BIOSYNTHESIS OF IRON OXIDE NANOPARTICLES FROM SIMPLE ASCIDIAN *PHALLUSIA ARABICA* AND THEIR TOTAL ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY

[1]H.Kohila Subathra Christy, [2]Dr. R. Jothibai Margret, [3]Dr. V.K. Meenakshi,
[1]Research Scholar, 9563, PG and Research Department of Chemistry, V.O.Chidambaram College, Thoothukudi
Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli – 627 012, TamilNadu
[1]Head and Assistant Professor of Chemistry, A.P.C. Mahalaxmi College for women, Thoothukudi, TamilNadu
[2]Associate Professor, Department of Chemistry, Pope's College, Sawyerpuram
[3]Associate Professor (Retired), Department of Zoology,
A.P.C. Mahalaxmi College for women, Thoothukudi

Abstract: The field of nanotechnology is one of the most active areas of research in modern materials science. The development of eco-friendly technologies in material synthesis is of considerable importance to expand their biological applications. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. In this paper, we have reported on biological synthesis of iron oxide nanoparticles and its total antioxidant and antibacterial activity. The synthesized rod shaped iron oxide nanoparticles (88.3 nm) were characterized by Ultra Violet - Visible spectrum (UV-Vis), Fourier Transform InfraRed spectroscopy (FT-IR) X-Ray diffraction (XRD), Energy dispersive X-ray analysis (EDAX), Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM). Antioxidant activity was found to be highly significant in iron oxide nanoparticles when compared to standard. The synthesized nanoparticles have an effective antibacterial activity against four pathogenic strains *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Shigella dysentriae*.

Keywords: Iron oxide nanoparticles, *Phallusia arabica*, AFM, Total antioxidant activity, Antibacterial activity

1. INTRODUCTION

In recent years, research in nanotechnology has attracted increasing interest because of its promising and revolutionary impacts on many areas, including medicine and technology (Zhang et al, 2011). Nanobiotechnology, which is an emerging and promising field of nanotechnology, is involved with different research fields of expertise, such as physics, chemistry, biology, medicine, engineering and material science (Duran and Seabra, 2012). Currently, a large number of physical, chemical, biological, and hybrid methods

are available to synthesize different types of nanoparticles. The nanoparticles formed using each method show specific properties. (Liu et al, 2001). Green synthesis of nanoparticles makes use of environmental friendly, non-toxic and safe reagents (Salam et al, 2012, Kumaran et al, 2017, Sankaravadivu et al, 2018).

Ascidians are marine sedentary organisms. They occur as the major components of fouling community settling on all kinds of surfaces, hard rocks, stone, hull of ships, branches, roots of trees, algae, floating objects, sand and muddy surface (Meenakshi, 2010). Some ascidians are widely enjoyed as food in Japan, particularly in Hokkaido and Tohoku districts because of the high amount of proteins, carbohydrates and other essential micronutrients (Margalino and Destefano, 1960). Like other marine food products, ascidians are delicious, relatively easily digestible and offer minerals, iodine and vitamins (Tamilselvi et al, 2010). Ascidians contain a wealth of interesting pharmacological substances (Davies-Coleman et al, 2000, Kohila Subathra Christy et al, 2014, 2015). The present study deals with biosynthesis, characterisation and applications of stable iron oxide NPs from *Phallusia arabica*.

II. MATERIALS AND METHODS

2.1. Collection and Identification of Animals

Phallusia arabica was collected from Green Gate area (8˚48'N and 78˚11'E) of Tuticorin Port, Tamil Nadu by SCUBA diving. 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. A label indicating date of collection, location, depth and colour of the specimen was pasted. A voucher specimen (AS 2276) was deposited in the Museum of the Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin 628002, Tamilnadu, India.

2.2. Extraction

Epibionts adhering to the surface of the test of *Phallusia arabica* were carefully removed. The specimen was washed several times with sterile sea water. It was 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 homogenized to get a coarse powder. The powder was stored in an air-tight container and used for all further investigations.

