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Green Synthesis, Characterization and Applications of Silver Nanoparticles of Valoniopsispachynema (G. Martens) Borgesen

A.Kingslin[1], P. Ravikumar[2]
[1]*PhD Research Scholar, R&D Centre, Bharathiar University, Coimbatore, Tamilnadu, India*
[2]*Principal In -charge, Government Arts College for Men, Krishnagiri, Tamilnadu, India.*

Abstract: The synthesis, characterization and application of biologically synthesized nanomaterials are an important aspect in nanotechnology. The present study deals with the synthesis of silver nanoparticles (AgNPs) using aqueous extract of green seaweed Valoniopsispachynema extract. The reduction of silver ions occurred when silver nitrate solution was treated with aqueous extract of seaweed at40^{0}C.The colour changes in reaction mixture (pale yellow to dark brown) is observed during the incubation period, because of the formation of AgNPs in the reaction mixture enables to produce particular colour due to their specific properties. The AgNPs obtained were characterized by UV-Visible Spectroscopy, FESEM, EDAX, XRD and FTIR techniques. The formation of AgNPs is confirmed by the appearance signatory dark brown colour of the solution and a characteristic peak at 434 nm in the UV-Vis spectrum. The AgNP lattice is unaffected by other molecules in the algal extract as revealed in the XRD pattern. FESEM images revealed that the synthesized NPs are spherical with size in the range of 68.79 to103.2 nm. FTIR spectrum indicated the presence of different functional groups in capping the nanoparticles. The seedling growth was positively affected by certain concentration of AgNPs. Earthworms provided an appropriate model for evaluating the environmental hazards of metals in soil and they are also excellent organisms for studying the process of regeneration. V.pachynema extract react with AgNPs of earthworm Lampitomauritii (Kinberg) in the mortality rate of 25% and 100% for 3minutes. The antioxidant activity of synthesized nanoparticles is evaluated performing 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the radical scavenging activities were highest in the concentration 500 ml (52.90%) and lowest in 100ml (47.76%).Thus it can be use as potential free radical scavenger. The approach of algal mediated synthesis appears to be cost efficient, eco-friendly and easy alternative to conventional methods of AgNPs. The outcome of this basic research piece of work is useful to determine the biocompatibility of AgNPs of V.pachynema.

Keywords: Green synthesis, Silver nanoparticles, Valoniopsispachynema, Characterization, Seed germination, Embryonic axis length, Lampitomauritii, Antioxidant activity.

I. INTRODUCTION

Nanotechnology is a fast emerging discipline not only in physics and chemistry but also in the field of biology. In view of the tremendous scope for application of nanotechnology in various fields, there is among scientists to carry out research in this most vital discipline. There has been increasing interest in the development of clean synthetic procedures(1)for nano products targeted at biomedical applications. An environmentally acceptable solvent system and eco-friendly reducing and capping agents are three essential elements for a completely “green” nanoparticles synthesis. The biological approach to materials synthesis is ideal in this respect. In the past few years, numerous microorganisms have been applied to synthesize inorganic nanostructures either intracellular or extracellular. While a number of biological species have been found to be capable of synthesizing AgNPs, the controllability of size and shape and the understanding of the principles involved are far from satisfactory.

There is a current drive to integrate all the approaches to design environmentally benign materials and processes. Such an approach will also be of advantage for the integration of metal and alloy quantum dots into biologically relevant systems. Recently, we had suggested an eco friendly method for the synthesis and stabilization of metal nanoparticles by suitable choice of materials and solvents(1). Silver is widely known as a catalyst for the oxidation of methanol to formaldehyde and ethylene to ethylene oxide(2).Colloidal silver is of particular interest because of distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activity(3). The silver particle is an important catalyst involved (4),which is used in the oil industry.

In the fields of nano science and nanotechnology, the largest activity has been focused on the synthesis of new nanoparticles with different sizes and new shapes, which have strong effects on their widely varying properties. Nanoparticles are attracting increase

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attention due to their unusual and fascinating properties, which are strongly influenced by their size, morphology and structure(5).Nanoparticles (NPs) are small in diameter, but large in surface area, and are important to many current exclusive medical and industrial applications such as biological engineering, catalysts, and electronic devices(6). Currently, a large number of physical, chemical, biological, and hybrid methods are available to synthesize different types of nanoparticles(7). Though physical and chemical methods are more popular for nanoparticles synthesis, the use of toxic compounds limits their applications. To overcome the problem of toxicity in synthesis, safe eco-friendly green methods have a major role for producing nanoparticles (8). Several methods have been used for the green synthesis of NPs using various biological materials as reducing agents such as microorganisms, marine organisms, micro fluids and plant extracts(9-13).Among the most important bio reductants are plant extracts, which are relatively easy to handle, readily available, low cost, and have been well explored for the green synthesis of other nanomaterials(14). Moreover, the biologically active molecules involved in the green synthesis of NPs act as functionalizing ligands, making these NPs more suitable for biomedical applications (15).Thus their phyto chemical include hydroxyl, carboxyl and amino functional groups which can serve both as effective metal-reducing agents and as capping agents to provide robust coating on the metal NPsin a single step(16).

AgNPs have been widely used during the past few years in various applications due to their well-known effectiveness in biomedical(17), electronic(18), catalysis(19) and optical applications(20). Apart from these antimicrobial activities, AgNPs are also known to possess antifungal, anti-inflammatory, antiviral,anti-angiogenesis and antiplatelet properties(21,22).Additionally, more recent developments have been AgNPs used in room spray,wallpaper gloves, laundry detergent, and wall paint formulations as well as in the textile industry for clothing manufacturing(23,24).

Earthworms represent a majorcomponent of the soil biomass. They can contribute extensively to soil formation through consumption of dead plant and animal matter, mixing of the particles during digestion, depositing their casts throughout the soil column and improving aeration and drainage of the soil by burrowing. This makes them one of the most suitable bioindicator organisms for testing chemicals in soils (25). Use of specific herbicides, fungicides and insecticides in the agricultural field can be highly toxic to earthworms and they will suppress or nearly eliminate earthworm population (26). In India *L. mauritti* was chosen as an indicator species for assessment of agro ecosystem contamination (27).

V.pachynema(G.Martens)Borgesen(Domain:Eukaryota,Kingdom:Viridiplantae,Phylum:Chlorophyta,Class:Ulvophyceae,Order:Cladophorales,Family:Valoniaceae,Genus:Valoniopsis,Species:Pachynema)isgreen algae,forms a rigid, densely tufted low mat on the exposed rocks in the middle and lower littorial belt almost the whole year around(Marine algae of Northen Taiwan by Young Meng Chiang).Valoniopsis algae growing seaweed in Coastal areas of South India.

II. MATERIALS AND METHODS

A. Collection and Identification

The brown seaweed *V. pachynema* (G.Martens) Borgesen (Valoniaceae) is collected from Kanyakumari Coast, Tamilnadu, South India. Samples were brought to laboratory in polythene bags and cleaned thoroughly with fresh water to remove adhering debris and associated biota. The algae is identified and authenticated by Botanical Survey of India, Southern Region, Coimbatore 641013 with the Voucher number SI/SRC/5/23/2014-15/Tech/851 Dated 22nd August 2014 and was dried in shade at room temperature until further use.

