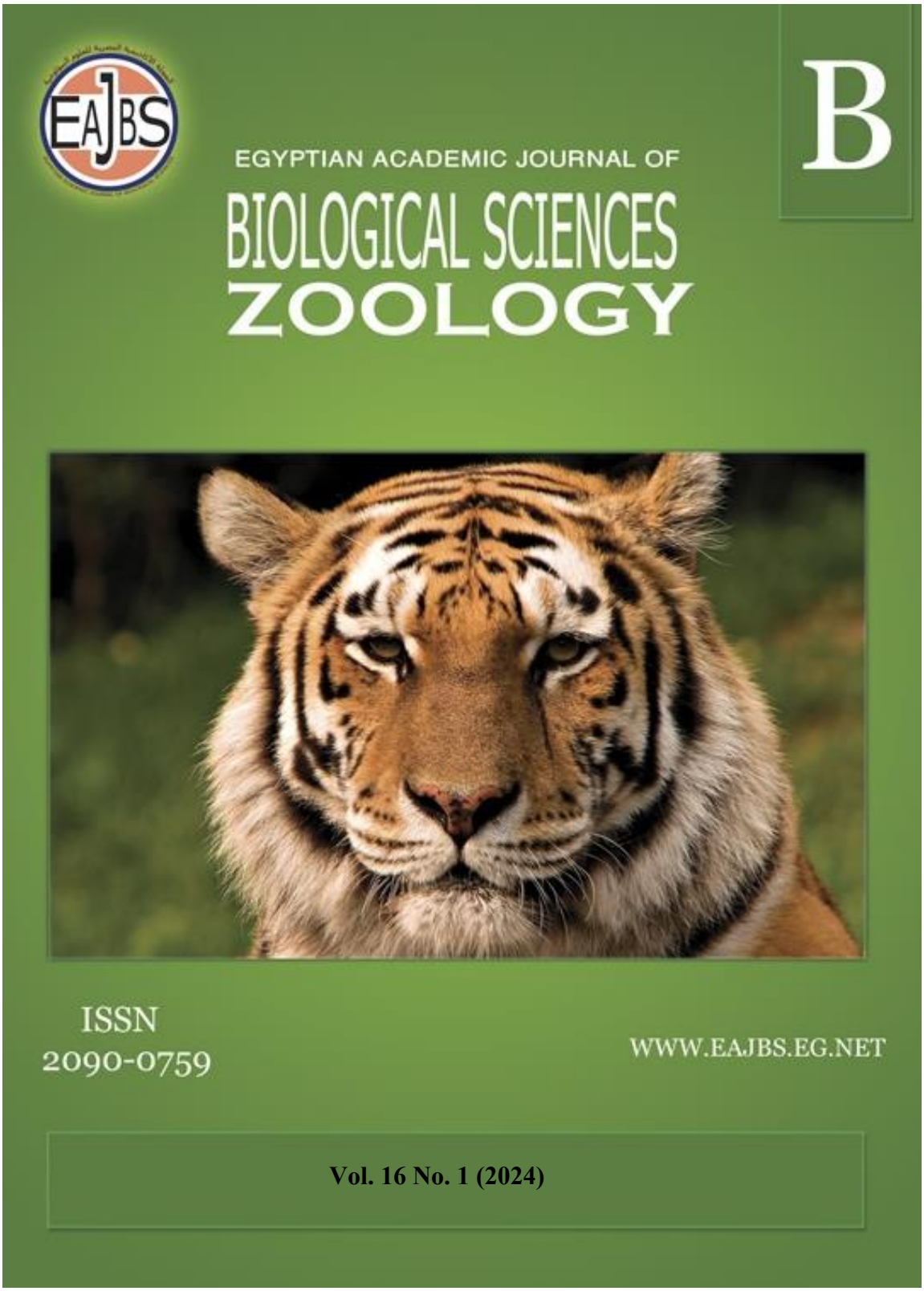

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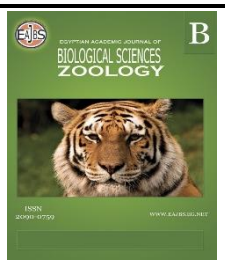

Antioxidant Potential of Silver Nanoparticles Synthesized from Ascidians

A. Arockia Jenecius Alphonse[1], M. Paripooranaselvi[2*], M.I. Delighta Mano Joyce[2], R.Sri Priya[3] and J. Antony Rajam[4]

