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Biosynthesis of Silver Nanoparticles Using *Phallusia Arabica* and Evaluation of Total Antioxidant and Antibacterial Activity

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ABSTRACT

Biosynthesis of nanoparticles is a current field of nanotechnology which has eco-friendly and economic benefits compared to chemical synthesis. In this study, the ascidian *Phallusia arabica* ethanol extract was acted as a reducing agent for the preparation of silver nanoparticles (Ag-NPs). Ag-NPs was confirmed by the colour change using UV-Visible Spectroscopy and the size and shape of the NPs were determined by the SEM and the functional groups was identified by using Fourier transform infrared spectroscopy. Synthesized Ag-NPs were characterized by X-ray diffraction studies and EDAX. Atomic force microscope measurements confirmed the size and morphology of the synthesized Ag-NPs. The antibacterial activity of synthesized silver nanoparticles was tested against five pathogenic strains *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Shigella dysentriae*

Keywords
Silver nanoparticles, Phallusia arabica, XRD, EDAX, FTIR, SEM, AFM, Antibacterial activity

INTRODUCTION

In current years, research in nanotechnology has attractive interest because of its overwhelming impacts on many areas, including technology and medicine[1]. Nanobiotechnology, is a promising and upcoming field of nanotechnology, which is associated with different research fields of expertise, such as physics, chemistry, biology, medicine, engineering and material science[2]. Recently, many chemical, physical, and biological methods are adopted to prepare nanoparticles. The synthesized nanoparticles exhibit defined properties[3]. Bio synthesis of nanoparticles makes use of non-toxic, pollution free and safe reagents[4-7].

Ascidians are marine sedentary organisms. Among the marine animals, ascidians are ranked third in overall activity next to sponges and bryozoans. Ascidians exhibit noticeable pharmacological activity and more than 130 natural products have been synthesized by them[8]. They found as the main components of fouling community settling on all kinds of surfaces, roots of trees, stone, hard rocks, branches, algae, floating objects, hull of ships, sand and muddy surface[9]. Some ascidians are used as a nutritious food item in Japan like other marine food products, particularly in Hokkaido and Tohoku districts because of the high amount of carbohydrates, proteins and other essential micronutrients[10,11]. Ascidians contain a wealth of interesting pharmacological substances[12-14]. Seasonal variation in the occurrence, distribution, Chemical screening, antimitotic, antimicrobial and antibacterial activity have been reported from the simple ascidian *Phallusia arabica*[15-19]. The present study deals with biosynthesis, characterisation and applications of stable silver NPs from *Phallusia arabica*.

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II. MATERIALS AND METHODS

2.1. Identification and Collection of Animals

Phallusia arabica was collected by SCUBA diving from Green Gate area (8˚48'N and 78˚11'E) of Tuticorin Port, Tamil Nadu. Small plastic containers or buckets with sufficient sea water to cover the collected specimens were used. Specimens were carefully dislodged from the substratum. Larger coral, rock fragments stick to the specimen was removed. First the animal's colour, appearance and habitat were noted then it is removed from the substratum. Date of collection, location, depth and nature of the species was pasted in a label. Specimen voucher (AS 2276) was preserved in the Museum of the Department of Zoology, A.P.C. Mahalaxmi College for Women, Thoothukudi 628002, Tamilnadu, India.

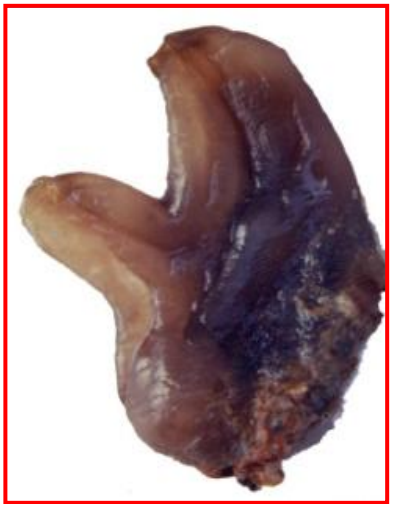

Figure - 1.*Phallusia arabica* Savigny, 1816 - External appearance

2.2. Extraction

The species was cleaned many times with sea water and dried under shade. The dry weight was taken after 48 hours and drying was continued till a constant weight was achieved. This ensures the complete removal of water from the samples. The dried animals were grinded to get powder and kept in an air-tight container for using further investigations.