Figure 1:Valoniopsispachynema(G.Martens) Borgesen

B. Preparation of Seaweeds Extract

The sample was washed thoroughly with Milli Q water to remove extraneous materials and washed seaweed was finely cut into small pieces and stirred with 100 mL sterile Milli Q water for 1min and kept in a water bath at 40^0C for 20 minutes. Finally the extract was filtered with Whatman filter paper no.1. The filtrate is used as reducing agent and stabilizer.

C. Biosynthesis of AgNPs

For the biosynthesis of AgNPs 50ml aqueous seaweed extract was mixed with 50ml of 1mM $AgNO_3$ solution, stirred well for 1 minute, kept in a water bath at 60^0C for 1 hour and then incubated in dark at room temperature under static condition. A control set up was also maintained without seaweed extract. The bio reduction of $AgNO_3$ into AgNPs can be confirmed visually by the change in colour from yellow to brown.

D. Characterization Techniques

1) *UV – Visible Spectroscopic Analysis:* The reduction process of the formation of AgNPs in solution was monitored on a Perkin – Elmer UV-VIS Spectrophotometer Lambda -35 to know the kinetic behaviour of the AgNPs. The reaction of the solution was analyzed at different reaction times in the wavelength ranges between 200 and 800 nm at a scan speed of 480nm/min. The Spectrophotometer was equipped with "UV Winlab" software to record and analyze data. Base line correction of the Spectrophotometer was carried out by using a blank reference. The UV –Vis absorption spectra of all the samples were recorded along with the resulting data recorded in graphical format headings were taken for all the concentrations mentioned. To study the effect of time duration on NPs formation, the reaction solution was incubated at specific time intervals of 0, 1, 6, 12 and 24 hours for each of the concentrations
2) *X-ray Diffraction Measurement (XRD):*This method is one of the most important non destructive tools to analyze all kinds of matter-ranging from fluids, to powders and crystals. XRD is an indispensable method for materials characterization and quality control. The phase evolution of cleaned powder as well as that of sintered samples was studied by X-ray diffraction technique (Philips PAN analytical, the Netherland) using Cu-Kα (= 1.54056 A^0). PAN analytical is an international scientific instrumentation and software supplier which develops and manufactures of XRD. It was formerly Philips Analytical, a division of Philips, priorto its sale in 2002.The generator voltage and current was set at 35 KV and 25 mA respectively. The Ag samples were scanned in the 2θ ranges 15 to 70^0C range in continuous scan mode. The scan rate was 0.04^0/sec
3) *Field Emission Scanning Electron Microscopy (FESEM):* FESEM was used to characterize mean particle size, morphology of the AgNPs. FESEM produces clear images with spatial resolution down to 10 nm that are electro statically less distorted (3 and 6 times better than conventional SEM). Smaller area contamination spots can be examined at electron accelerating voltages compatible with energy dispersive X–ray spectroscopy. Reduced penetration of low kinetic energy electrons probes closer to the immediate material surface. High quality, low voltage images are obtained with negligible electrical charging of samples (accelerating voltages range from 0.5 to 30 kV). The powder sample and freeze dried sample of the AgNPs solution was sonicated with distilled water; small drop of this sample was placed on glass slide allowed to dry. A thin layer of platinum was coated to make the samples conductive (Jeol. JSM-6480 L V FESEM) machine was operated at a vacuum of the order of 10-5 torr. The accelerating voltage of the microscope was kept in the range 10-20kv.
4) *Fourier Transform Infrared (FTIR) Measurement:* FTIR measurements were carried out to investigate and predict any physicochemical interactions between different components in a formulation in the dried biomass of the extract treated with $AgNO_3$ to find out the compound responsible for the synthesis of $AgNP_S$. FTIR is a measurement technique that allows one to record infrared spectra. Infrared light is guided to compounds with small energy differences in the possible vibration and rotational states. For a molecule to absorb IR, the vibrations or rotations within a molecule must cause a net change in the dipole moment of the molecule. These measurements were carried using a FTIR PERKIN ELMER instrument with a wavelength range of 400 to 4000 nm where the samples were incorporated with KBr pellets to acquire the spectra. The results were compared for shift in functional peaks.
5) *Energy Dispersive X ray (EDX) Analysis observation of AgNPs:* EDX was carried using JEOL 2100 High Transmission Electron Microscope to confirm the presence of Ag in the particles as well as to detect other elementary compositions of the particles
6) *Particle Size Analysis (Diffuse Light Scattering (DLS) method):*A laser diffraction method with a multiple scattering technique has been used to determine the particle size distribution of the powder. It was based on Mie-scattering theory (Thiele and French, 1998). This theory provides vigorous solutions for light scattering by an isotropic sphere embedded in a homogeneous

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medium. In order to find out the particles size distribution the Ag powder was dispersed in water by horn type ultrasonic processor (Vibronics, VPLPI). The data on particle size distribution were extracted in Zeta sizer version 6.20 Mall052893, Malvern Instruments.

E. Applications

1) *Seed Germination Assay (International seed testing Association(ISTA)Rules(2014):*Experiment was made to evaluate the effect of AgNPs V.pachynema on the germ inability of the candidate seeds (Vignaunguiculata(L.) Walp, Vignaradiata (L.) R.Wilczek and Cicerarietinum (L.) in a completely randomized design with duplicates as per ISTA Rules 2014. The treatments in the experiment were taken in 25%, 50%, 75% and 100% concentration of AgNPs. This setup was kept in room temperature of 28^0C for 24 hrs. Before the treatment the seeds were pre-soaked in AgNPs solution for 12 hr
2) *Embryonic Axis Length (EAL) Study:*The embryonic axis length, fresh and dry weight of the embryonic axis and % increase /decrease over the control germinated seeds of V. unguiclata, V. radiata and C. arietinum were calculated in 25%, 50%, 75% and 100% concentrated AgNPs solution of V.pachynema.
3) *Toxicology Study Of LampitomauritiiL.* The AgNPs was allowed to interact with earthworm, *L. mauritii* at 25%, 50%, 75% and 100% concentrations of AgNPs solution and incubated at a room temperature of 28^0C. The time taken for the earthworm's mortality was noted and tabulated. Methanol, Ethanol, Recitified sprit and silver nitrate solution served as controls.
4) *Antioxidant activity*

B. Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Activity.

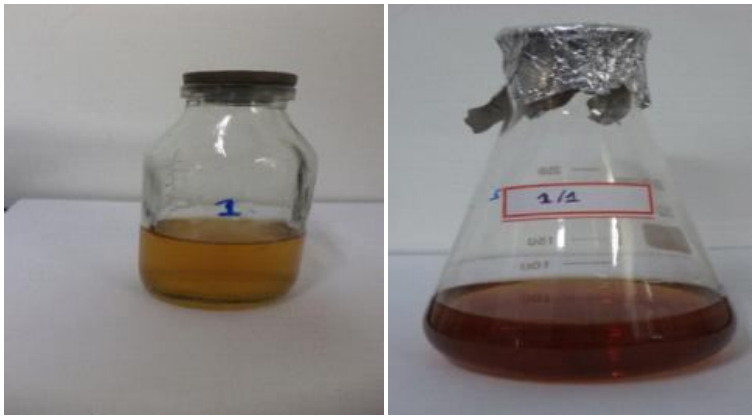
The DPPH radical scavenging activity of different concentration (100, 200, 300, 400 and 500 µg/ml/OD value) of green synthesized AgNPs of *V.pachynema* was measured according to the method described earlier by Kulkarni*et al. (*2004). The test samples (100-500mL) were mixed with 0.8mL of Tris-HCL buffer (pH 7.4) to which 1mL DPPH (500 mM in ethanol) was added. The mixture was shaken vigorously and left to stand for 30min. Absorbance of the resulting solution was measured at 517 nm in a UV-Visible Spectrophotometer (UV-160A; Shimadzu Co.). Radical scavenging potential was expressed as IC50 value, which represents the concentration, which scavenged 50% of the DPPH radicals.