[1]Department of Botany, St.Mary's College (Autonomous), Thoothukudi, Tamilnadu, India.
[2]Department of Zoology, St. Mary's College (Autonomous), Thoothukudi. Tamilnadu, India.
[3]Department of Zoology, Sadakathullah Appa College (Autonomous), Tirunelveli,Tamilnadu, South India
[4]Department of Chemistry, St.Mary's College (Autonomous), Thoothukudi, Tamilnadu, India
*** E-mail** : mparipooranaselvi@gmail.com

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ABSTRACT

The investigation of nanotechnology is currently one of the most exciting fields in materials science. The subject of nanotechnology has made tremendous strides in recent years, with a variety of approaches created to produce nanoparticles of a given form and size depending on particular requirements. Nanotechnology is described as the production, characterization, study and use of nanoscale substances for the advancement of science. As a type of chemical defence, ascidians produce a lot of secondary metabolites. Attempts have been made to synthesise silver nanoparticles from *Phallusia nigra*. The antioxidant activity of silver nanoparticles from *Phallusia nigra* was evaluated based on their ability to scavenge DPPH free radicals. Silver nanoparticles from *Phallusia nigra* exhibited significant, dose-dependent scavenging activity with 48.38%, 56.45%, 69.35%, 75.64 % and 84.51% at concentrations of 20, 40, 60, 80 and 100 µg/ml respectively. The scavenging activity of AgNPs produced from *Didemnum psammatode* was substantial, with values of 30.48%, 39.83%, 50.80%, 64.03 % and 67.74% at 20, 40, 60, 80 and 100 µg/ml concentrations respectively.

INTRODUCTION

Several abiotic stresses cause the overproduction of reactive oxygen species (ROS) in plants and animals which are extremely reactive and toxic causing damage to proteins, lipids, carbohydrates and DNA, resulting in oxidative stress. This oxidative stress causes damage to tissues and results in a large number of diseases [Sindhi *et al*., 2013]. Antioxidants are compounds that may protect cells from the harm produced by free radicals, which are unstable molecules. Antioxidants interact with and stabilise free radicals, perhaps preventing some of the harm that free radicals can do. Antioxidants are compounds that may protect cells from the harm produced by free radicals, which are unstable molecules. Antioxidants interact with and stabilise free radicals, perhaps preventing some of the harm that free radicals can do. Free radicals react quickly with membranes, causing cellular degradation and, eventually, death. Cancer may result from free radical damage.

Free radicals react quickly with membranes, causing cellular degradation and, eventually, death. Cancer may result from free radical damage. Antioxidants neutralize the effects of ROS and thus help in preventing diseases.

Silver nanoparticles are produced via a variety of processes, including physical, chemical and biological synthesis. It is important to remember that every approach has benefits and drawbacks. The organism functions as a capping, reducing, or stabilising agent during the biological manufacture of silver nanoparticles, reducing Ag^{+} to generate Ag^{0} [Zhao and Stevens, 1998]. Due to their appealing physicochemical characteristics and biological activity, such as their strong antibacterial efficacy and a broad range of bactericidal effects that are comparatively non-toxic, silver nanoparticles are crucial in the field of nanomedicine [Durán *et al*., 2016].

Ascidians, often known as sea squirts, are a fascinating group of marine sedentary creatures that develop secondary metabolites with distinct structural patterns for chemical defence, something that does not exist in terrestrial plants. They grow on all underwater marine constructions, are considered a nuisance, and are normally discarded. These wastes may include a variety of natural goods. Ascidians have also been demonstrated to have bioactive peptides with unique architectures [Chakraborty and Ghosh, 2010]. Ascidians are second on the list of potential drug sources [Azumi *et al*., 1990]. Marine organisms, particularly those that are environmentally hazardous, such as biofoulers, can be used in the research. Various species of ascidians have shown activities like antioxidant, antimicrobial, antiproliferative, antitumor, immunomodulatory, antiinflammatory, antifertility, wound healing, CNS depressant and cardioprotective, etc [Priya *et al*., 2018; Gopalakrishnan *et al*., 2012; Meenakshi *et al*., 2012a; Meenakshi *et al*., 2014a; Meenakshi *et al*., 2012b; Meenakshi *et al*., 2013a; Meenakshi *et al*., 2013b; Delighta Mano Joyce *et al*., 2015a; Meenakshi *et al*., 2014a; Delighta Mano Joyce *et al*., 2015b; Meenakshi *et al*., 2013c; Meenakshi *et al*., 2014b; Alphonse *et al*., 2023]. In India, studies on the anti-oxidant potential of silver nanoparticles synthesised from the ascidians *Phallusia nigra* and *Didemnum psammatode* are lacking. As ascidians are available along the Tuticorin coast, an attempt has been made to make silver nanoparticles, which were then studied using UV-visible spectrophotometry, FT-IR spectral analysis, and to assess their anti-oxidant potential.

