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Sargassum Polycystum C.Agardh Mediated Synthesis of Gold Nanoparticles Assessing its Characteristics and its Activity against Water Borne Pathogens

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Abstract

This paper reports on the eco-friendly synthesis of gold nanoparticles (AuNPs) using the seaweed *Sargassum polycystum* C. Agardh extract. Biological synthesis for nanoparticle using plants is gaining considerable interest among researchers as an eco-friendly alternative to conventional physical and chemical methods, as this approach eliminates the use of toxic chemicals. Synthesized AuNPs was monitored by UV-Vis spectroscopy and was found to be complete within 30 min. Confirmation of elemental gold was carried out by elemental mapping using different physical techniques such as Fourier transform infrared (FT-IR) spectroscopy, X-ray diffraction (XRD) and energy dispersive X-ray spectroscopy (EDX), Scanning electron microscopy (SEM). The bio reduced AuNPs exhibited remarkably good anti-bacterial activity against pathogens specifically *Pseudomonas aeruginosa* (20 mm) which is more susceptible. The elaborate experimental evidences support that the seaweed *Sargassum polycystum* C. Agardh extract can provide an environmentally benign rapid route for synthesis of AuNPs that can be applied for various purposes.

Keywords: X-ray diffraction; Optical properties; Nano structures

Introduction

Synthesis of gold nanoparticles (AuNPs) has gained immense significance during the last few years due to their catalytic, optical, and electrical properties [1]. Nanoparticles exhibit atom-like behaviours due to high surface energy resulting from high and large specific surface area, high fraction of surface atoms and wide gap between valence and conduction band when divided to near atomic size. The synthesis of AuNPs using an eco-friendly method is important to address the growing concerns on the overall toxicity of nanoparticles for medical and biotechnological applications. AuNPs have been considered important due to their unique and tunable surface plasmon resonance (SPR) property and their applications in biomedical science including drug delivery, tissue/tumour imaging, photo thermal therapy, immune chromatography, and identification of pathogens in clinical specimens [2]. Most of the available chemical processes for synthesis of gold nanoparticles (AuNPs) involve toxic chemicals that get adsorbed on the surface, leading to adverse effects in medical applications. Presently there is a growing need to develop environmentally benign process for rapid synthesis of nanoparticles [3]. The choice of an environmentally compatible solvent system, an eco-friendly reducing agent, and non-hazardous capping agents for the stabilization of the nanoparticles are three main criteria for a totally "green" nanoparticle synthesis. Concerning the biological application of nanoparticles it has been emphasized that methods of synthesis through biological system via, micro-organisms include bacteria, yeasts, fungi and diatoms synthesizing inorganic materials either intra or extracellularly would make the nanoparticles more biocompatible [4]. The uses of marine algae include traditional cosmetics, as antipyretic and antiseptic compounds, vermifuges and as treatments for sunstroke, coughs, haemorrhoids, stomach ailments, nose-bleeds, goitre and urinary diseases [5]. *Sargassum polycystum* belongs to Phaeophyta species. It is reported to be the most abundant brown algae and chief source of alginic acid. It has been suggested that environmental factors such as temperature, salinity, rainfall and p^H influences the seasonal growth in *Sargassum* species. During recent years *Sargassum* from coastal water have been subjected to repeated annual [6]. Herein, we report the biogenic synthesis of AuNPs using aqueous extract of *S.polycystum* for reduction of Au ions. We demonstrated its antibacterial activity against various water borne pathogens.

Materials and Methods

Collection of seaweed and extract preparation

Sargassum polycystum was collected from the Gulf of Mannar, Rameshwaram (9.2800° N, 79.3000° E), Tamil Nadu, India. The seaweed sample was washed in seawater and then fresh water to remove the epiphytes and other contamination. The sample was authenticated by Botanical Survey of India (BSI), Coimbatore (BSI/SRC/5/23/2014-15/Tech.169). The dry leaves were ground into fine powder in electric blender (Bajaj Model GX 11, Mumbai, India). 5 g of this powder was suspended in 100 mL of distilled water in a 300 mL Erlenmeyer flask followed by boiling for 15 min. The extract obtained was filtered through Whatmann filter paper No.1 (50 mm; Sigma, Bangalore, India). The filtrate was collected and stored at 4°C which was used throughout all the experiments. The reaction was carried out at room temperature [7]. The reagents were of analytical grade obtained either from Merck (Mumbai, India) and Sisco Research Laboratories (Mumbai, India).

