ISSN 2063-5346
Section A-Research paper

BIOSYNTHESIS, CHARACTERIZATION AND CORROSION INHIBITORY EFFICIENCY OF SILVER NANOPARTICLES USING ASCIDIANS

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Submission Date: 31-07-2023; Accepted Date: 14-08-2023

doi: 10.48047/ecb/2023.12.10.741

Abstract

Background: One of the most active study fields in contemporary materials science is nanotechnology. There have been impressive developments in the field of nanotechnology in recent years, with numerous methodologies developed to synthesize nanoparticles of particular shapes and sizes depending on specific requirements. The number of new uses for nanoparticles and nanomaterials is growing quickly. Secondary metabolites are abundant in ascidians as a kind of chemical defense. **Objectives:** An attempt has been made to synthesize and characterize silver nanoparticles by using ascidians - *Phallusia nigra* and *Didemnum psammatode* and their corrosion inhibitory action. **Methods**: The nanoparticles were synthesized biologically and characterized by Ultraviolet-Visible (UV-Vis) spectrophotometry, Fourier Transform Infrared (FTIR) Spectroscopy and Scanning Electron Microscopy (SEM). **Results**: For *Phallusia nigra* and *Didemnum psammatode*, the corrosion rates in 24 and 72 hours were recorded as 3.62, 5.05% and 7.32, 11.92 % respectively. **Conclusion**: The Ag nanoparticles performed efficiently in bringing down the corrosion property of carbon steel in the medium of seawater with ascidian extract.

Keywords: *Phallusia nigra, Didemnum psammatode,* silver nanoparticles, corrosion inhibition

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1. INTRODUCTION

Metals are used extensively in human activities because of their superior electrical and mechanical qualities. Maintenance of these metals from undesirable corrosion is essential, since corrosion makes the metal weaker. Corrosion is a natural process that involves interactions between a substance and its surroundings and results in modifications to the metal's properties and features [1]. It gradually deteriorates the materials and converts a refined metal into a more chemically stable oxide by electrochemical reaction with their environment. Corrosion causes metal loss from the surface and changes its physical and chemical properties. Costs might increase even with routine maintenance to remove and repair corrosion. There are numerous steps to reduce rust. A corrosion inhibitor is required to reduce corrosion based on cost, effectiveness and environmental friendliness. Nanotechnology is one of the newest fields of study in the modern discipline of material science. Nanoparticles display entirely new or better characteristics such as size, particle dispersion and shape. Novel applications of nanoparticles and nanomaterials are quickly developing in various fields [2]. Natural products from marine organisms especially ascidians rank second as the most promising source of drugs for cancer [3]. They are an interesting group of marine sedentary organisms commonly called 'sea squirts' found to occur on the Thoothukudi coast. The study can make use of marine organisms, particularly those that cause environmental problems as biofoulers. Numerous species of ascidians have shown activities like antimitotic, antiproliferative, antiviral, antimicrobial, antiproliferative, antitumor, immunomodulatory, anti-inflammatory, antifertility, wound healing, CNS depressant and cardioprotective etc [4-19]. Silver nanoparticles were studied to reduce oxygen by electrochemically [20]. A number of studies on anticorrosion activity of green inhibitors have been carried out [21,22]. An attempt has been made to synthesize silver nanoparticles of *tunicates - Phallusia nigra* and *Didemnum psammatode* and to characterize by UV-Visible spectrophotometry, FT-IR Spectroscopy, X-ray Diffraction, Scanning Electron Microscopy and subjected to application.

2. MATERIALS AND METHODS

Animal material: Samples of simple ascidian *Phallusia nigra* savigny, 1816 were collected from the undersurface of the barges of Thoothukudi harbour. The specimens of colonial ascidian *Didemnum psammatode* Sluiter, 1895 were collected from the intertidal rocky shore area of Hare island, Thoothukudi, Tamilnadu. Identification up to the species level was carried out based on the key to the identification of Indian ascidians by Meenakshi, 1997 [23].

Systematic Position: *Phallusia nigra* belongs to Phylum: Chordata; Subphylum: Urochordata; Class: Ascidiacea; Order: Enterogona; Family: Ascidiidae; Genus: *Phallusia;* Species: *nigra Didemnum psammatode* belongs to Phylum: Chordata; Subphylum: Urochordata; Class: Ascidiacea; Order: Enterogona; Family: Didemnidae; Genus: *Didemnum;* Species: psammatode

Preparation of Powder: The specimens were dried under shade. The dried animals were homogenized to get a coarse powder. The dried powder of *Phallusia nigra* and *Didemnum psammatode* was used.