MATERIALS AND METHODS

Collection and Identification of Specimens:

SCUBA diving was used to gather *Phallusia nigra* from Tuticorin Port. The specimens of colonial ascidian *Didemnum psammathodes* were collected from the intertidal rocky shore area of Thoothukudi north breakwater, Tamilnadu. At the collection site, samples were washed with seawater to eliminate sand, dirt, and overgrowth before being transported to the laboratory. The identification process was completed up to the species level using the key to verify the identity of Indian ascidians [Meenakshi, 1997].

Systematic Position:

Phallusia nigra belongs to Phylum: Chordata; Subphylum: Urochordata; Class: Ascidiacea; Order: Enterogona; Suborder: Phlebobranchia; Family: Ascidiidae; Genus: *Phallusia*; Species: *nigra.*

Didemnum psammatode belongs to Phylum: Chordata; Subphylum: Urochordata; Class: Ascidiacea; Order: Enterogona; Suborder: Aplousobranchia; Family: Didemnidae; Genus: *Didemnum*; Species: *psammathodes*

Chemicals Used:

Silver nitrate was purchased from Sigma Aldrich. All other chemicals were analytical grade (99%), and used without further purification. The extract was prepared by

using sterile distilled water.

Powder and Extract Preparation:

The specimens were dried under shade. The dried animals were ground into a coarse powder. The dried powder of the tunicate *Phallusia nigra* was used.

Phallusia nigra dry powder (25 g) was weighed, mixed with 100 ml of sterile distilled water, and filtered with Whatman No. 1 filter paper (0.45 m pore size). After that, the filtrate was passed through 0.22 mm filters. The extract was kept at 40° C for future use. For the generation of silver nanoparticles, an aqueous solution of 1mM $AgNO_3$ was produced. 90 ml of 1 mM silver nitrate aqueous solution was paired with 10 ml of *Phallusia nigra* extract for reduction of Ag^+ ions, and the mixture was left to incubate at ambient temperature for 15 hours. The filtrate functions as a reducing agent and stabilizer for 1 mM $AgNO_3$. The exact same procedure was employed for *Didemnum psammatode*.

Characterization of Silver Nanoparticles:

UV-Vis Analysis:

The Ag nanoparticles were characterized in a Perkin-Elmer UV-VIS spectrophotometer, Lambda-19 to know the kinetic behavior of synthesized Ag nanoparticles. The scanning range for the sample was 200-800 nm at a scan rate of 480 nm/min. The spectrophotometer was equipped with "UVWinlab" software to record and analyze data. Baseline correction of the spectrophotometer was carried out by using a blank reference. The UV-Vis absorption spectra of all the samples were recorded and numerical data were plotted in the "Origin 6.5".

FT-IR Analysis:

The chemical composition of the synthesized silver nanoparticles was studied by using an FTIR spectrometer (perkin-Elmer LS-55-Luminescence spectrometer). The solutions were dried at 75^{o}C and the dried powders were characterized in the range 4000–400 cm^{-1} using KBr pellet method.