III. RESULTS AND DISCUSSION

A. Visual and UV –Vis Spectrophotometer Analysis

The formation of AgNPs is confirmed through visual assessment. The reaction mixture turned to dark brown colour from brownish yellow colour within 20 min indicated the synthesis of AgNPs(Figure 2)

Figure 2: Colour intensity of the *V. pachynema* extract incubated with Silver ions



a) Before the synthesis of AgNPs(b)after synthesis of AgNPs

The appearance of dark brown colour may be due to the excitation of surface plasmon resonance (SPR) effect and reduction of $AgNO_3$(28).UV-Vis spectrum of reaction mixture at different wavelengths ranging from 213to 434 nm showed strong absorption peak with centering at 434 nm(Figure3)indicated the formation of AgNPs formed by different methods(29). The wide absorption peak may be induced by the wide size distribution of AgNPs.

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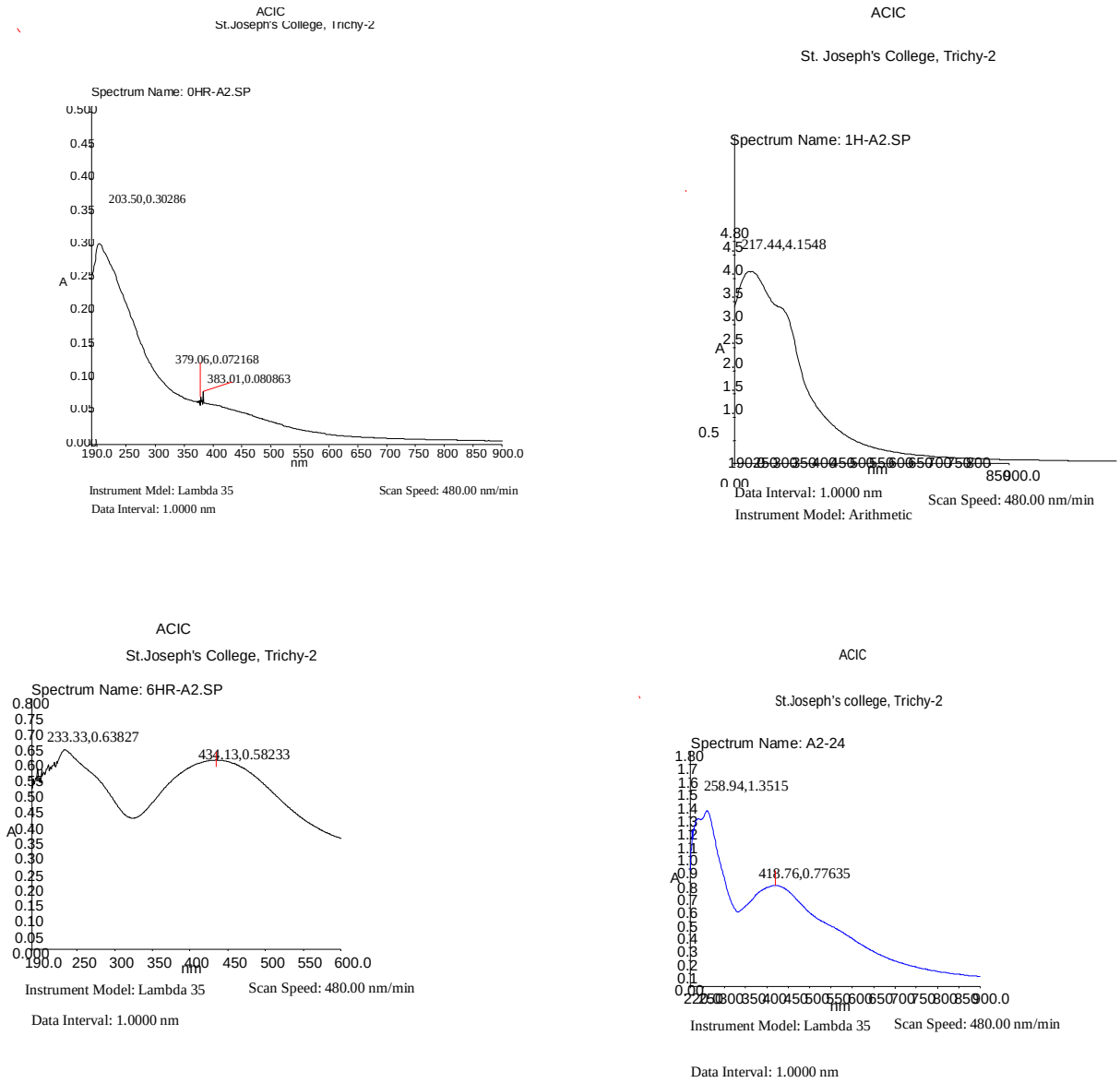
Figure 3:UV – Visible Spectrum of AgNPs formed using *V.pachynema*



The frequency and width of the surface plasmon absorption depends on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium (30-32).

B. Field Emission Scanning Electron Microscopy(FESEM):

FESEM has been used to identify the size, shape and morphology of NP_S. The morphology and size of the green synthesized AgNPs were studied by FESEM (Fig.4) shows as synthesized NPs were spherical in morphology with a size range of 68.79 to 103.2 nm in which few NPs were agglomerated. The agglomeration of NPs decreased as the algal extract concentration was increased which is also evidenced by the UV-Vis Spectroscopy results. Control of the size and structure of the resultant nanoparticles can be related to the interactions between bio-compounds such as polysaccharides, proteins, polyphenols and phenolic compounds and metal atoms (30).

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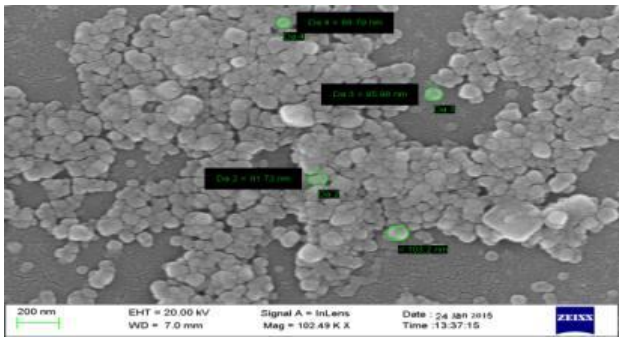
Figure 4: FESEM images of AgNPs at different magnifications



C. Energy dispersive X-ray Spectroscopy (EDX)analysis of Silver nanoparticles:

The elemental composition of the green synthesized sample was also determined by EDX on the FESEM. Analysis through EDX spectrometers confirmed the presence of elemental silver signal of the AgNPs(Fig5). The intense signal at 3kev strongly suggests that Ag was the element, which has an optical absorption in this range due to the surface plasmon resonance (SPR). Notably, the other signals in the range of 0-4kev represent the typical absorption of Chlorine, Potassium, Calcium, Sodium, Oxygen, Magnesium, Silver and Aluminium thus indicates the presence the algal extract(as a capping ligand) on the surfaces of the NPs. Identification lines for the major emission energies for silver (Ag) are displayed and there correspond with peaks in the spectrum, thus giving confidence that silver has been correctly identified.