Synthesis of silver nanoparticles: 25 g of the dry powder of *Phallusia nigra* was weighed, mixed with

100 ml sterile distilled water and filtered through Whatman No.1 filter paper (pore size 0.45 µm) and was further filtered through 0.22 µm sized filters. The extract was stored at 40°C for further experiments. The aqueous solution of 1 mM silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 10 ml of *Phallusia nigra* extract was added to 90 ml of the aqueous solution of 1 mM silver nitrate for the reduction of Ag^+ ions and kept for an incubation period of 15 hours at room temperature. Here the filtrate act as a reducing and stabilizing agent for 1 mM of $AgNO_3$. The same procedure was followed for *Didemnum psammatode* also.

Characterization of Silver Nanoparticles:

UV-Vis Analysis: To understand the kinetic behaviour of silver nanoparticles (AgNPs), they were examined in a Perkin-Elmer Lambda-19 UV-Vis spectrophotometer. The samples were scanned between 200 and 900 nm at a rate of 480 nm per minute. To record and analyze data, "UVWinlab" software was installed on the spectrophotometer. Using a blank reference, the spectrophotometer's baseline correction was performed. All materials' UV-Vis absorption spectra were captured, and numerical data were displayed in "Origin 6.5."

FT-IR analysis: The synthesized silver nanoparticles' chemical composition was studied using an FT-IR spectrometer (Perkin-Elmer LS-55-Luminescence spectrometer). The solutions were dried at 75°C and the powders were characterized in the range 4000 – 400 cm^{-1} using the KBr pellet method.

Scanning electron microscopy (SEM): SEM was used to study the details of the specimen's surface. It uses a focused beam of high-energy electrons to generate signals across the surface of the specimen. The electronic signals emitted from the interaction between the specimen and the electron beam formed three-dimensional images. When the electron beam interacts with the surface of the specimen, the signal produced can be low-energy secondary electrons and high-energy backscattered electrons and characteristic X-rays.

Anticorrosion Activity: Ascidians – *Phallusia nigra* and *Didemnum psammatode* were used for the corrosion tests. Small plates of carbon steel (10 × 15 mm) were used for corrosion tests, polished using silicon carbide grinding paper with grit P400, degreased with sodium carbonate, rinsed in distilled water and dried with ethanol. After that, the samples were weighed accurately using an analytical balance (Kern KB, KERN & SOHN GmbH, Balingen, Germany). They were then suspended entirely in glass vessels containing 25 mL of seawater, without and with ascidian extract. The gravimetric method examined the corrosion experiments for 7 days at room temperature (22 ± 2°C) using one sample for each test. After the corrosion time ended, samples were taken out, washed with distilled water, dried at room temperature and weighed accurately. The surface morphology of the carbon steel, before and after corrosion tests, was examined using a digital optical microscope (MT4096, USB, Media-Tech, Bratislava, Slovakia, magnification 300X).

3. RESULTS AND DISCUSSION

Silver nanoparticles were synthesized by using the extracts of *Phallusia nigra* and *Didemnum psammatode* successfully. The UV-Vis absorption spectra of the synthesized AgNPs using the two species of ascidians were shown in Figures 1 and 2. Absorption spectrum of AgNPs using *Phallusia nigra* formed in the reaction media has absorbance maxima at 270 and 320 nm. Absorption spectrum of AgNPs using *Didemnum psammatode* formed in the reaction mixture in the range of 200-300 nm showed two peaks at 250 and 270 nm indicating the formation of silver nanoparticles using *Didemnum psammatode*.