DPPH (2,2-diphenyl- 1-picrylhydrazyl) Radical Scavenging DPPH Assay:

The radical scavenging efficiency of DPPH is used to examine free radicals in the antioxidant component [Blois, 1958]. DPPH was produced in methanol (0.1 mM). 1 ml of DPPH in methanol was added to 3 ml of ethanolic extract of *Phallusia nigra* at different concentrations (20, 40, 60, 80 and 100 µg/ml). After vigorous shaking, the mixture was left to stand at room temperature for 30 minutes. The absorbance at 517 nm was determined using a UV-Vis spectrophotometer. Ascorbic acid was employed as a standard. The percentage of inhibition was determined by comparing the absorbance values of the test samples to those of the control (which had not been treated with extract). The inhibition percentage was calculated as radical scavenging activity as follows,

$$\boldsymbol{I = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \; x \; 100}$$

Where, I - Inhibition percentage; $Abs_{control}$ - Absorbance of control;

Abs_{sample} - Absorbance of sample

RESULTS AND DISCUSSION

AgNPs from the extract of *Phallusia nigra* and *Didemnum Psammatode* were synthesized successfully. The UV-Vis absorption spectra of the Ag NP are shown in Figures 1 and 2. Absorption spectra of Ag nanoparticles formed in the reaction media have absorbance maxima at 270 nm.

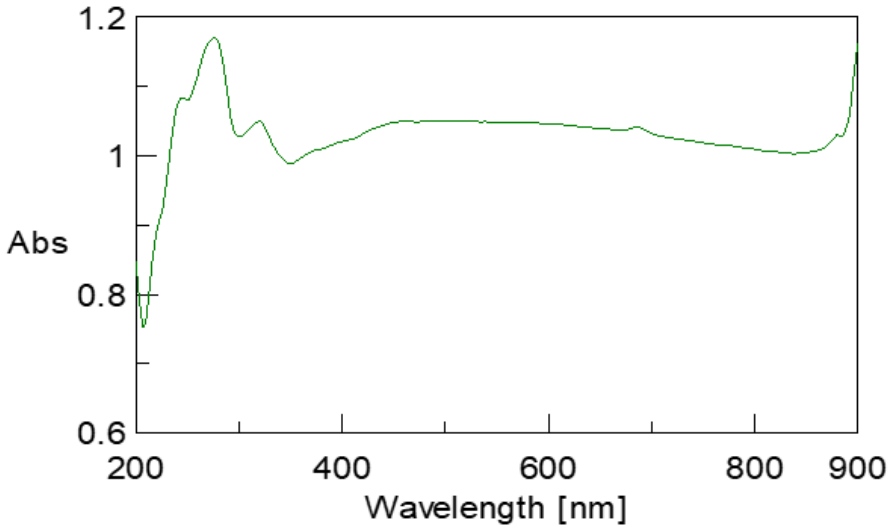

Fig. 1: UV-vis spectrum for Ag nanoparticles of *Phallusia nigra.*

Absorption spectra of Ag nanoparticles formed in the reaction mixture in the range of 200-300 nm. The absorption spectrum has shown two peaks at 250 and 270 nm indicating the formation of silver nanoparticles using *Didemnum psammathodes*.

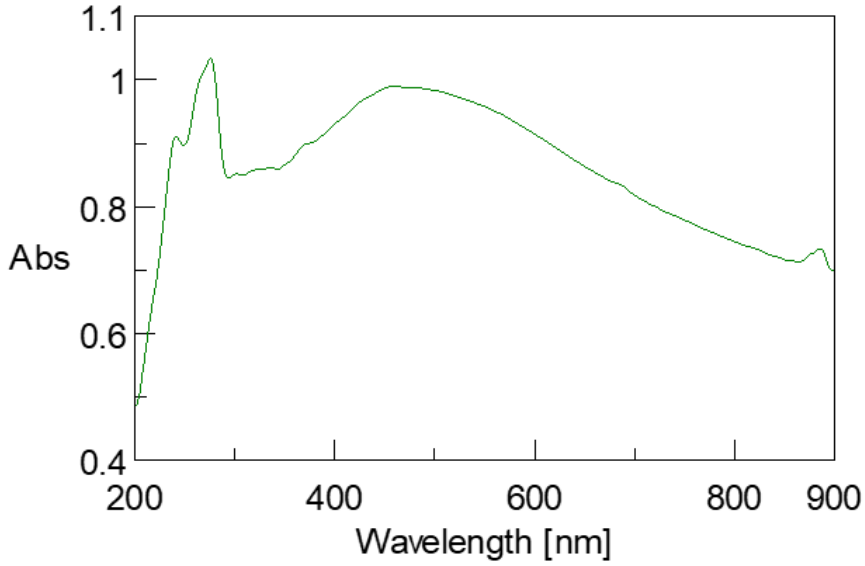

Fig. 2: UV-vis spectrum for Ag nanoparticles of *Didemnum psammathodes*

FT-IR Analysis:

FT-IR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticles synthesized. The FT-IR spectra of silver nanoparticles are shown in Figures 3 and 4.