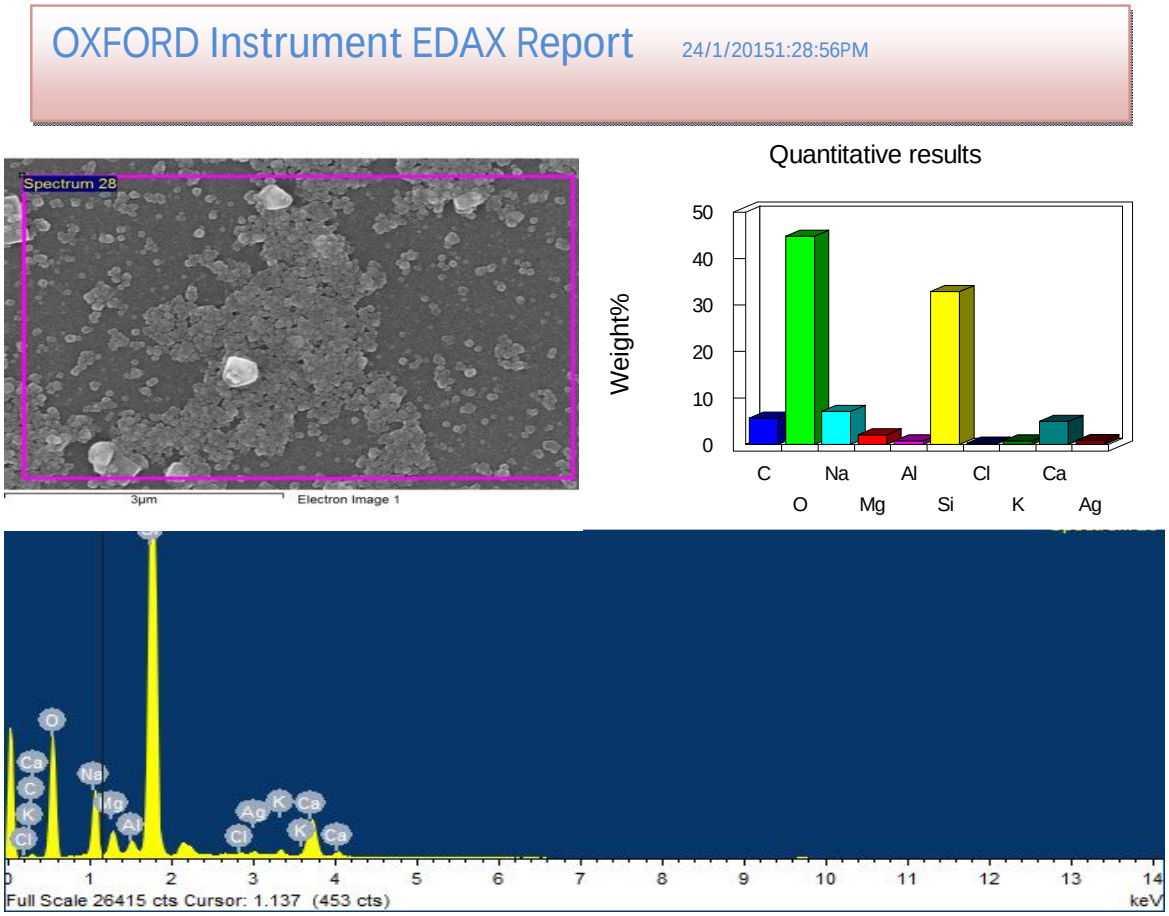


Figure 5:EDX spectrum of synthesized AgNPs

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D. Particle size Analyzer (Diffuse light scattering, DLS method)

A laser diffraction method with a multiple scattering technique has been used to determine the particle size distribution of the powder. It was based on Mie-Scattering theory (Thiele and French,1998).The data on particle size distribution were extracted in Zetasizer Ver.6.20 (Mal1052893, Malvern instruments) (Tables 1&2) and (Fig.6).

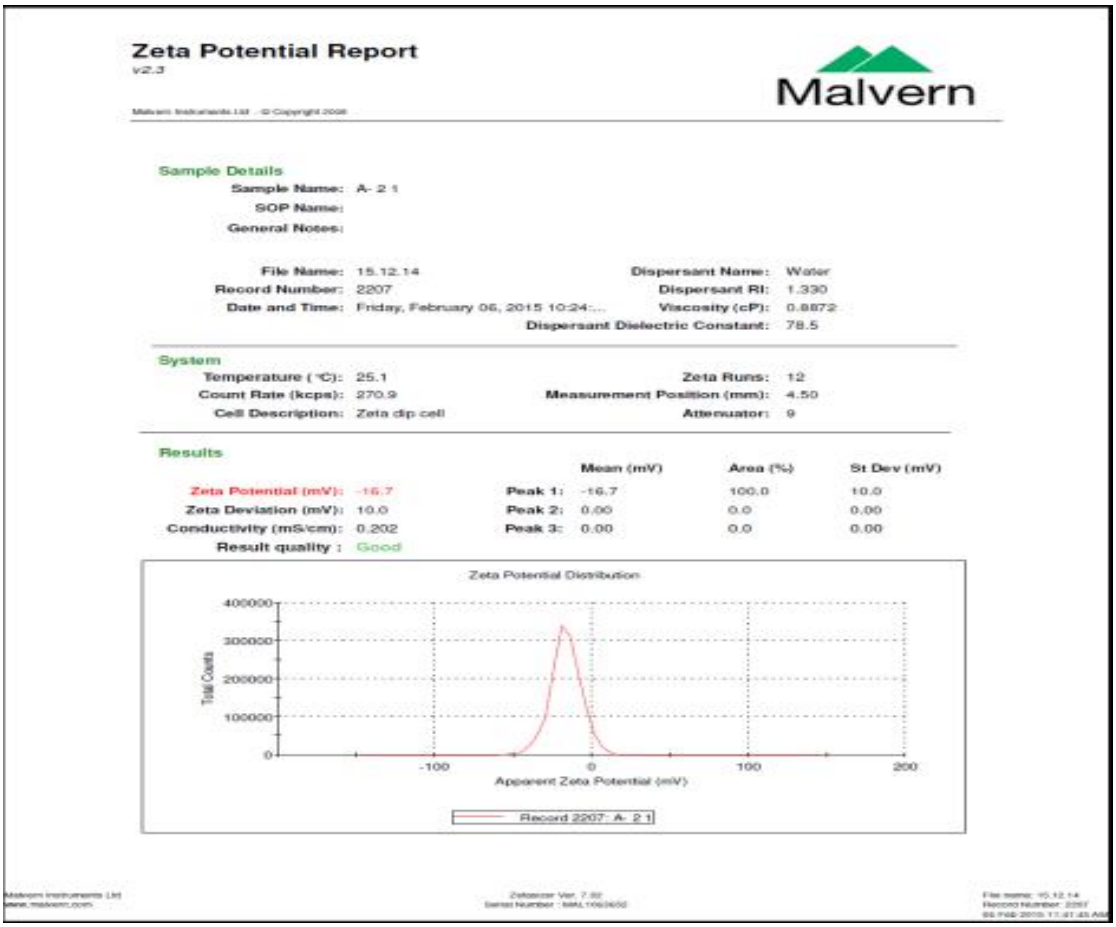
Table 1: Summary of particle size analysis (Ref. Figures)