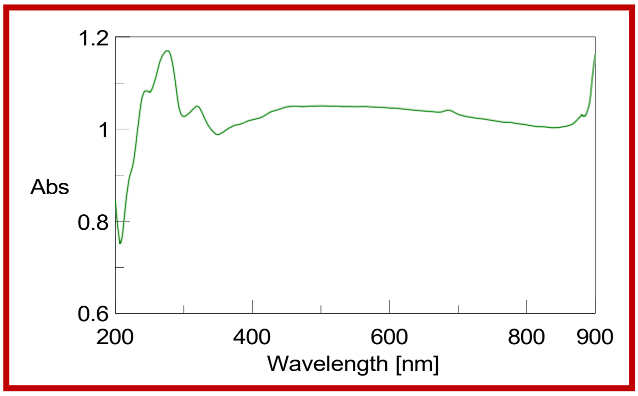


Fig (1). UV-Vis spectrum for AgNPs using *Phallusia nigra*

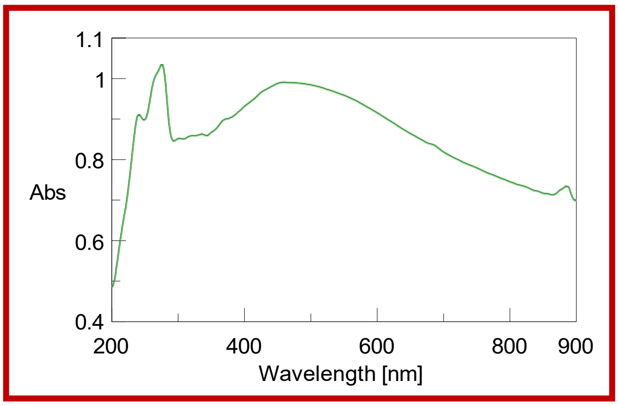


Fig (2). UV-Vis spectrum for AgNPs using *Didemnum psammatode*

FT-IR Analysis

FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the synthesized metal nanoparticles. The FTIR spectrum of silver nanoparticles using *Phallusia nigra*was showed in Figure 3. It revealed the presence of prominent peaks at 3424, 2923, 2106, 1627, 1422, 1384, 1120, 1021, 874, 675, 610, 513 and 466 cm^{-1} corresponding to different functional groups. The peak corresponding to 3424 cm^{-1} indicates -NH stretching band of amino group. The peak at 2923 cm^{-1} responds to C-H stretching of alkanes and alkyl groups and 1384 cm^{-1} indicates the C-H bending of alkanes. C-C multiple bonds stretching of the alkyne (mono-substituted) was observed at 2106 cm^{-1} and the methylene scissoring vibration from the protein was observed at 1422 cm^{-1}. The carbonyl stretching groups such as acids, ketones and amides were noted at the peak of 1623 cm^{-1}. The plausible peaks at 1120 and 1021 cm^{-1} revealed the functional group of C-O stretching of esters and ethers. The following peaks at 874, 675 and 610 cm^{-1} indicated the C-

X stretching halogen compounds. The peak at 466 cm^{-1} confirms the metal-oxygen bond which evidenced the formation of Ag nanoparticles.

The spectrum for AgNPs using *Didemnum psammatode* (Figure 4) revealed the presence of prominent peaks at 3984, 3455, 2923, 1632, 1384, 1121, 1050, 639, 609 and 467 cm^{-1} corresponding to different functional groups. -OH stretching of alcohols and phenols was indicated from the peak at 3984 cm^{-1}. The peak corresponding to 3455 cm^{-1} indicates -NH stretching band of amino group. The peak at 2923 cm^{-1} responds to C-H stretching of alkanes and alkyl groups and 1384 cm^{-1} indicates the C-H bending of alkanes. The carbonyl stretching groups such as acids, ketones and amides were noted at the peak of 1632 cm^{-1}. The plausible peaks at 1121 and 1050 cm^{-1} showed the functional group of C-O stretching of esters and ethers. The following peaks at 639 and 609 cm^{-1} indicated the C-X stretching halogen compounds. The peak at 467 cm^{-1} confirms the metal-oxygen bond which evidenced the formation of Ag nanoparticles.