The spectrum for Ag nanoparticles of *Phallusia nigra* revealed the presence of prominent peaks at 3424, 2923, 2106,1627, 1422, 1384, 1120, 1021, 874, 675, 610, 513 and 466 and cm^{-1} corresponding to different functional groups.

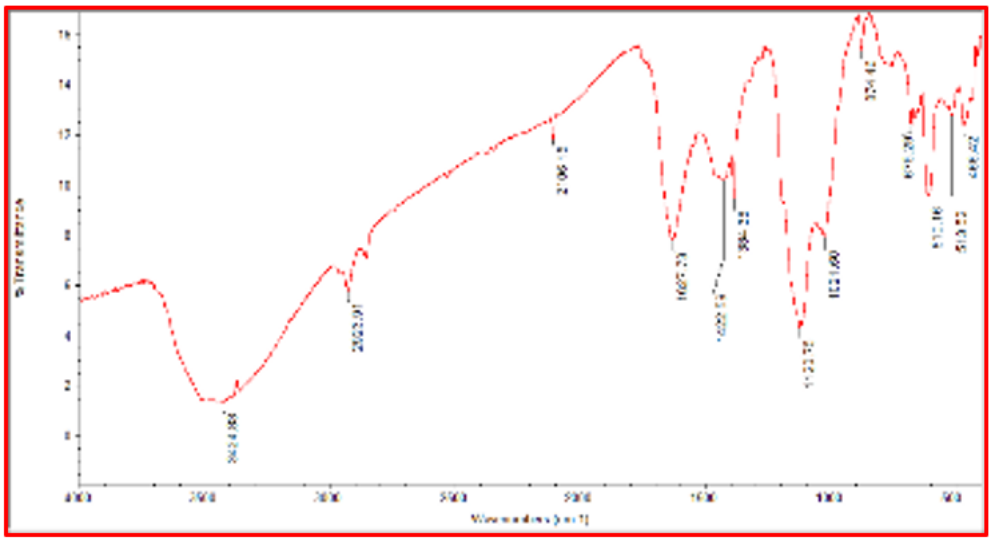

Fig. 3: FT-IR results for silver nanoparticles of *Phallusia nigra.*

The spectra for Ag nanoparticles of *Didemnum psammathodes* 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. O-H stretching of alcohols and phenols was indicated from the peak at 3984 cm^{-1}. The peak corresponds to 3455 cm^{-1} indicating N-H stretching (primary) functional 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 bond stretching of alkyne (mono-substituted) and aromatic functional groups were observed at 2106 and 1422 cm^{-1}. 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} revealed the functional group of C-O stretching of esters and ethers. The following peaks at 639 and 609 were 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.