Synthesis of AuNPS

The reaction mixture was initially light brown and there was no

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turbidity formation during the course of time. Synthesis of AuNPs was carried out at the concentration of 50% against 1mM $HAuCl_4$ in a total volume of 100 ml made up with double distilled water. Reduction of Au^{3+} ions was initiated by addition of 5 mL of extract to 95 mL of 10^{-3} M aqueous chloro auric acid solution in a 500 mL Erlenmeyer flask. The resulting mixtures were placed on a magnetic stirrer at the rate of 150 rpm for 25 min and initial colour change was observed from light brown to ruby red colour.

Characterization of AuNPs

The color change in reaction mixture (metal ion solution+ alga extract) was recorded through visual observation. The bioreduction of gold ions in aqueous solution was monitored by periodic sampling subsequently measuring UV-Vis spectra of the solution at 500-600 nm using a 3-5 mm quartz cuvette. UV–Vis spectra of these aliquots were monitored as a function of time of the reaction. All the measurements were carried out at room temperature. All UV-visible (UV–vis) spectroscopic measurements of the synthesized AuNPs were carried out on a Cary 100 BIO UV–vis spectrophotometer (Varian, Palo Alto, CA, USA). AuNPs synthesized after 20 min of reaction between 1 mm chloroauric acid solution and extract were centrifuged at 10,000 rpm for 15 min at room temperature, following which the pellet was re-dispersed in sterile distilled water to remove any uncoordinated biological molecules. The chemical composition of the gold nanoparticles was characterized by FT-IR (Bruker Tensor 27). The phase formation of bio-reduced AuNPs was studied with the help of XRD. Bio reduced gold chloride solution was air dried. XRD patterns were recorded by a SEIFERT X-ray diffractometer with Cu K α radiation. The samples were scanned in the 2θ range of 10°C-70°C. Energy-dispersive X-ray (EDX) analysis was done using LEO 1430 VP, Carl Zeiss AG, Oberkochen, Germany to confirm the presence of gold in the particles, as well as to detect any other components, if present, performed on a SEM instrument(JEOL JSM 7500F Field Emission Scanning Electron Microscope).

Antibacterial activity

Antimicrobial activity of the crude extracts was determined by the agar well diffusion method [8]. All test organisms were inoculated in Mueller Hinton broth. Isolates were seeded on Mueller Hinton agar plates by using sterilized cotton swabs. Agar surface was bored by using sterilized gel borer to make wells (7 mm diameter). 100 µl of the test extract and 100 µl of sterilized distilled water (negative control) were poured in to separate wells. The standard antibiotic tetracycline was placed on the agar surface as positive control. Plates were incubated at 37°C for 48 hours. Triplicate plates were maintained for each organism. The plates were examined for evidence of zones of inhibition which appear as a clear area around the wells. The diameter of such zones was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimeter [9].