2.3. Synthesis of Silver Nanoparticles

5 mL of ethanolic extract of *Phallusia arabica* was added to 45 mL of 1 mM silver nitrate. This solution was carried to continous stirring for 3 h using a magnetic stirrer[20]. The reaction mixtures were monitored for its colour change (brown). This shows the presence of silver nanoparticles. The filtered solid product was powdered at room temperature.

2.3 Characterisation of Nanoparticles

The above synthesised NPs were characterised by FT-IR, UV, XRD, SEM, AFM and Redox potential.

(i) FT-IR spectral studies

Silver NPs was determined using a Fourier Transform Infrared Spectrometer (Thermo scientific Nicolet is 5) iD5 ATR/ Attenuated Total Reflectance) – Znse (zinc selenide) Accessory system. Finely powdered 1 mg nanoparticle was added with about 100 mg of dried potassium bromide (IR grade) powder. The mixture was then pressed to make a transparent disc. It was examined under IR spectrometer and the resulting IR spectrum was recorded.

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(ii) UV-Visible spectroscopic studies

JASCO V-650 (UV-VIS spectrophotometer) was used to identify the absorption spectrum of the prepared nanoparticles. The synthesized nanoparticles were analysed with a Philips CM 200 model using 200kV electron acceleration voltage and with a resolution of $2.4A^0$. UV-VIS spectral behaviour was studied by using Computer controlled JASCO V-650[21].

(iii) X-**Ray Diffraction studies**

X-ray diffraction spectroscopy was used to determine the phase variety and grain size of synthesized silver NPs. Various phases present in the prepared samples were analysed by X′ pert high score software with match and search facility. Scherrer′s equation was used to calculate the particle size of the synthesized samples.

(iv) SEM and EDAX studies

Morphological features of the synthesized silver NPs were analysed by Scanning Electron Microscope (JSM-6480 LV). A thin layer of platinum was coated to make the samples conductive. Then the samples were studied in the SEM at an accelerating voltage of 20 KV. Inorganic metals present in the samples were identified by EDAX characterisation.

(v) Atomic Force Microscopy studies

A thin film was prepared on a silica glass plate by dropping a few drops of silver NPs. It was permitted to dry at room temperature in the dark (to avoid NPs diameter growth due to temperature and/or light). Atomic Force Microscopy was used to scan the deposited film on silica glass plate.

(vi) Redox potential

Cyclic voltammogram was used to detect the transfer of electrons during an oxidation-reduction (redox) reaction. The electrode potential in solution is linearly cycled from a starting potential to final potential and back to the starting potential. Here, the current is measured as a function of potential. This process, in turn, cycles the redox reaction. Multiple cycles can takes place. The system starts off with an initial potential at which no redox can takes place.

Applications

(vii) Total Antioxidant activity

The total antioxidant activity of the ethanolic extract of *Phallusia arabica* and synthesised silver NPs were studied according to the method described by Prieto et al[22]. 3mL of Phosphomolybdenum solution was added with 0.3 mL of sample solution of different concentrations (50, 100, 150, 200 and 250 µg/mL). Mixture of 0.3 mL of water and 3mL Phosphomolybdenum solution was acted as control and Ascorbic acid was acted as reference standard. For the completion of the reaction the test tubes were supported at 95 °C for 10 min. Sample absorbance was recorded at 695 nm using a spectrophotometer against a control solution after cooling at room temperature. By using the standard ascorbic acid, the antioxidant capacity was denoted as Ascorbic acid equivalent (AAE). The % of total antioxidant activity was determined using the following formula,

Total antioxidant activity % = Ac-As/Ac×100

Where Ac is the absorbance of the control

As is the absorbance of the sample

(viii)Antibacterial assay

The antibacterial activity was measured by agar cup plate method[23]. The pathogens like *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumonia*, *Shigella dysentriae* and *Serratia marcescens* were collected from clinical laboratory and stored in 4°C. The overnight culture inoculum (100µl) of each bacteria was individually swabbed on Mueller Hinton agar plates. In each plate sterile discs of 5 millimeter

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diameter were impregnated with the known concentration of the nano silver (15 µl) and standard antibiotic were placed and the plates were incubated at 37°C for 24 hrs. The antibacterial activity of the nanoparticles were recorded as the mean diameter of the resulting inhibition zone of growth measured in millimetres.