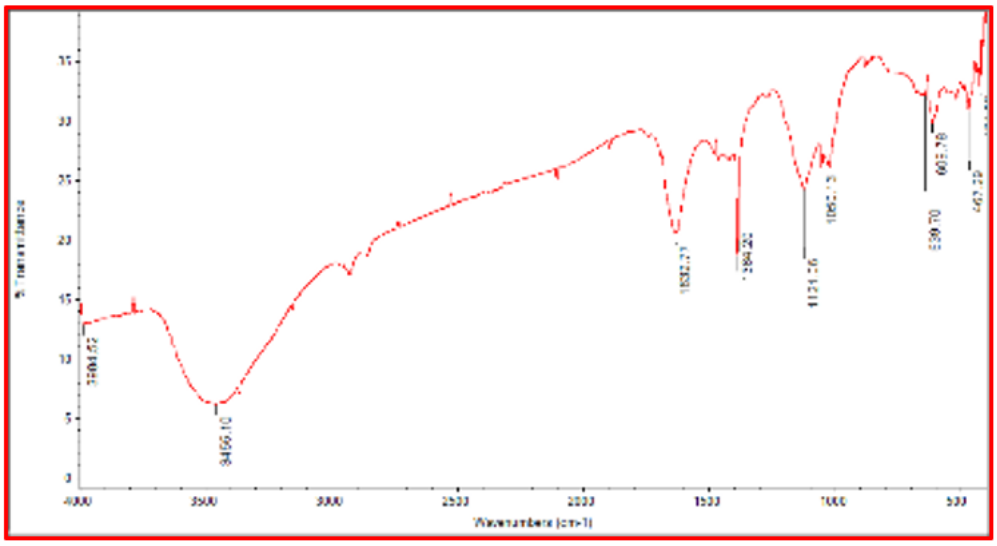

Fig. 4: FTIR result for silver nanoparticles of *Didemnum psammathodes.*

The capacity of silver nanoparticles generated from *Phallusia nigra* and *Didemnum psammatode* to scavenge DPPH free radicals was used to test the antioxidant activity. Tables 1 and 2 and Figures 5 and 6 show the findings of the DPPH radical scavenging efficiency of various concentrations of ethanolic extracts of *Phallusia nigra* and *Didemnum*

psammatode. At 20, 40, 60, 80, and 100 µg/ml concentrations, AgNPs produced from *Phallusia nigra* displayed substantial scavenging action with 48.38%, 56.45%, 69.35%, 75.64%, and 84.51%, respectively. The scavenging activity of AgNPs generated by *Didemnum psammatode* was significant at 20, 40, 60, 80, and 100 µg/ml concentrations, with values of 30.48%, 39.83%, 50.80%, 64.03%, and 67.74%, respectively. The chemical structure of an antioxidant determines its intrinsic reactivity towards free radicals and other ROS and hence the antioxidant activity. The efficiency of antioxidants also depends on their concentration and location in the system [Shahidi and Zhong, 2015].

Flavonoid-rich plants may be an excellent antioxidant source, increasing an organism's total antioxidant capacity and protecting it against lipid peroxidation [Koleva *et al*., 2002]. The higher radical scavenging activity of the leaf extract might be attributed to its high quantity of phenolic compounds, which were the principal antioxidant elements, and their entire content was directly proportionate to their antioxidant efficiency [Esmaeili *et al*., 2015]. According to Priya *et al*., 2016, the existence of flavonoids and phenolic components in the ethanolic extract of *Eudistoma viride* may be accountable for the highest antioxidant activity [Priya *et al*., 2016]. Preliminary chemical screening of *Phallusia nigra* ethanolic extract revealed the existence of alkaloids, flavonoids, glycosides, terpenoids, phenolic compounds and tannins [Azumi *et al*., 1990]. Hence the presence of the phenols and flavonoids may play a major role in the antioxidant activity.

Table1: *In-vitro* Antioxidant activity of silver nanoparticles synthesised from *Phallusia nigra*

Concentration (µg/ml)	Absorbance	Percentage of Scavenging Activity
20	0.32	48.38
40	0.27	56.45
60	0.19	69.35
80	0.151	75.64
100	0.096	84.51
Ascorbic acid	0.044	92.90
Control	0.62	-