2.3. Synthesis of Iron oxide Nanoparticles

Iron oxide NPs were synthesised according to the method described by Mahdavi et al. Dried *Phallusia arabica* (1 g) was taken in a 500 ml conical flask added with 100 ml ethanol. It was kept under soaking for 6 hours and then filtered through Whatman no. 1 filter paper. 5 ml of the above prepared extract was dripped

slowly into the $FeCl_3$ solution with constant stirring at room temperature with normal atmosphere pressure. After 3 mins visible colour changes were observed. The yellow colour ethanolic solution of $FeCl_3$ turned to brownish black.

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 analysis

The FT-IR spectra of iron oxide NPs was determined using a Fourier Transform Infrared Spectrometer (Thermo scientific Nicolet is 5) iD5 ATR/ Attenuated Total Reflectance) – Znse (zinc selenide) Accessory system. One mg of finely powdered nanoparticle was mixed with about 100 mg of dried potassium bromide (IR grade) powder. The mixture was then pressed in a special dye to yield a transparent disc. The disc was then held in the instrument beam for spectroscopic examination and the resulting IR spectrum was recorded.

(ii) UV-Visible spectroscopic studies

The Surface Plasmon resonances (SPR) of synthesized NPs have been studied by UV-Vis double-beam bio-spectrophotometer. To the ethanolic extract of *Phallusia arabica* aqueous $FeCl_3$ was added. The reaction mixture was filled in a glass cuvette of path length 10 mm and spectral analysis has been done in the range of 300 to 700 nm. DI water was used as blank.

(iii) XRD analysis

The phase variety and grain size of synthesized iron oxide NPs were determined by X-ray diffraction spectroscopy (Philips PAN analytical). Different phases present in the synthesized samples were determined by X′ pert high score software with search and match facility. Particle size of the prepared samples were determined by using Scherrer′s equation.

(iv) SEM and EDAX analysis

Morphological features of the synthesized iron oxide NPs were studied by Scanning Electron Microscope (JSM-6480 LV). After 24 hrs of the addition of $FeCl_3$ solutions, the SEM slides were prepared by making a smear of the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the SEM at an accelerating voltage of 20 KV. Inorganic metals present in the samples were identified by EDAX characterisation.

(v) AFM analysis

A thin film of iron oxide NPs were prepared on a silica glass plate by dropping a few drops and allowed to dry at room temperature in the dark (to avoid NPs diameter growth due to temperature and/or light). The deposited film on silica glass plate was then scanned with the Atomic Force Microscopy

(vi) Redox potential

Cyclic voltammograms trace the transfer of electrons during an oxidation-reduction (redox) reaction. The potential of an electrode 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.

2.5 Applications of Iron oxide Nanoparticles

(i) Total Antioxidant activity

The total antioxidant activity of the ethanolic extract of *Phallusia arabica* and synthesised iron oxide was measured according to the method described by Prieto et al. 3mL of Phosphomolybdenum reagent was mixed with 0.3 mL of sample solution of varying concentrations (50, 100, 150, 200 and 250 µg/mL). Mixture of 0.3 mL of water and 3mL Phosphomolybdenum reagent was used as control and Ascorbic acid was used as reference standard. The test tubes were incubated at 95 °C for 10 min for the completion of the reaction. After cooling at room temperature, sample absorbance was measured at 695 nm using a spectrophotometer against a control solution. The antioxidant capacity was expressed as Ascorbic acid equivalent (AAE) by using the standard Ascorbic acid. The % of total antioxidant activity was calculated 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

(ii) Antibacterial assay

The antibacterial activity was measured by agar cup plate method (Khalid et al, 1999). 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. Sterile discs of 5 mm diameter were impregnated with the known concentration of the iron oxide (15 µl) and standard antibiotic were placed in each plate. 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.