Record No	Count rate (kcps)	Z-Average (d.nm)	Size (d.nm)	Intensity (%)	St Dev (d.nm)	Pdl / Intercept
1	311.4	100.2	115.9	98.2	40.94	0.344/0.870
2	317.3	104.3	140.7	100.0	64.54	0.283/0.867
3	309.1	105.5	152.3	100.0	77.33	0.284/0.870

Table 2: Summary of Zeta Potential analysis

Record No	Count rate (kcps)	Z-Potential (mV)	Mean (mV)	Area (%)	St Dev (mV)	Z-Deviation (mV)	Conductivity (ms/cm)
1	270.9	-16.7	-16.7	100.0	10.0	10.0	0.202

Figure 6: Zeta Potential



E. XRD analysis of Silver nanoparticles

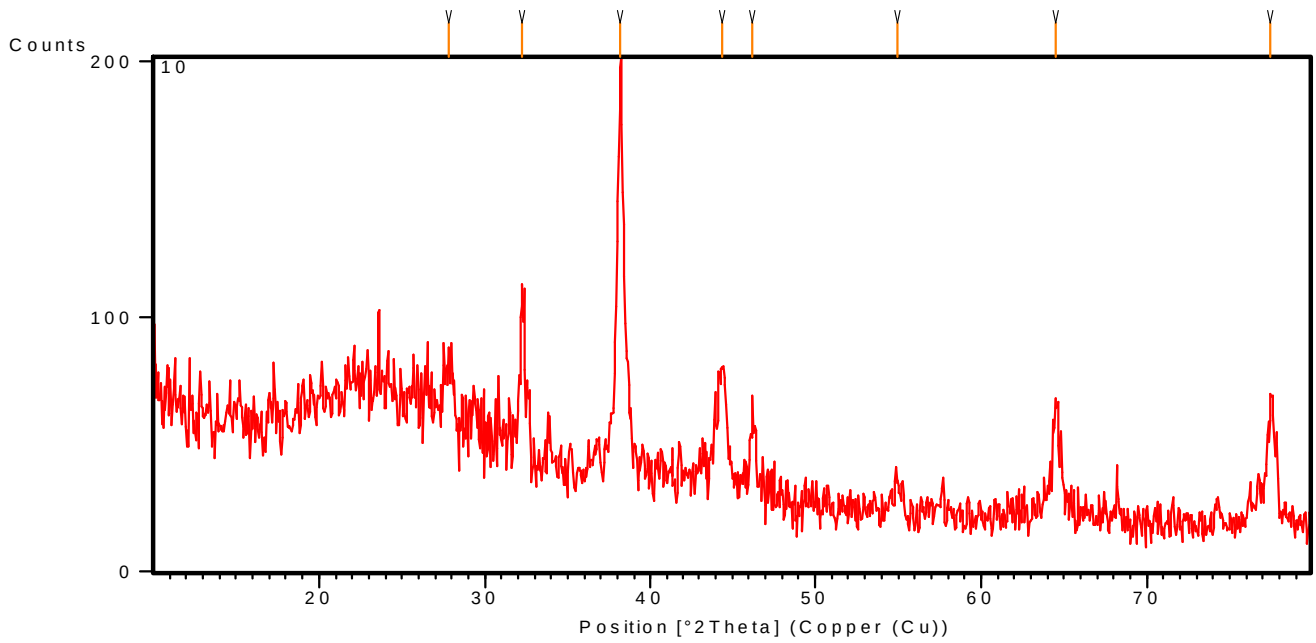
The crystalline nature of AgNPs was further confirmed from X-ray diffraction (XRD) analysis. The XRD spectrum (Fig.7) indicates the face –centered cubic structure of the AgNPs. There are six distinct reflections in the diffractogram at 32.26^0(45), 38.16^0(109),

44.4^0(30), 46.23^0(20), 64.50^0(26) and 77.47^0(32). The intense reflection at 109, in comparison to the other five, may indicate the growth direction of the nanocrystals. On the basis of the half-width (^) of the 109 reflection in the powder pattern, the average grain size, L-determined by broadening of the 109 reflection by the Debye – Scherer formula

$$D=K\lambda/\beta\cos\Theta$$

Where K is the Scherrer constant and its value is .094, λ is the wavelength of the X-ray, β is the full width at half maximum and Θ is the Bragg angle. From the Scherrer equation the average crystalline size of $AgNP_S$ is found to be 42nm. The absence of any additional reflections other than the reflections belonging to the Ag lattice clearly suggests that the green synthesized AgNPs lattice was unaffected by other molecules in the algal extract

Figure 7: XRD pattern of AgNPs



Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
28(1)	15(110)	0.6(2)	3.20647	13.98
32.26(3)	45(28)	0.3(3)	2.77279	40.78
38.16(3)	109(83)	0.4(3)	2.35665	100.00
44.4(3)	30(78)	1(1)	2.04074	27.68
46.23(4)	20(16)	0.3(1)	1.96232	18.64
54.9(3)	8(53)	1(2)	1.67024	7.71
64.50(4)	26(29)	0.4(1)	1.44351	23.57
77.47(3)	32(28)	0.5(2)	1.23107	29.52