The Active Index (AI) and Proportion Index (PI) were calculated using the following formulae,

$$\text{Active Index (AI)} = \frac{\text{Inhibition zone of the test sample}}{\text{Inhibition zone of the standard}}$$

$$\text{Proportion Index (PI)} = \frac{\text{Number of positive results obtained for individual extract}}{\text{Total number of tests carried out for each extract}}$$

III. RESULTS AND DISCUSSION

3.1. UltraViolet-Visible Spectrum of silver nanoparticles

Fig.2. Shows the presence of UV-Vis spectrum of silver nanoparticles formed with *Phallusia arabica.* The UV- Visible spectrum exhibited significant absorption peak at 301 nm for silver nanoparticles, 223 nm for silver nitrate[24]. The colour change (brown) was monitored by UV-Vis spectroscopy. It shows the reduction of silver ions to silver nanoparticles by exposing the silver nitrate to *Phallusia arabica* extract.

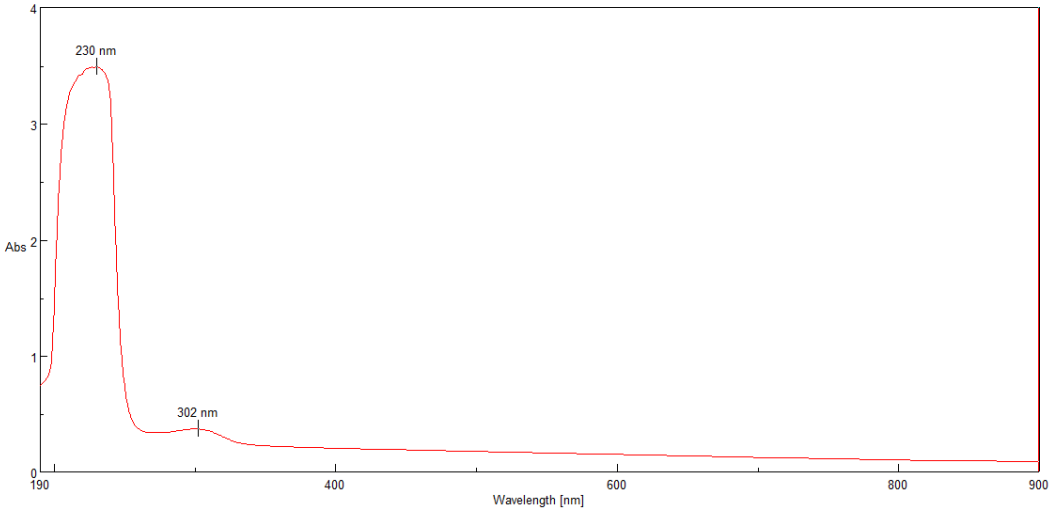

Figure - 2. UltraViolet-Visible Spectrum of silver nanoparticles synthesized using ethanol extract of *Phallusia arabica*

3.2. FT-IR spectrum of silver nanoparticles

Fig.3. indicates the possible interaction of molecules for capping and stabilization of Ag nanoparticles observed at 3444, 2426, 1651, 1633, 1384, 827, 598 cm^{-1}. The peak at 1384 cm^{-1} shows the C–H bending vibration of alkane compounds. The peak at 3,444 cm^{-1} shows the presence of phenols and alcohols with free O–H group[25]. The additional peaks at 2426, 1651, 1384, 827, and 598 cm^{-1} are associated to Ag-NPs. Similar results has been reported by aqueous extract of brown marine macroalga, *Sargassum Muticum*[26].

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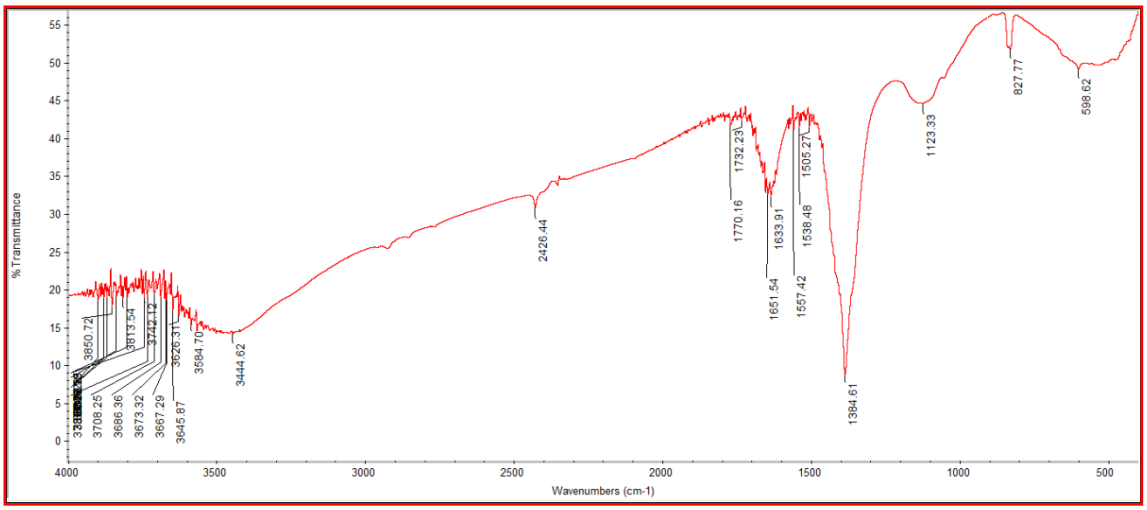