From the results, 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. UV-Visible Spectrum of iron oxide Nanoparticles

UV–Vis absorption spectrum of the *Phallusia arabica* are depicted in Fig. 1. The characteristic surface plasmon resonance band of Fe_3O_4 occurs at wavelength in the range of 200-500 nm as a function of different concentration of metal ion with different volume of *Phallusia arabica* extracts at room temperature. The maximum SPR band centered at 239 nm, which indicates the reduction of $FeCl_3$ into Fe_3O_4 (Latha et al, 2014). The characterization of iron oxide Nanopartilce under the UV-Visible spectroscopy analysis shows the peak at 355nm, which is due to charge transfer spectra (Behera et al, 2012).

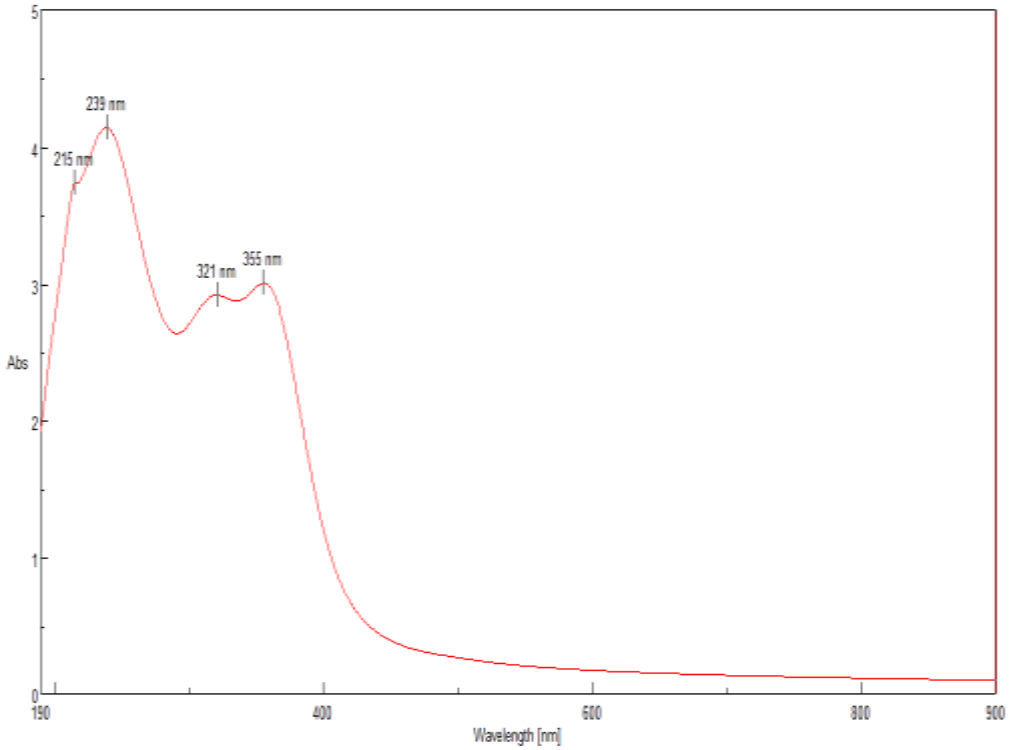

Fig. 1. UV-Visible Spectrum of Iron oxide nanoparticles synthesized using ethanol extract of *Phallusia arabica*

3.2. FTIR spectrum of iron oxide Nanoparticles

FT-IR spectrum of the *Phallusia arabica* exhibited a broad band at 1636.92 cm^{-1} which is due to the presence of carbonyl groups in the ketones (Silverstein et al, 1991). The strong absorption peak at 3414.34 cm^{-1} is assigned to O-H stretching of alcohol and phenolic compounds (Fig. 2.). The band at 1400.81 cm^{-1} shows the presence of CH bending vibration (Kohila Subathra Christy et al, 2012). The formation of Fe_3O_4 is

characterised by the absorption band at 424 cm^{-1} is corresponds to the Fe–O band (Wang and Chen, 2006). From the FTIR result, the soluble elements present in *Phallusia arabica* extract could have acted as capping agents preventing the aggregation of nanoparticles in solution, and thus playing a relevant role in their synthesis and shaping.