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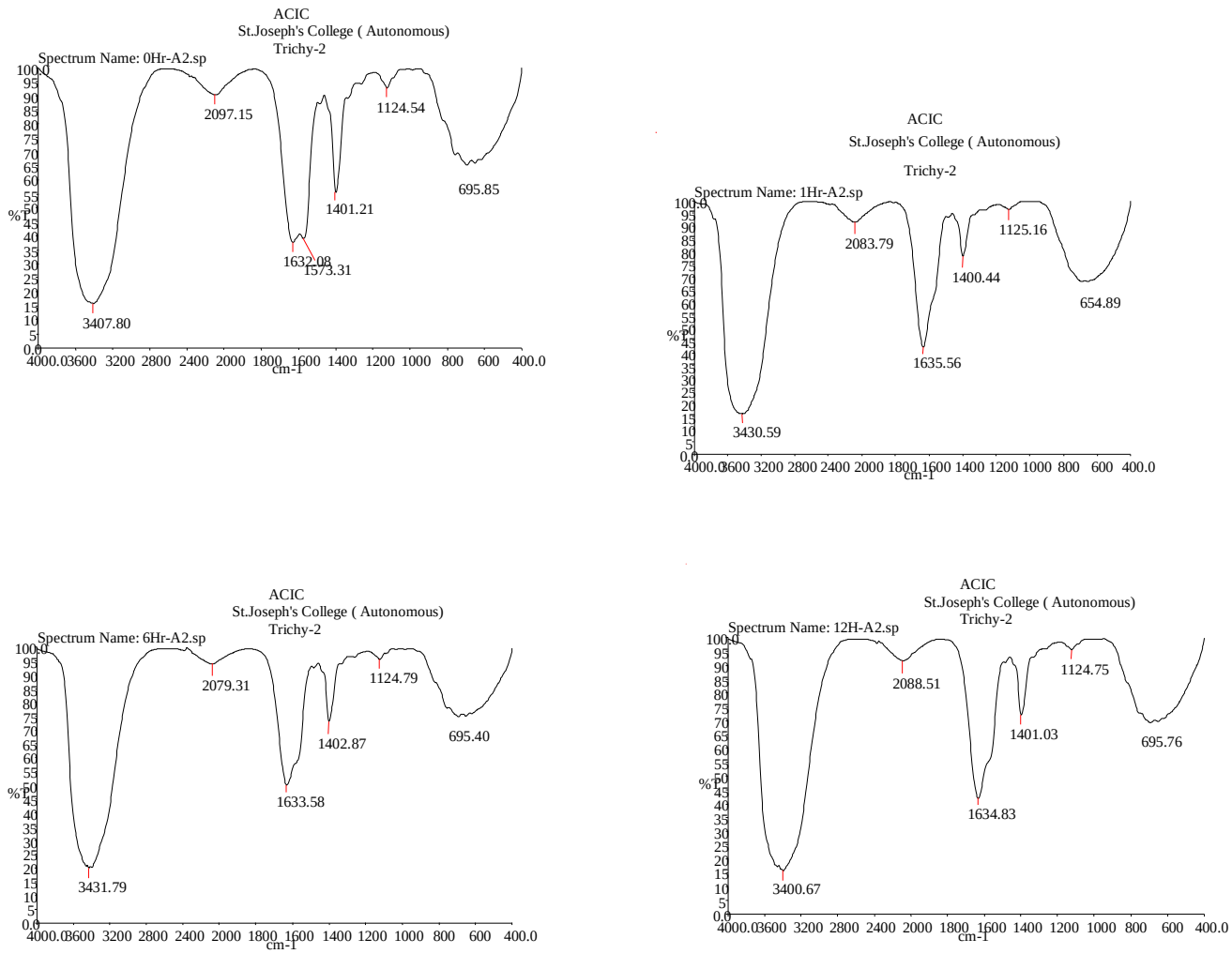
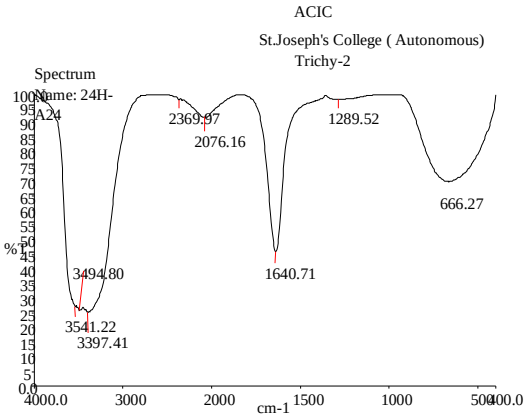
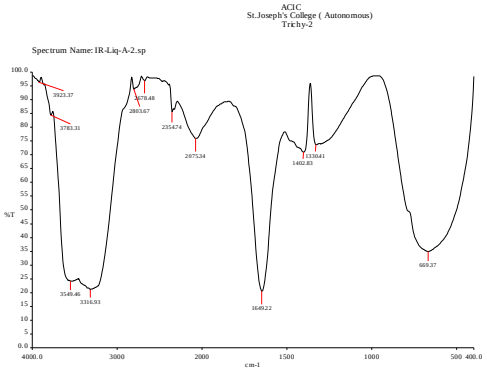
F. FTIR Analysis of Silver Nanoparticles:

FTIR measurements were carried out to identify the possible bimolecule in V.pachynemaextract which a responsible for capping leading to efficient stabilization of the AgNPs. The IR spectrum (Fig.8) of AgNPs synthesized using V.pachynema manifests prominent absorption bands located at 3923, 3783, 3549, 3316, 2803, 2678, 2354, 2075, 1649,1402, 1330,669cm^{-1}. The broad spectrum at 3929cm^{-1} shows the O-H stretch, free hydroxyl of alcohols and Phenols. The very strong absorption band at 3549cm^{-1} corresponds to the N-H stretch of amides and that at 1649cm^{-1} corresponds to the NH bend of primary amines.IR spectroscopic study confirmed that the V.pachynema extract has the ability to perform dual functions of reduction and stabilization of silver nanoparticles.

Figure8:FTIRAnalysis







Summary of FTIR analysis (Ref. Figures)

S.N	Hour of reaction	frequency, cm–1	Band	functional group
1	0	3908.15 – 97.51	O–H stretch, free hydroxyl	alcohols, phenols
		3434.33 – 12.60	O–H stretch, H–bonded	alcohols, phenols
		2362.81 – 91.49	C(triple bond)N stretch	Nitriles
		2081.61 – 86.68	–C(triple bond)C– stretch	Alkynes
		1636.44 – 33.84	–C=C– stretch	Alkenes
		1383.76 – 94.95	C–H rock	Alkanes
		670.22 – 64.29	C–Br stretch	alkyl halides
2	1	3909.46 – 98.57	O–H stretch, free hydroxyl	alcohols, phenols
		3435.42 – 12.60	N–H stretch	Amides
		2362.74 – 96.38	C(triple bond)N stretch	Nitriles
		2338.25 – 96.45	H–C=O: C–H stretch	Aldehydes
		1636.34 – 40.68	N–H bend	primary amines
		1384.09 – 96.20	C–H bend	Alkanes
		666.95 – 66.08	–CC–H: C–H bend	Alkynes
	6	3435.41 – 15.86	N–H stretch	Amides
		2363.98 – 91.82	H–C=O: C–H stretch	Aldehydes
		2337.88 – 92.59	C(triple bond)N stretch	Nitriles
		1636.78 – 38.86	–C=C– stretch	Alkenes
		1385.13 – 95.55	C–H rock	Alkanes
		672.81 – 66.39	C–Br stretch	alkyl halides
4	12	3898.10 – 70.28	O–H stretch, free hydroxyl	alcohols, phenols
		3772.78 – 56.58	O–H stretch, H–bonded	alcohols, phenols
		3452.95 – 15.84	N–H stretch	Amides
		2938.91 – 38.41	C–H stretch	Alkanes
		2732.67 – 45.16	H–C=O: C–H stretch	Aldehydes
		2624.49 – 46.56	H–C=O: C–H stretch	Aldehydes
		2517.87 – 46.91	C–H stretch	Alkanes
		2359.33 – 35.34	C(triple bond)N stretch	Nitriles
		2205.34 – 48.40	–C(triple bond)C– stretch	Alkynes
		2104.73 – 48.55	–C(triple bond)C– stretch	Alkynes
		1618.38 – 42.18	N–H bend	primary amines

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		1390.37 – 57.22	C–H bend	Alkanes
		1051.58 – 57.01	C–N stretch	aliphatic amines
		663.41 – 48.71	N–H wag	secondary amines
5	24	3436.41 – 15.87	N–H stretch	Amides
		2361.05 – 94.67	C(triple bond)N stretch	Nitriles
		2074.39 – 92.01	–C(triple bond)C– stretch	Alkynes
		1636.58 – 46.66	–C=C– stretch	Alkenes
		1384.10 – 96.47	C–H rock	Alkanes
		669.72 – 72.68	C–Br stretch	alkyl halides
6	Px	3910.71 – 97.57	O–H stretch, free hydroxyl	alcohols, phenols
		3436.02 – 3.17	N–H stretch	Amides
		2363.68 – 94.54	H–C=O: C–H stretch	Aldehydes
		2336.45 – 94.63	C(triple bond)N stretch	Nitriles
		2074.74 – 84.92	C=O stretch	carbonyls
		1636.20 – 22.59	N–H bend	primary amines
		1384.03 – 94.20	C–H rock	Alkanes
		670.81 – 55.39	C–Br stretch	alkyl halides
7	$AgNO_3$	3435.01 – 15.85	N–H stretch	Amides
		2362.75 – 96.65	C(triple bond)N stretch	Nitriles
		2339.24 – 96.89	–C(triple bond)C– stretch	Alkynes
		2076.02 – 92.94	–C(triple bond)C– stretch	Alkynes
		1639.38 – 48.20	N–H bend	primary amines
		1384.29 – 96.73	C–H bend	Alkanes