Figure - 3. FTIR Spectrum of silver nanoparticles synthesized using ethanol extract of *Phallusia arabica*

3.3. X-Ray Diffraction analysis of silver NPs

The X-Ray Diffraction pattern of *Phallusia arabica* used silver NPs showed a number of Bragg reflections at 2θ = 29.4069, 32.2707, 46.2802, 76.8546, (Fig.4.). It shows three main diffraction features corresponding to (1 1 1), (2 0 1) and (2 2 0) planes and all the three peaks can be indexed to standard cubic phase of silver (JCPDS File No 04-0783). The received reflections are sharp and high in intensity which proves that the synthesized nanoparticles are well crystalline. The X-Ray Diffraction pattern observed in this study was consistent with previous reports[27].

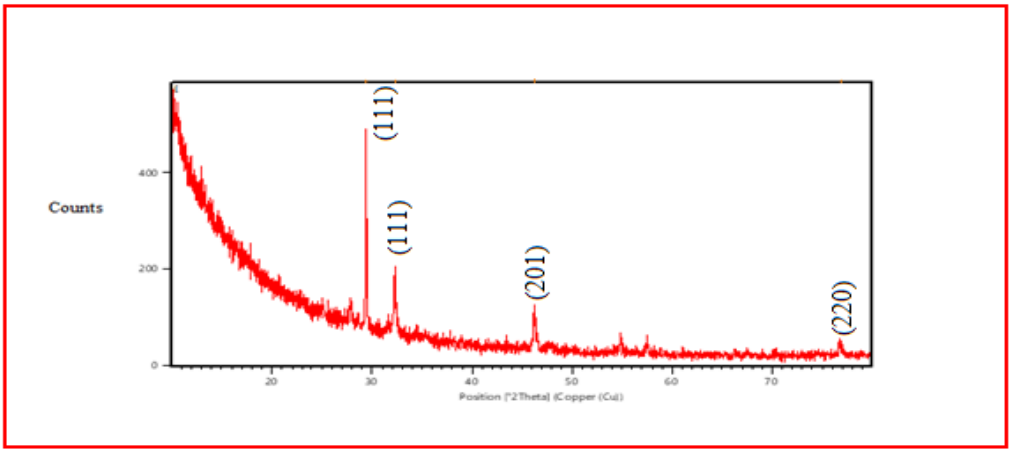

Figure - 4. X-Ray Diffraction patterns of silver nanoparticles synthesized using ethanol extract of *Phallusia arabica*

3.4. EDAX analysis of silver nanoparticles

Elemental silver signal of the silver nanoparticles was proved by Energy dispersive X-ray (EDAX) spectrometers.