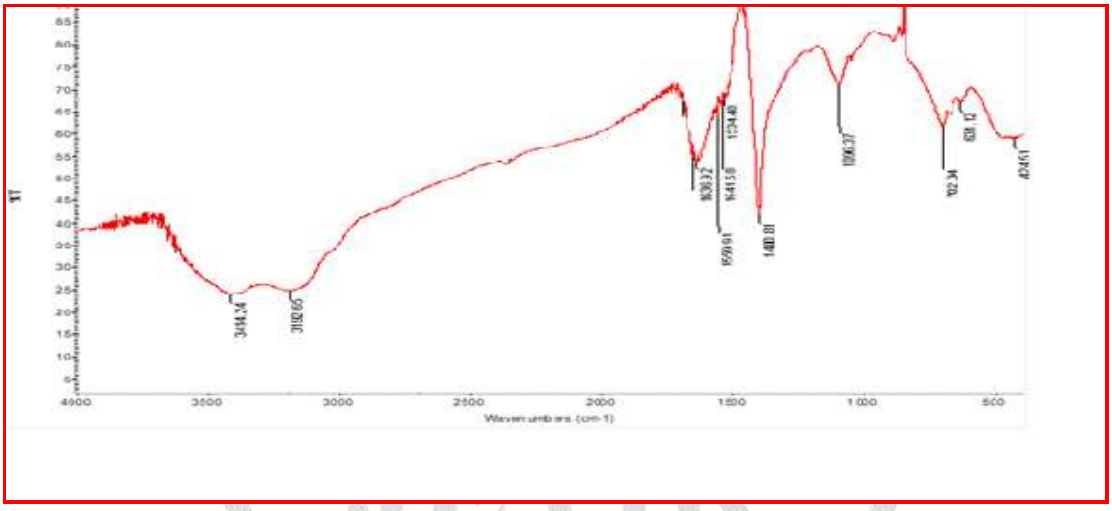

Fig. 2. FTIR Spectra of iron oxide nanoparticles synthesized using ethanol extract of *Phallusia arabica*

3.3. XRD analysis of iron oxide Nanoparticles

Fig. 3. shows the XRD pattern of iron oxide NPs prepared by using *Phallusia arabica* extract. All the diffraction peaks in this pattern were found to be in good agreement with JCPDS No: 36-1451. The sample showed the major characteristic peaks for prepared crystalline iron oxide nanoparticles at 2θ values of 31.94, 32.76, 45.79, 56.62 and 75.35 degrees corresponding to (002), (110), (002), (112), and (002) respectively.

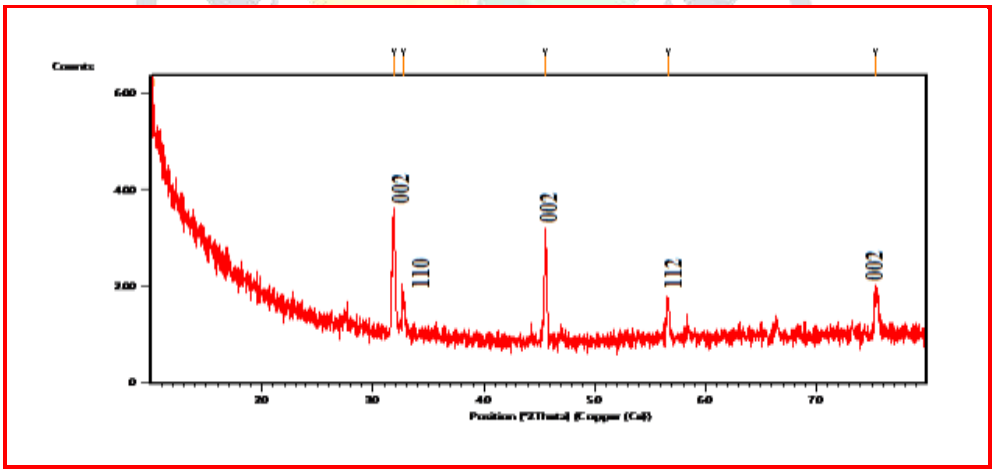

Fig. 3. XRD patterns of iron oxide nanoparticles synthesized using ethanol extract of *Phallusia arabica*

3.4. EDAX analysis of iron oxide Nanoparticles

Atomic percentage values could be helpful in reflecting the atomic content on the surface and near surface regions of the NPs (Fig. 4.). The atomic percentages obtained from EDAX quantification were 18.85 % of O, 11.73% of Fe.