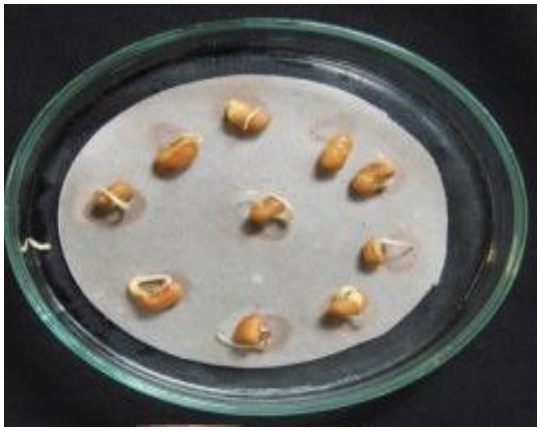
IV. APPLICATIONS

A. Seed Germination Assay

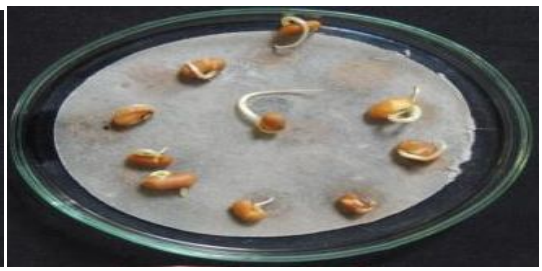
The highest germination percentage and germination rate were observed at concentration (Table.3) (Fig.9). Based on studies of NP_S effects on seed germination mechanism; it is possible that NP_S increase water absorption by seeds (31). The impact of AgNPs root length and shoot length, exposure to specific concentrations of AgNPs could enhance germination compared with non-exposed plants, whereas higher and lower concentrations could affect growth negatively (32).

Figure9:Seed Germination Assay
Vignaunguiculata (L.)Walp

25% *50%*

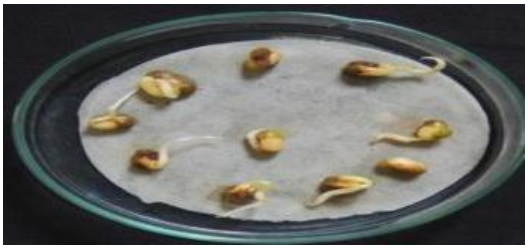


75%100%



Vignaradiata (L.)R.Wilczek

25%



50%

75%

100%

Cicerarietinum (L.)

25%

50%

75%

100%

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Table - 3 -Seed germination assay

Algal name	Seed name	Concentration	Germination (%)	Increase / Decrease over C
V.pachynema	Vignaunguiculata(L.) Walp	Control	60	
		25%	90	+ 50
		50%	70	+ 16.6
		75%	80	+33.3
		100%	90	+ 50
	Vignaradiata (L.)R.Wilczek	Control	80	
		25%	10	-87.5
		50%	80	0
		75%	80	0
		100%	80	0
	Cicerarietinum(L.)	Control	50	
		25%	80	+60
		50%	40	-20
		75%	60	+20
		100%	50	0

B. Embryonic axis length study

Embryonic axis length observed at different concentration (Table - 4). Exposure to specific concentrations of AgNPs could enhance plant growth compared with non-exposed plants, whereas higher and lower concentrations could affect growth negatively (33).

Table - 4 – Embryonicaxis length study

Seed	Conc. of NPs/ Mean	EAL cm Mean	% Increase / Decrease of EAL over C	FWt g Mean	DWt g Mean	Water g Mean	% Increase / Decrease of DWt over C
Vignaunguiculata(L.)Walp	Control	3.5		0.150	0.062	0.088	
	25%	3.06	+12.57	0.172	0.059	0.113	-0.48
	50%	2.62	-25.14	0.129	0.052	0.077	-16.12
	75%	2.26	-35.42	0.196	0.048	0.148	-22.58
	100%	1.9	-45.71	0.128	0.057	0.071	-8.06
Vignaradiata (L.) R.Wilczek var.	Control	2.96		0.175	0.035	0.140	
	25%	2.26	-23.64	0.175	0.045	0.130	+28.57
	50%	2.92	-1.35	0.142	0.041	0.101	+17.14
	75%	2.24	-24.32	0.138	0.041	0.097	+17.14
	100%	2.48	-16.21	0.106	0.038	0.068	+8.57
Cicerarietinum (L.)	Control	1.9		0.585	0.252	0.333	
	25%	2.54	+33.68	0.721	0.334	0.387	+32.53
	50%	2.16	+13.68	0.743	0.340	0.403	+34.92
	75%	2.6	+36.84	0.553	0.253	0.300	+0.39
	100%	1.96	+3.15	0.579	0.277	0.302	+9.92

C. Toxicology study of Lampito Mauritii

Earthworms provided an appropriate model for evaluating the environmental hazards of metals in soil and they are also excellent organisms for studying the process of regeneration. *V.pachynema* extract react with AgNPs of earthworm *Lampitomauritii* (Kinberg) in the mortality rate of 25% and 100% for 3minutes.(Table - 5) and (Fig.10).

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Figure 10: Earthworm control

25%50%

75%

100%

Table - 5 - Earthworm

Algal name	Death time (min)				
	25%	50%	75%	100%	C
V.pachynema	3	2.25	4	3	110

Solvent death rate

S.No	Name	Death time (min)
1	Methanol	1.39
2	Sprit	2.05
3	Ethanol	2.25
4	$AgNO_3$	3

D. Antioxidant Activity

The antioxidant activity of silver nanoparticles from *V.pachynema* provides the great expectations in pathogenic microbe controlling in advance technology. The silver nanoparticles were reacted with concentration decreased level (Table.6) and (Fig.11).The observed scavenging effect AgNPs and standard on the total antioxidant activity decreases.

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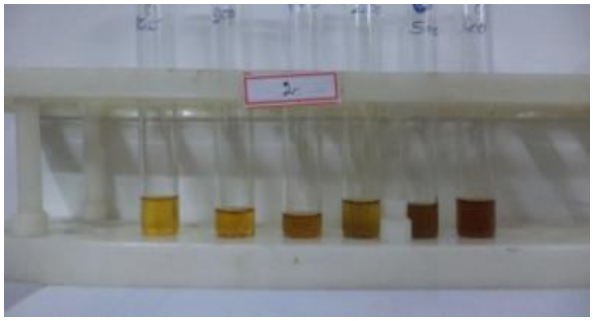
Figure 11: Antioxidant Activity

Control



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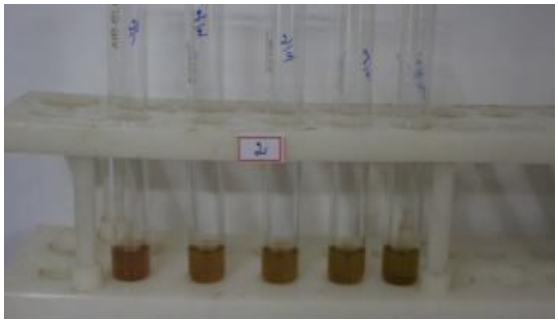