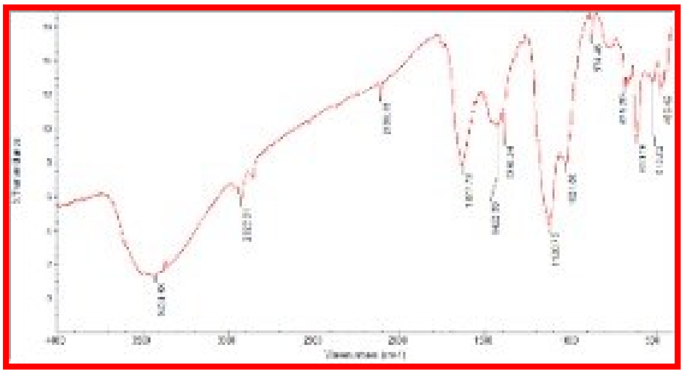


Fig (3). FTIR spectrum for AgNPs using *Phallusia nigra*

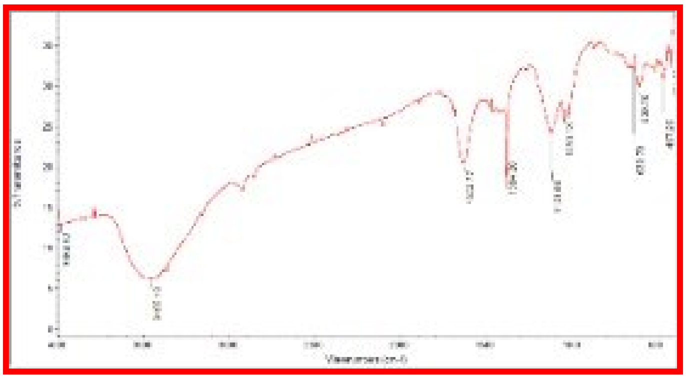
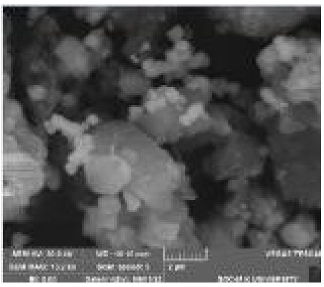
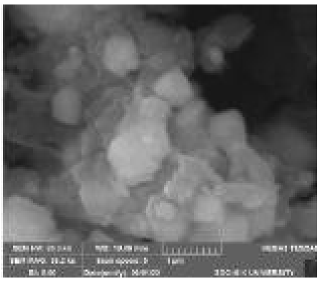
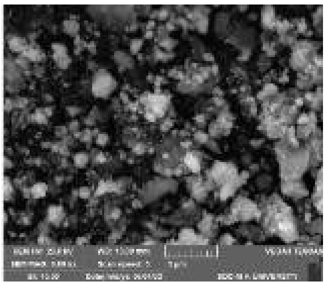
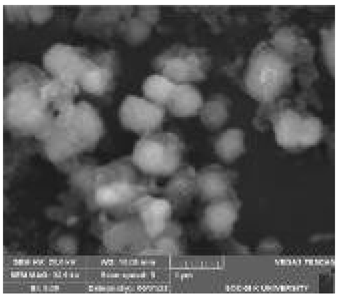
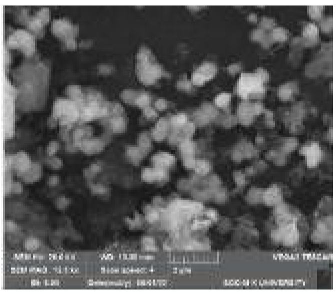
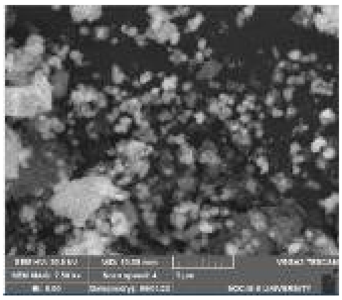


Fig (4). FTIR spectrum for AgNPs using *Didemnum psammatode*

The typical SEM images of biosynthesized silver nanoparticles using *Phallusia nigra* were depicted in Figure 5. These images provide information on topographical, morphological, chemical compositional and grain or crystallographic orientation [24, 25]. The SEM results revealed the regular spherical shape with a range size of approximately at 20 kV confirming the formation of nanoparticles by *Phallusia nigra*. SEM micrograph clearly indicates the biofabrication of NPs with relatively monodispersed spherical-shaped particles and diameters of 1, 2 and 5 µm. However, due to sample preparation, slight aggregation of particles has arbitrarily arisen in discrete spots.

The nanoparticles are quite polydispersed and a layer of the organic material surrounding the synthesized nanoparticles could explain the good dispersion of the nanoparticles in the solution. Generally, the nanoparticles synthesized using extract of ascidian *Didemnum psammatote* are well dispersed although some were noted to be agglomerated. Notably, the majority of the particles in the SEM images are not in physical contact with each other but appeared separated by the organic layer. Therefore, SEM images clearly indicate the coating of nanoparticles with an organic layer. The presence of several polyphenolic compounds including flavonoids and terpenoids facilitated the reduction of ions and also stabilized the surface of the resultant nanoparticles. The quantity of metal ions influenced the size of the particles. When the metal salt concentration is increased to 5 mM, obvious changes in the size distribution of nanospheres were observed.

