2 ensuring its proper handling and usage.

CRediT authorship contribution statement

Ramesh Vinayagam: Methodology, Data Curation and original draft preparation; **Vasundra Nagendran:** Editing– Characterisation, Results and discussions; **Louella Concepta Goveas:** Validation & Visualization; **Manoj Kumar Narasimhan:** Validation & Visualization. **Thivaharan Varadavenkatesan:** Software and data analysis; **Narendhar Chandrasekar:** Writing - Reviewing and editing. **Raja Selvaraj:** Conceptualizing with Supervision – Reviewing and Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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