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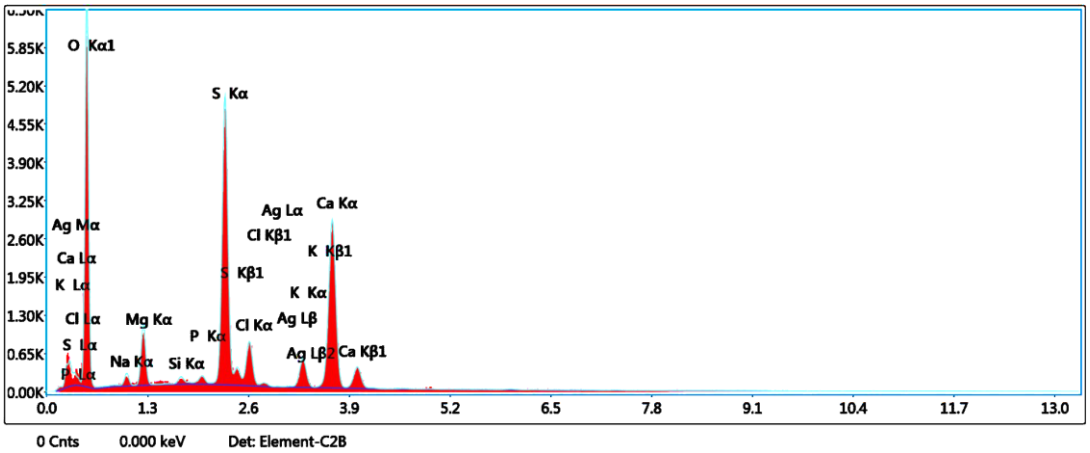
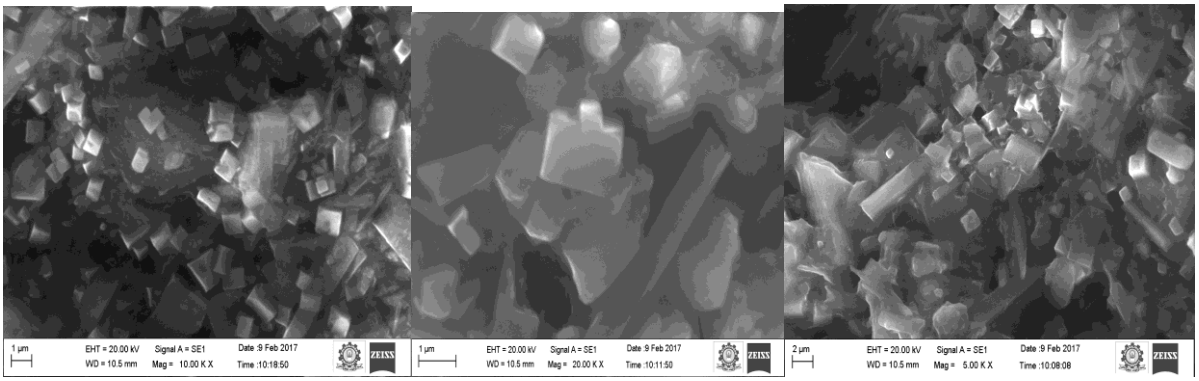


Figure- 5. EDAX Spectrum of silver nanoparticles synthesized using*Phallusia arabica* extract

3.5. SEM studies of silver NPs

Fig.6 (a-c) indicates the results of the silver nanoparticles formed from the *Phallusia arabica* extract captured by means of Scanned electron microscopy. Silver nanoparticles synthesized using *Phallusia arabica* were monodispersed with cubic structures with mean sizes of 200 nm. Similar results have been reported from extract of *Padina tetrastromatica* leaf[28].



(a) (b) (c)

Figure – 6 a-c. SEM images exhibiting the morphological characteristics of silver nanoparticles synthesized using the ascidian *Phallusia arabica*

3.6. AFM studies of silver NPs

AFM was used to investigate the surface morphology and size of the Ag nanoparticles.Fig.7. shows three dimensional images of the nanoparticles. A well separated spherical particles are noted from the 2D view. The three Dimensional view indicated that the growth direction of all the particles was almost same. The particle size measured from AFM images matches well agree with the values obtained from the corresponding XRD pattern. Similar results have been reported from the marine seaweed *Sargassum wightii*[27].

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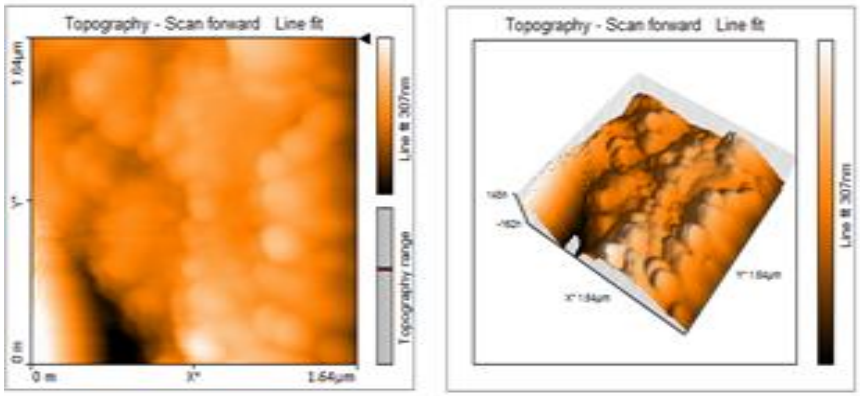

Figure - 7. AFM of silver NPs synthesized using ethanol extract of*Phallusia arabica*

3.7. Cyclic voltammogram of silver NPs

Cyclic voltammograms were recorded in the scan rate 0.1 V/s for 1.0 mL of silver nanoparticles synthesised using *Phallusia nigra* extract. It showed one oxidation peak (0.094). The background current was recorded from –0.4 to 0.4V was shown in Fig. 8. It confirms the presence of nanosilver.