Anticorrosion activity

Anticorrosion activity and inhibition activity of synthesized silver nanoparticles in 24 hours are shown in Table 1. Weight loss and Rate of corrosion are calculated by using the following formulae.

Weight loss = Initial weight – Final weight

$$\text{Rate of Corrosion (\%)} = \frac{\text{Weight Loss}}{\textit{Initial weight}} \times 100$$

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From the weight loss data obtained, the inhibition efficiencies (% I) were calculated using

$$\% \text{I} = \frac{CR_{blank} - CR_{inh}}{CR_{blank}} \text{ x } 100$$

where CR_{blank} and CR_{inh} are the corrosion rate in the absence and presence of the inhibitor respectively. The results obtained in 24 hours are shown in Table 1. The results indicate that silver nanoparticles synthesized using *Phallusia nigra* show a significant corrosion rate and inhibitive effect than *Didemnum psammatote*. The variation of inhibition efficiency of silver nanoparticles synthesized by using two ascidians in 72 hours is also depicted in Table 2.

The surface morphology images show that the corrosion reaction does not take place homogeneously over the surface of carbon steel in the absence of ascidians in seawater.

Weight loss in 24 hours was calculated. It was 0.708 g which is higher for *Didemnum psammatode* and the rate of corrosion was 3.62 g/cm^2 h. Minimum weight loss was noted as 0.4306 g for *Phallusia nigra* and the rate of corrosion was 2.23 g/cm^2 h. Extracts of ascidians inhibit the corrosion of carbon steel in 0.5 M H_2SO_4 and the inhibition efficiency of silver nanoparticles synthesized using *Phallusia nigra* and *Didemnum psammatode* was 55.84 and 28.32% respectively. The weight loss was noted after 72 hours. Rate of corrosion and inhibition efficiency of *Phallusia nigra* was 5.11 g/cm^2 h and 57.13% whereas *Didemnum psammatode* exhibits 7.32 and 38.59 % respectively. The inhibition is due to the adsorption of the inhibitor molecules on the steel surface and the blocking of its active sites [26].

Table 1: Corrosion inhibition efficiency of silver nanoparticles in 24 hours

Samples	Weight of inhibitor (g)	Weight of plate (g)		Weight loss (g/cm^2)	Corrosion rate (g/cm^2 h)	Inhibition efficiency (% I)
		Before immersion	After immersion for 24 hours			
Phallusia nigra	0.06	19.2626	18.8320	0.4306	2.23	55.84
Didemnum psammatode	0.06	19.5526	18.8446	0.708	3.62	28.32
Blank		20.0995	19.0840	1.0155	5.05	-

Table 2: Corrosion inhibition efficiency of silver nanoparticles in 72 hours

Samples	Weight of inhibitor (g)	Weight of plate (g)		Weight loss (g/cm^2)	Corrosion rate (g/cm^{2}h)	Inhibition efficiency (% I)
		Before immersion	After immersion for 72 hours			
Phallusia nigra	0.06	19.2626	18.2767	0.9859	5.11	57.13
Didemnum psammatode	0.06	19.5526	18.1209	1.4317	7.32	38.59
Blank	-	20.0995	17.7027	2.3968	11.92	-

Statistical Analysis The data were analyzed with two-way ANOVA. A statistical difference of $P<0.7591$ and $P<0.2398$ were considered significant in all cases.

CONCLUSION

Using the extracts of *Phallusia nigra* and *Didemnum psammatode,* silver nanoparticles were produced biologically in a simple and effective manner. The synthesized nanoparticles act as inhibitors for mild carbon steel plate and significant inhibition efficiency was noted. From a technological standpoint, the generated silver nanoparticles have prospective uses in the biomedical industry and this process offers several benefits, including low cost, compatibility with medical and pharmaceutical applications and large-scale commercial manufacturing. UV-Vis spectroscopy and FT-IR investigations were used to characterize the nanoparticles in detail. Once the effect of these ascidian extracts on inhibiting corrosion has been established, a more thorough investigation using inhibitive assay-guided isolation and surface analytical techniques will allow for the characterization of the active compounds in the adsorbed layer and help in determining which compounds are the most active.

LIST OF ABBREVIATIONS

UV-Vis - Ultraviolet-Visible; FTIR - Fourier Transform Infrared Spectroscopy; SEM - Scanning Electron Microscopy

FUNDING

The authors sincerely acknowledge the financial assistance funded by DBT, New Delhi for the successful completion of the work.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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