Results and Discussion

Reduction of Au^{3+} to AuNPs could be followed by colour change from yellow to ruby red and further by UV-Vis spectroscopy. The peak observed at 540 nm confirmed the synthesis of AuNPs as it is in agreement with the previous reports [10]. Various techniques used for characterization produced characteristic results for the AuNPs. *Sargassum polycystum* aqueous extract to 1mM $HAuCl_4$ solution lead to the appearance of ruby red colour indicating the formation of gold nanoparticles. These colours arise due to the excitation of Surface Plasmon Vibrations in the metal nanoparticles. The absorption maxima for the biosynthesized nanoparticles were noted in the visible range of 500-560 nm. The gold Surface Plasmon Resonance (SPR) band occurred at 532 nm and steadily increased in intensity as the different concentration of reaction medium without any shift in the peak (wave-length). Reduction of Au^{3+} to AuNPs by GGFE could be followed by colour change from yellow to ruby red and further by UV-Vis spectroscopy. The peak observed at 532 nm confirmed the synthesis of AuNPs as it is in agreement with the previous reports. The presence of carboxylic, amine, phosphate and hydroxyl functional groups was confirmed by FT-IR measurements (Figure 1). FT-IR is an important parameter to check the functional groups present and stabilization of nanoparticles. The range of Au nanoparticles falls between 800-1400 which confirms the presence of Au nanoparticles. These peaks present in the synthesis of nanoparticle may also indicate that seaweed extract may play a role in stabilizing AuNPs by adsorbing on the surface of bio reduced AuNPs. Analysis via Energy Dispersive X-ray (EDX) spectrometers confirmed the presence of elemental gold signals of the Au-NPs (Figure 2). The phase formation of the synthesized AuNPs was analysed employing X-ray diffraction which confirmed that the bio reduced metal nanoparticles are of elemental gold existence of peaks (111), (200), (220) and (311) matched with the standard Joint Committee for Powder Diffraction Set (JCPDS). In similar works, the presence of intense peaks of nanoparticles (111), (200) and (211) appeared which are indexed as crystalline silver centered cubic phase, the XRD pattern thus clearly shows that the silver nanoparticles formed by the reduction of Ag^{2+} ions by *S. polycystum* extract are crystalline [11] (Figure 3). The XRD patterns of green-synthesized *Turbinaria conoides* nanoparticle revealed that AuNPs corresponded to the crystalline gold fcc phase. The diffraction peaks obtained at 2θ=38.36° (111), 44.13° (200), 64.78° (220), and 77.98° (311) are identical with those reported for standard gold metal (Joint Committee on Powder Diffraction Standards-JCPDS, USA) [12]. Similarly, the XRD patterns of green-synthesized *Gnidia glauca* flower extract revealed existance of peaks (111), (200), (220) and (311) matched with the standard Joint Committee for Powder Diffraction Set (JCPDS) data-04784. This confirmed face centered cubic structured AuNPs formation. Peak broadening indicated restricted particle size. Enlarged pattern of (111) peak is shown in the inset of XRD plot [13]. Seaweed extract alone does not bring any effects in the shape and size of the particles, the phytochemicals present in the extracts may have the role of synthesizing the nanoparticles. Using the Scherrer's formula the size of the nanoparticle has been calculated. $d=0.9\lambda/\beta \cos\theta$ Here 0.9 is the shape factor, generally taken for a cubic system, λ is the X-ray wavelength, typically 1.54 Å, β is the full width at half the maximum intensity (FWHM) in radians, and θ is the Bragg angle. Using the above formula the crystallite size calculated is ~15 nm. The surface of gold nanoparticles and the elements present in the extract were analysed by EDX (Figure 2). The AuNPs were mono dispersed as spherical shaped. The average size of the particles is between 30-60 nm (Figure 4). A few agglomerated particles were also observed due to the spectral shift. Antibacterial activity of *S. polycystum* extract, gold nanoparticles and their combination were tested against *E.coli* and *P.aeruginosa* organism and the activity is shown in Figure 5. Antibacterial activity of *S. polycystum* extract, silver nitrate and their combination were tested against human pathogens also.

The crude extract of *S. polycystum* and silver nanoparticles showed cytotoxicity against MCF 7 breast cancer cell line in dose dependent manner. The IC50 for seaweed extract at 300 mg mL^{-1} for 72 h was higher than other concentration and the IC50 for Silver nanoparticles at 135 mg mL^{-1} for 72 h was higher than other concentration [14,15].

