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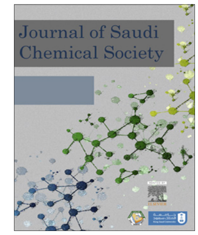

ORIGINAL ARTICLE

Green synthesis of silver nanoparticles by bloom forming marine microalgae *Trichodesmium erythraeum* and its applications in antioxidant, drug-resistant bacteria, and cytotoxicity activity

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KEYWORDS

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Abstract In the present study, we developed an eco-friendly method of stable silver nanoparticles (AgNPs) production using the aqueous extract of *Trichodesmium erythraeum*. The biosynthesized AgNPs were characterized using UV–Vis spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), Energy-Dispersive X-ray (EDX), and X-ray diffraction (XRD). The results affirmed that synthesized AgNPs were crystalline in nature, cubical in shape, and the average size of *T. erythraeum* silver nanoparticles (TENPs) was 26.5 nm. The antioxidant potential of synthesized AgNPs (500 µg/ml) was 77.01 ± 0.17% in DPPH, 67.5 ± 0.22% in Deoxy-ribose, 52.77 ± 0.42% in ABTS and 88.12 ± 0.26% in nitric oxide radical scavenging assays. The antibacterial results showed excellent inhibition against the clinical strains (*Staphylococcus aureus* and *Proteus mirabilis*) and drug-resistant bacterial strains such as *E. coli* (Amikacin^R), *S. aureus* (Tetracycline^R) and *S. pneumoniae* (Penicillin^R). The maximum anti-proliferative effect of TENPs was seen using 50 µg concentration against He La and MCF-7 cell lines, and IC_{50} values were 25.0 ± 0.50 µg/ml and 30.0 ± 0.50 µg/ml, respectively, at 24 h.

1. Introduction

Nanoparticles (NPs) are gaining importance due to their unique characteristics, such as electronic, mechanical, optical, magnetic, and chemical properties [1,2]. Microalgae have been shown to produce not only silver nanoparticles but also of other metal ions, such as gold, cadmium, and platinum [3,4].

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Amongst numerous metal nanoparticles, AgNPs have unique importance since they have antifungal, antibacterial, antineoplastic, wound healing, and conductivity properties. Thereby, the utility of these nanoparticles in biomedical, sensing, imaging, and drug delivery applications is increasing continuously [5]. They are one of the most widely used nanomaterials in industrial products and medical needs, as well as added as active source in detergents [6]. However, due to strict regulations the simple potential conventional biosynthesis are the focussed on green based reducing and stabilizing agents intense research [7–10]. Compared to native metal nanoparticles, biosynthesized nanoparticles are vastly valuable in therapeutics because of their substantial antioxidant potential [11].

In contrast to developing countries, the prevalence of cancer is very high in developed countries [12]. Treatment approaches such as chemotherapy and radiotherapy are not only expensive but have detrimental side effects on the normal cells [13]. In the area of nanomedicine, AgNPs can act as remarkable therapeutic agents due to their anticancer potential, in addition to being relatively safe in the medical usage [14,15].

Silver nanoparticles can be synthesized by various methods such as chemical, physical, and biological ones. Green synthesis of nanoparticles using naturally benign resources is an emerging branch in nanotechnology, and several kinds of biologically assisted synthetic methods are available for its production [16]. As of now, plant extracts, microbes, and algae have been used for the synthesis of nanoparticles. The algae-based biosynthesis of nanoparticles is an emanating trend in recent years.

Similar to other biological organisms like fungi, yeast, and bacteria, algae also have significant results for the synthesis of nanoparticles. The algae-based nanosynthesis has evolved as a distinct branch called phyco-nanotechnology [17]. Various investigations have been done on the biosynthesis of nanoparticles using seaweed extracts; but, the use of microalgae for nanoparticle synthesis is very less. In this context, some recent studies have demonstrated promising applications of microalgae for the synthesis of metal nanoparticles [18].

Marine microalgae are the microscopic plant life in the sea comprising diatoms, dinoflagellates, and blue green algae. *Trichodesmium* sp. is nitrogen-fixing, non-hetero cystous diazotrophic blue-green algae, which occur in oligotrophic waters throughout tropical and subtropical oceans. The morphology of the genus shows cylindrical trichomes and form colonies [19]. The species is found in the stable water at a depth lower than 50 m. *Trichodesmium erythraeum* is one of the most common bloom-forming species, and the blooming of this species is exhibited as greenish yellow colour patches in the open sea [20,21] *Trichodesmium* sp. has been extensively studied due to its imperative role in bio-geochemical cycling and ecological reputation; but, potential biotechnological applications of this species are not well understood. Furthermore, studies related to the anticancer activity of silver nanoparticles screened from marine cyanobacteria are meager.

In the current research, an attempt was made to synthesize AgNPs from the aqueous extracts of *T. erythraeum*, which was collected from the intense bloom observed near Tuticorin coast, India. Optimally synthesized AgNPs were tested for their antioxidant, antibacterial, and cytotoxic activities.

2. Materials and methods

2.1. Chemicals

All analytical reagents and media components were purchased from Hi-Media, Mumbai, (India) and Sigma-Aldrich (St. Louis, USA). All chemicals were of analytical grade with maximum purity.

2.2. Collection of marine microalgae

Dense *T. erythraeum* bloom was observed at Tuticorin harbor (lat: 8°45′59.67″N, long: 78°13′21.71″E), southeast coast of India, on March 2017. Algal samples were collected from the surface water using plankton net made up of bolting silk (with No. 25 mesh size 48 μm). Samples were transported to the laboratory in an icebox and later cleaned thoroughly with Milli-Q water to remove all adhering epiphytes. The specimen was fixed in $\geq$2% formalin solution, and the microalgal density was estimated using Sedgwick Rafter Counting Chamber [22] with the aid of an inverted microscope. The microalgae identification was done based on the standard taxonomic monograph [23]. Later, the algal biomass was centrifuged at 3000 rpm for 20 min, and the supernatant was removed and the algal biomass were stored at $\leq$ 4 °C for further use.

2.3. Preparation of algal extract and biosynthesis of AgNPs

The algal extract was prepared using following method with slight modifications [24] and the details were given in the supplementary.

2.4. Characterization of AgNPs

The bio-reduction of silver ions was periodically monitored using UV–Visible spectrophotometer SHIMADZU, UV-1800 (Asia Pacific PVT LTD, Singapore) at a wavelength of 300–700 nm. The identification of functional molecules in the biosynthesized nanoparticles was done using FTIR system PERKIN ELMER-SPECTRUM RX-1 (Norwalk, USA). The structural analysis of synthesized sample was done using bench top X-ray diffractometer INEL EQUINOX 1000 (Artenay, France). XRD was performed for purpose of the dimension of purely synthesized TENPs with (h), (k), and (l) values. The particle size (L) of TENPs was calculated using Debye-Scherrer's formula ($L = 0.9\ \lambda/\beta \cosh \theta$). SEM analysis was done by JEOL JSM-5610LV (Tokyo, Japan), Energy Dispersive X-ray Spectroscopy (EDX) analysis was carried out using INCAx-sight (EDX) (Oxford instruments, Oxfordshire, UK) and Atomic Force Microscopy (AFM) AGILENT-5500 (Santa Clara, USA) was used to investigate the morphology and size of the synthesized nanoparticles.

2.5. Antioxidant assays

2.5.1. Scavenging capability on DPPH radicals

The free radical-scavenging ability of TENPs was determined using DPPH radical [25] included in the supplementary file.

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