.

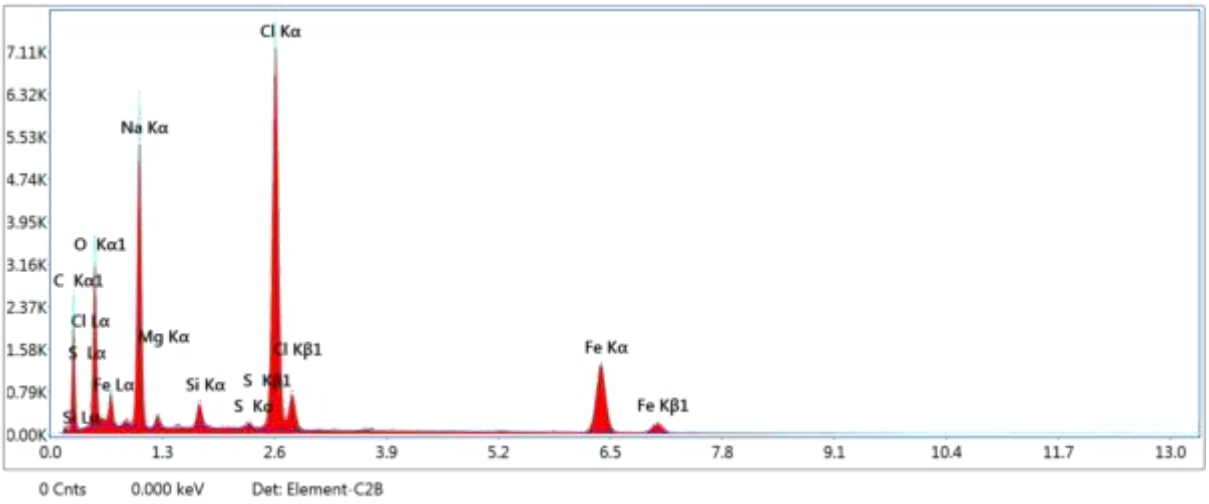

Fig. 4. EDAX Spectrum of Iron oxide nanoparticles synthesized using *Phallusia arabica* extract

3.5. SEM analysis of iron oxide Nanoparticles

SEM image shows (Fig. 5.) the clear morphology of iron oxide nanoparticles. The SEM image of iron oxide nanoparticles synthesized using *Phallusia arabica* exhibited cubic structure and was found to be in the nanometer range.

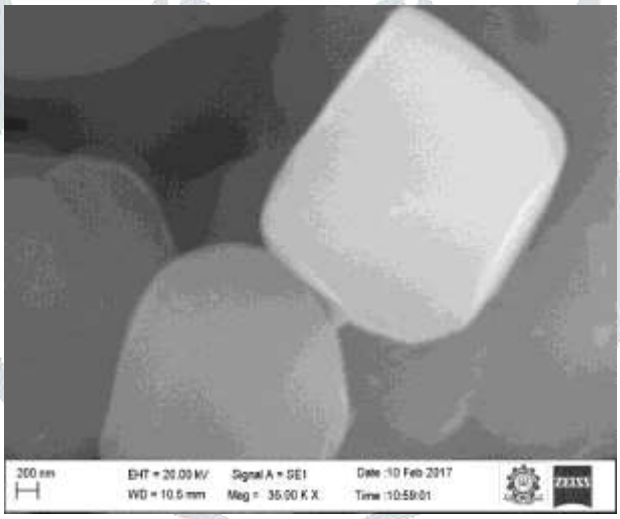

Fig. 5. SEM image of iron oxide nanoparticles synthesized using the extract of *Phallusia arabica*

3.6. AFM analysis of iron oxide Nanoparticles

The surface morphology and size of the iron oxide nanoparticles were studied by AFM. The three dimensional images of the nanoparticles are shown in Fig. 6. From the 2D view, rod shaped particles are seen. The sizes of the particles are in the range of 88.3 nm. The 3D view revealed that the growth direction of all the particles was almost same. Iron oxide NPs exhibited remarkable tendency to form uniformly sized and shaped agglomerates.