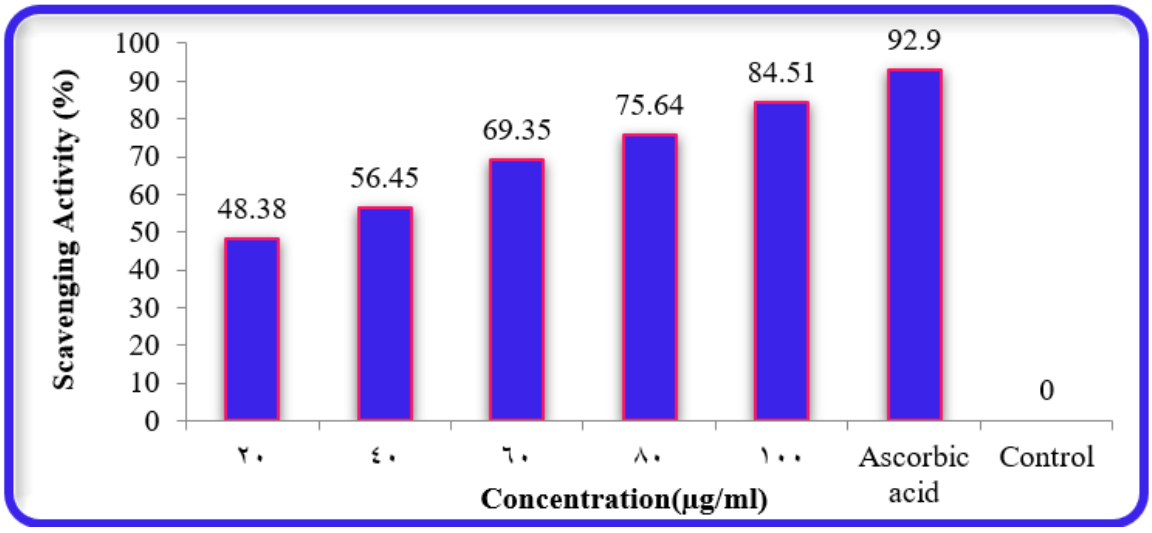

Fig. 5: *In-vitro* Antioxidant activity of silver nanoparticles synthesized from *Phallusia nigra.*

Table 2: *In-vitro* Antioxidant activity of silver nanoparticles synthesised from *Didemnum psammatode*.

Concentration (µg/ml)	Absorbance	Percentage of Scavenging Activity
20	0.431	30.48
40	0.381	39.83
60	0.305	50.80
80	0.203	64.03
100	0.200	67.74
Ascorbic acid	0.044	92.90
Control	0.62	-

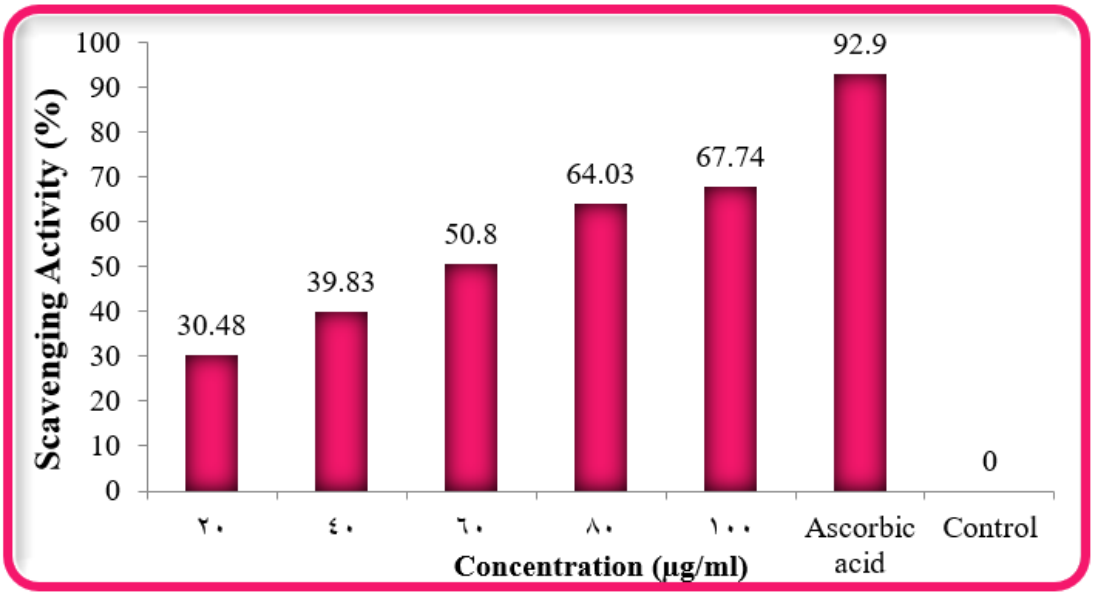

Fig. 6: *In-vitro* Antioxidant activity of silver nanoparticles synthesised from *Didemnum psammatode*.

CONCLUSION

The quick biological production of silver nanoparticles using *Phallusia nigra* and *Didemnum psammatode* extracts is an ecologically beneficial, easy and efficient technique for nanoparticle synthesis. From the perspective of technology, generated silver nanoparticles have potential biological applications as well as various benefits, including cost-effectiveness, suitability for use in the pharmaceutical and healthcare industries and extensive commercial production. The structure determination studies were carried out using UV-visible and FT-IR spectra. The antioxidant activity was evaluated by measuring the ability of silver nanoparticles produced from *Phallusia nigra* and *Didemnum psammatode* to scavenge DPPH free radicals and it was substantial.

Declarations:

Ethical Approval: Ethical Approval is not applicable.

Competing interests: The authors declare no conflict of interest.

Authors Contributions: I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

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Availability of Data and Materials: All datasets analysed and described during the present study are available from the corresponding author upon reasonable request.

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