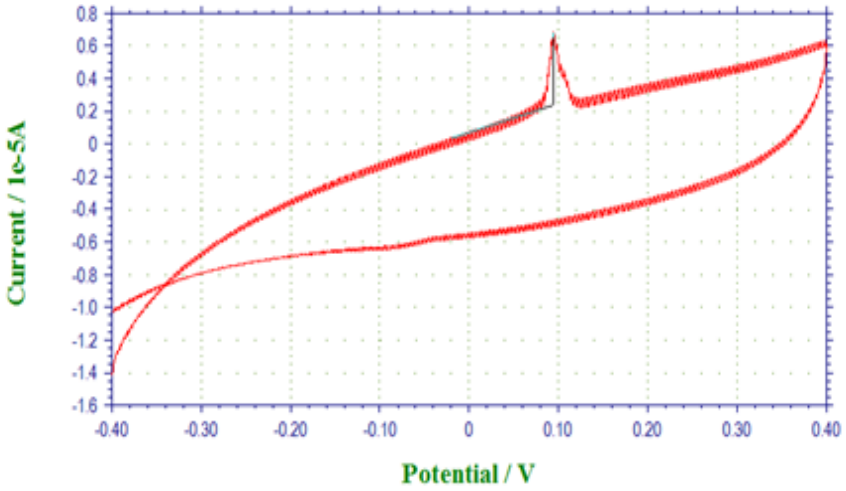

Figure – 8. Cyclic Voltammogram of silver NPs

Applications of Nanoparticles

3.8. Total Antioxidant activity

The antioxidant potential of the ethanolic extract and silver NPs of *Phallusia arabica* was determined from their capacity to reduce the reduction of Mo (VI) to Mo (V) by the antioxidant-enriched fractions and subsequent formation of a green phosphate/Mo (V) complex at acidic pH. Antioxidant activity is mainly due to the presence of its bio-active compounds mainly polyphenols, carotenoids, and vitamin E and C[29]. This reveals that the concentration of the bioactive compounds present in the extract is responsible for its antioxidant activity (Table 1). Thus, higher concentration of silver nanoparticles indicates significant antioxidant activity. Silver nanoparticles exhibited a significant total antioxidant activity that increased with increasing concentration.

Table 1. Total antioxidant activityof *Phallusia arabica* at varying concentrations

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Concentration (µg/ml)	Ethanol extract	Silver NPs	Standard Ascorbic acid
50	18.50	21.16	20.31
100	20.10	24.58	23.54
150	24.15	28.96	27.56
200	26.50	33.67	31.40
250	33.40	36.79	35.57

3.9. Antibacterial activity

When compared to standard drug (Table 2) silver nanoparticles showed significant inhibition zone against *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Shigella dysentriae*. Minimum zone of inhibition was noted against *Bacillus cereus* (17 mm) and maximum zone of inhibition was found against *Shigella dysentriae* (29 mm) and it was higher than that of the positive control gentamycin (26 mm). The possible reason for the antibacterial activity of silver is that Ag NPs may attach to the surface of the cell membrane disturbing permeability and respiration functions of the cell[30]. Smaller Ag NPs having the large surface area available for interaction would give more antibacterial effect than the larger Ag NPs. It is also possible that Ag NPs not only interact with the surface of membrane, but can also penetrate inside the bacteria[31].

Table 2. Antibacterial activityof *Phallusia arabica*

Pathogens Names	**Silver NPs**		**Standard (Gentamycin)**
	DIZ*	**AI#**	
Bacillus cereus	17	1.0	17
Bacillus subtilis	22	1.37	16
Staphylococcus aureus	19	1.05	18
Enterococcus faecalis	20	1.11	18
Escherichia coli	19	0.79	24
Klebsiella pneumoniae	18	0.64	28
Shigella dysentriae	29	1.11	26
Serratia marcescens	21	0.84	25

*DIZ- Diameter of Zone Inhibition; #AI- Active Index

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