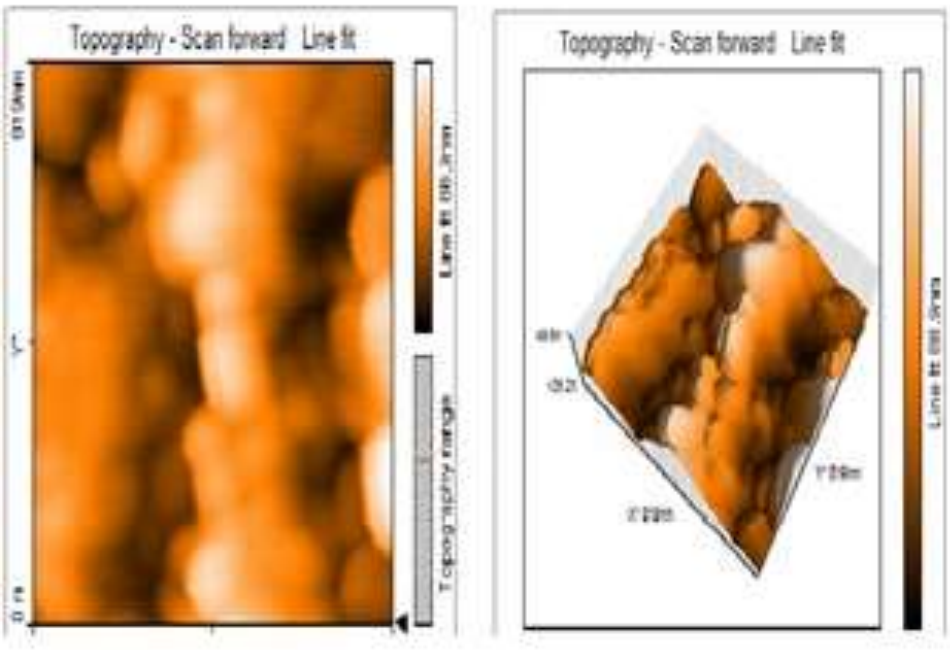

Fig. 6. AFM image of iron oxide nanoparticles synthesized using ethanol extract of *Phallusia arabica*

3.7. Cyclic voltammogram of iron oxide Nanoparticles

The voltammogram of nanoparticles exhibited one sharp anodic peak (-0.212 V) and one cathodic peak (0.05 V) (Fig. 7.). It confirms the presence of iron oxide nanoparticles.