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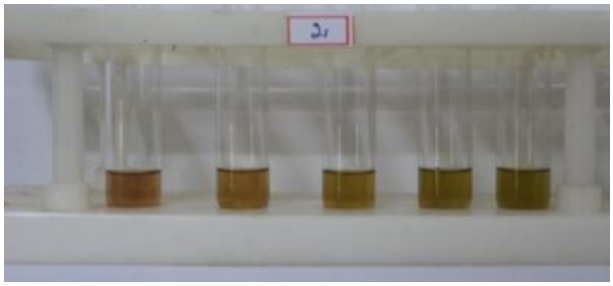

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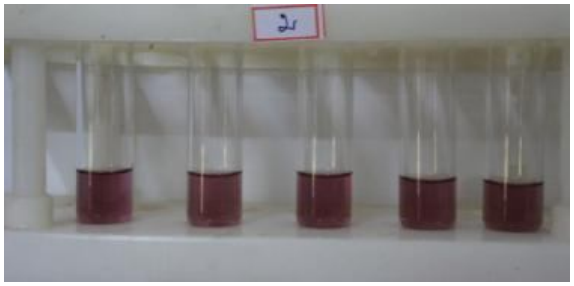

Table - 6 - Antioxidant activity

Algae no	Components	Concentration (µg/ml) / OD value					
		100	200	300	400	500	IC50
1	AgNPs	1.122	1.078	1.061	0.057	1.011	1.065
	%	47.76	49.81	50.60	50.79	52.90	50.37
	Extract	1.331	1.271	1.170	1.137	1.118	1.194
	%	38.03	43.34	45.53	47.06	47.95	44.38
	C	2.148					

V. CONCLUSION

This work clearly shows that the possibility of enhancement in the application of alga to the synthesis of AgNPs. In the present study, we have reported a simple biological process for synthesizing AgNPs using alga V.pachynema. In this study, a simple, ecofriendly and economic biological procedure has been developed to synthesize AgNPs. We have characterized the synthesized AgNPs using several techniques. The characteristic absorption peak at 434nm in UV-Visible spectrum confirmed the formation of AgNPs. The biosynthesized AgNPs one expected to have remarkable applications in pharmaceutical and biomedical fields. Such cheap source of material given an opportunity to cost-effective preparation of various silver-based nanostructures. Crystalline nature of AgNPs is evident from the characteristic peaks in the XRD pattern. FESEM images revealed that the synthesized NP_S are nearly spherical with size in the range 68.79-103.2nm. The Characteristic peaks in the FTIR spectrum revealed the presence of alcohols and phenols in seaweed extract which is responsible for the formation of AgNPs. Seaweeds are cost-effective, renewable marine resources. Their abundance and case of availability also make them good green reagents for the green synthesis of novel $AgNP_S$. Exposure to non-materials can encourage earlier seed germination and improve plant production our results indicated that exposure to AgNPs had significant effect. Hence the present study aims to establish a novel environmentally safe method for the preparation of AgNPs using aqueous extract of brown seaweed V.pachynema. The seaweed extract has a dual effect as it act as reducing agent of silver ions and as stabilizing agents for the formed AgNPs had significant effescts on the seed germination and embryonic axis length study of V. unguiculata, V. radiate and C. arietinum. The outcomes of this study useful for determining the biocompatibility of AgNPs.

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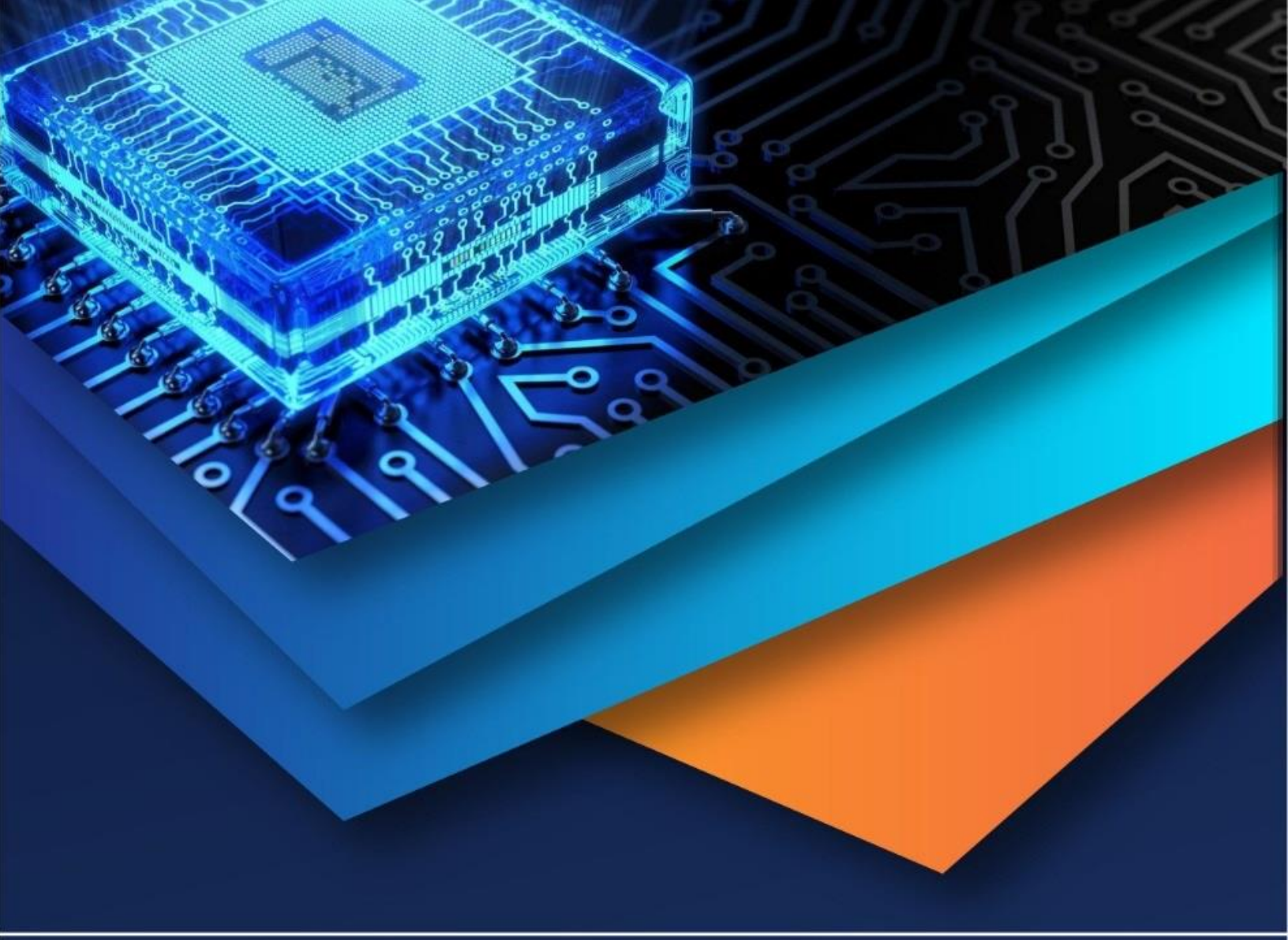

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