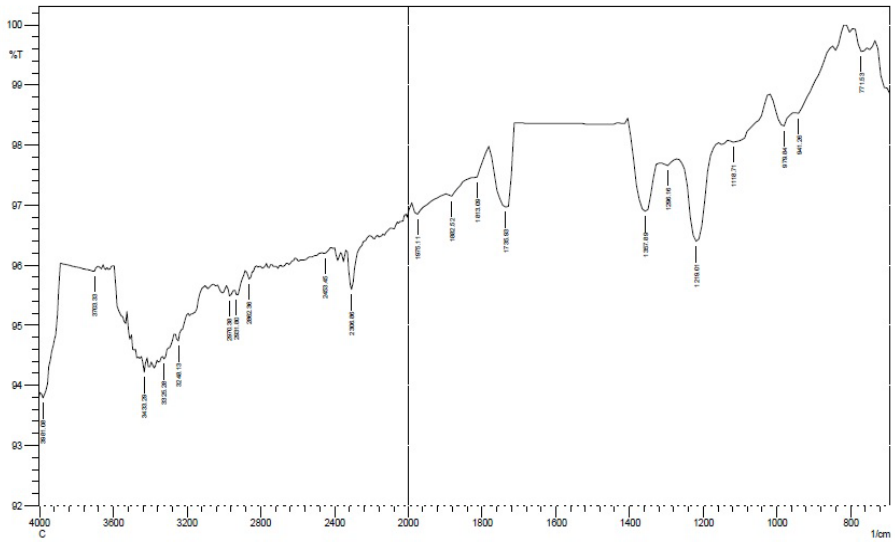

Figure 1: FT-IR spectra of gold nanoparticles.

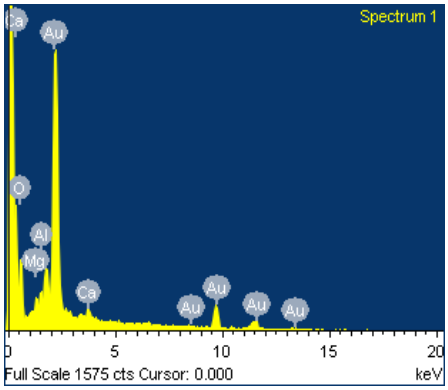

Figure 2: EDX analysis of Gold Nanoparticles.

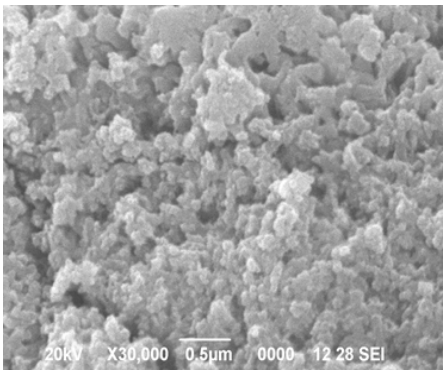

Figure 4: SEM image of gold nanoparticles.

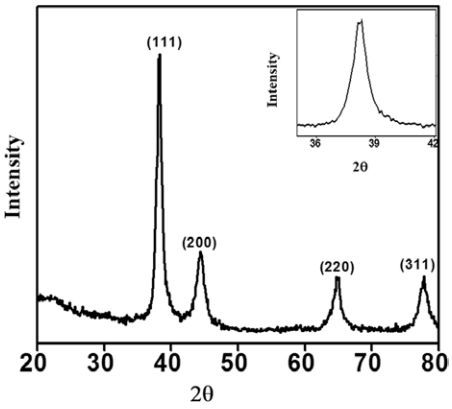

Figure 3: XRD image of gold nanoparticles.

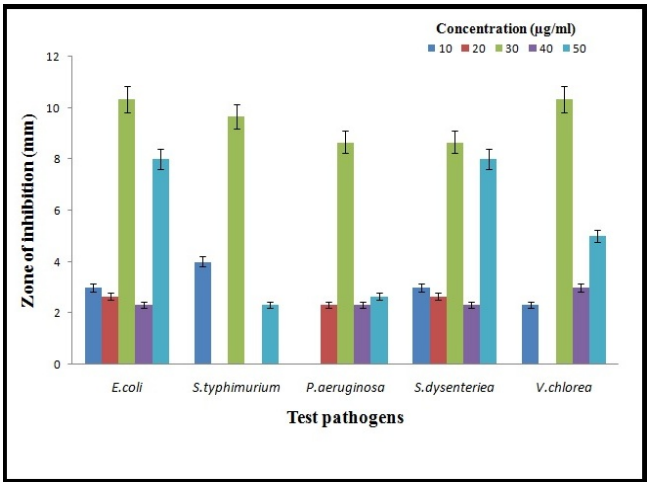

Figure 5: Values are expressed as mean ± standard deviation of thethree replicates.

Conclusion

Green synthesis of spherical shaped AuNPs has been achieved using extract of *S. polycystum* thus bringing into light yet another use

of the plant, besides its usual utilities. The method stands out primarily due to the fact that it is eco-friendly and shuts down the demerits of conventional physical and chemical methods. These particles are anticipated to have an extensive application in various biomedical applications. Thus this rapid, eco-friendly and economical route can be used to synthesize AuNPs with wide biotechnological and chemical applications.

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