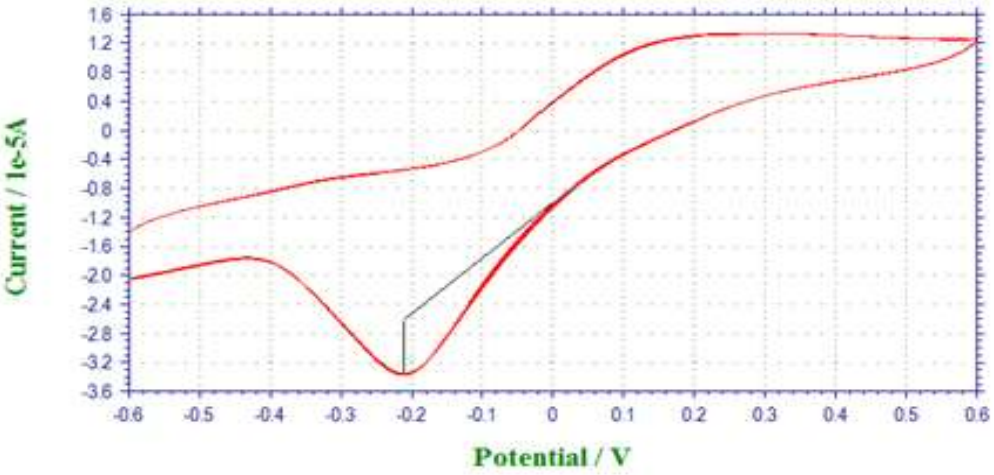

Fig. 7. Cyclic Voltammogram of nano iron oxide

Applications

3.8. Total Antioxidant activity

Antioxidant activity depends on the presence of its bio-active compounds mainly polyphenols, carotenoids, vitamin E and C (Oktay et al, 2003). The concentration of the bioactive compounds present in the extract is important to showing antioxidant activity. Thus, higher concentration of extracts shows higher antioxidant activity. Iron oxide showed a good total antioxidant activity that increased with increasing concentration (Table 1.1). Antioxidant activity was found to be highly significant in iron oxide nanoparticles when compared to standard.

Table 1.1: Total antioxidant activity of *Phallusia arabica*

Concentration (µg/ml)	Ethanol extract	Iron oxide NPs	Standard Ascorbic acid
50	18.50	21.55	20.31
100	20.10	24.90	23.54
150	24.15	29.15	27.56
200	26.50	33.98	31.40
250	33.40	37.94	35.57

3.9. Antibacterial assay

The antibacterial activities of the iron oxide nanoparticle evaluated against eight pathogenic bacteria are presented in Table 1.2. The result of antibacterial activity of iron oxide nanoparticle showed significant antibacterial activity against four pathogenic strains *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Shigella dysentriae* with zone of inhibition ranging from 18 mm to 27 mm when compared to standard drug.

There are many factors responsible for the antibacterial activity of iron oxide nanoparticles. Some authors have demonstrated that the small size of nanoparticles can also contribute to bactericidal effects. For example, Lee et al. reported that the inactivation of *Escherichia coli* by zero-valent iron nanoparticles could be because of the penetration of the small particles into *E. coli* membranes. Nano scale zero valent iron (NZVI) could then react with intracellular oxygen, leading to oxidative stress and eventually causing disruption of the cell membrane.

Table 1.2: Antibacterial activity of *Phallusia arabica*

Pathogens Names	Iron oxide NPs		Standard (Gentamycin)
	DIZ*	AI#	
Bacillus cereus	16	0.94	17
Bacillus subtilis	20	1.25	16
Staphylococcus aureus	18	1.0	18
Enterococcus faecalis	19	1.05	18
Escherichia coli	17	0.71	24
Klebsiella pneumoniae	10	0.36	28
Shigella dysentriae	27	1.04	26

Serratia marcescens	20	0.8	25

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

IV. REFERENCES

[1] Zhang, X. Yan, S. Tyagu, R.D. and Surampalli, R.Y. 2011. Synthesis of nanoparticles by microorganisms and their application in enhancing microbiological reaction rates. Chemosphere, 2011, 82: 489-494.

[2] Durán, N. and Seabra, A.B. 2102. Metallic oxide nanoparticles: state of the art in biogenic syntheses and their mechanisms. Appl. Microbiol. Biotechnol, 95: 275-288.

[3] Liu, J. Qiao, S.Z. Hu, Q.H. and Lu, G.Q. 2001. Magnetic nanocomposites with mesoporous structures: synthesis and applications. Small, 7: 425-443.

[4] Salam, H.A. Rajiv, P. Kamaraj, M. Jagadeeswaran, P. Gunalan, S. and Sivaraj, R. 2012. Plants: Green route for nanoparticle synthesis. Int. J. Biol. Sci, 1: 85-90.

[5]Kumaran, N. Sri, Vijayaraj, R. Kumaresan, M. and Jayaprakashvel, M. 2017, Eco-friendly synthesis of silver nanoparticles from marine ascidian, Didemnum psammathodes and its in vitro anti-inflammatory properties. Journal of Bionanoscience, 11(6): 560-566.

[6] Sankaravadivu, S. Jothibai Margret, J. and Meenakshi, V.K. 2018. Syhthesis of iron oxide Nanoparticles from colonial tunicate, Journal of Emerging Technologies and Innovative Research, 5(7): 393-397.

[7] Meenakshi, V.K. 2010. Indian ascidians-potential candidates for research. A review. International Journal of Biological Technology, Special issue 29-33.

[8] Margalino, G.A and Destefano. M. 1960. Contributo alla conoscenza della digeribilita delle Ascidie Eduli. Thalassia jonica, 3:69-82.

[9] Tamilselvi, M. Sivakumar, V. Abdul Jaffar Ali, H. and Thilaga, R.D. 2010. Preparation of pickle from *Herdmania pallida*, simple ascidian, World Journal of Dairy and Food Sciences, 5(1): 88-92.

[10] Davies-Coleman, M.T. Cantrell, C.L. Gustafson, K.R. Beutler, J.A. Pannell, L.K. and Boyd, M.R. 2000. Stolonic acids A and B, New cytotoxic cyclic peroxides from an Indian Ocean ascidian *Stolonica* species. Journal of Natural Products, 63: 1411-1413.

[11] Kohila Subathra Christy, H. Jothibai Margret, J. and Meenakshi, V.K. 2014. Antipyretic and Analgesic activity of *Phallusia arabica* Savigny, 1816, International Journal of Medicinal Chemistry and Analysis, 4(3): 162-165.

[12] Kohila Subathra Christy, H. Jothibai Margret, J. and Meenakshi, V.K. 2015. Evaluation of Wound healing activity of *Phallusia arabica*, World Journal of Pharmaceutical Research, 4(7): 1202-1213.

[13] Mahdavi, M. Namvar, F. Ahmad, M.B. and Mohamad, R. 2013. Green biosynthesis and characterization of magnetic iron oxide (Fe_3O_4) Nanoparticles using seaweed (*Sargassum muticum*) aqueous extract, Molecules, 18: 5954-5964.

[14] Prieto, P. Pineda, M. and Aguilar M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem, 269: 337-41.

[15] Khalid, F. Siddiqi, R. and Mojgani, N. 1999. Detection and Characterization of a Heat Stable Bacteriocin (Lactocin LC-09) Produced by a Clinical Isolate of *Lactobacilli.* Medical Journal of Islamic Academy of Science, 12(3): 67-71.

[16] Latha, N. and Gowri, M. 2014. Bio Synthesis and Characterisation of Fe_3O_4 Nanoparticles Using Caricaya Papaya Leaves Extract. International Journal of Science and Research, 3(11): 1551-1556.

[17] Behera, S.S. Patra, J.K. Pramanik, K. Panda, N. and Thatoi, H. 2012. Characterization and Evaluation of Antibacterial activities of chemically synthesized iron oxide nanoparticles. World Journal of Nanoscience and Engineering, 2: 196-200.

[18] Silverstein, R.M. Clayton Bassler, G. and Morrill, T.C. 1991. Spectrometric identification of organic compounds. John Wiley & Sons, Inc, Fifth Edition, 101-131.

[19] Kohila Subathra Christy, H. Jothibai Margret, J. and Meenakshi, V.K. 2013. Infrared and gas chromatogram-mass spectral studies of the ethanolic extract of *Phallusia arabica* Savigny, 1816. Archives of Applied Science and Research, 5(4): 17-23.

[20] Wang, H.R. and Chen, K.M. 2006. Colloids and Surfaces A: Physicochem. Eng. Aspects. 281, 190.

[21] Oktay, M. Gülçin, I. and Küfrevioglu, O.I. 2003. Determination of in vitro antioxidant activity of fennel (Foeniculum vulgare) seed extracts. LWT-Food Sci Technol, 36: 263-271.

[22] Lee, C. Kim, J.Y. Lee, W.I. Nelson, K.L. Yoon, J. and Sedlak, D.L. 2008. Bactericidal Effect of Zero-Valent Iron Nano- particles on *Escherichia coli*, Environmental Science & Technology, 42(13): 4927-4933.