Green Synthesis of Silver Nanoparticles Using Aqueous extract of *Selaginella moellendorffii,* a Himalayan Pteridophyte and its Catalytic activities

Thesis submitted in partial fulfilment of requirement

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In

BIOCHEMISTRY

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Above all, I express my indebtedness to **Almighty God** whose blessings helped me to complete my work successfully.

Eiyaa
13/08/18

DECLARATION

I hereby declare that the work being presented in the dissertation entitled "Green Synthesis of Silver Nanoparticles Using Aqueous extract of *Selaginella moellendorffii,* a Himalayan Pteridophyte and its Catalytic activities" in partial fulfilment of the requirements for the award of the degree of Masters of Biochemistry, School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala is my own work during the period of January to June 2018, under the supervision of Dr. Diptiman Choudhury. My thesis has not previously formed the basis for award of any degree, or other similar title or recognition.

Date: 13/08/2018

13/8/18
Riya Gupta

Place: Patiala

This is to certify the above statement made by the candidate is correct and true to the best of our knowledge.

13/8/18

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CERTIFICATE

This is to certify that the thesis entitled "Green Synthesis of Silver Nanoparticles Using Aqueous extract of *Selaginella moellendorffii,* a Himalayan Pteridophyte and its Catalytic activities" by Riya Gupta (301607008) submitted to School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala for the Degree of Master of Science is a record of bonafide research work, carried out by her under my supervision. I believe that the thesis fulfils part of the requirements for the award of Master of Science. The results in the thesis have not been submitted for the award of any other degree.

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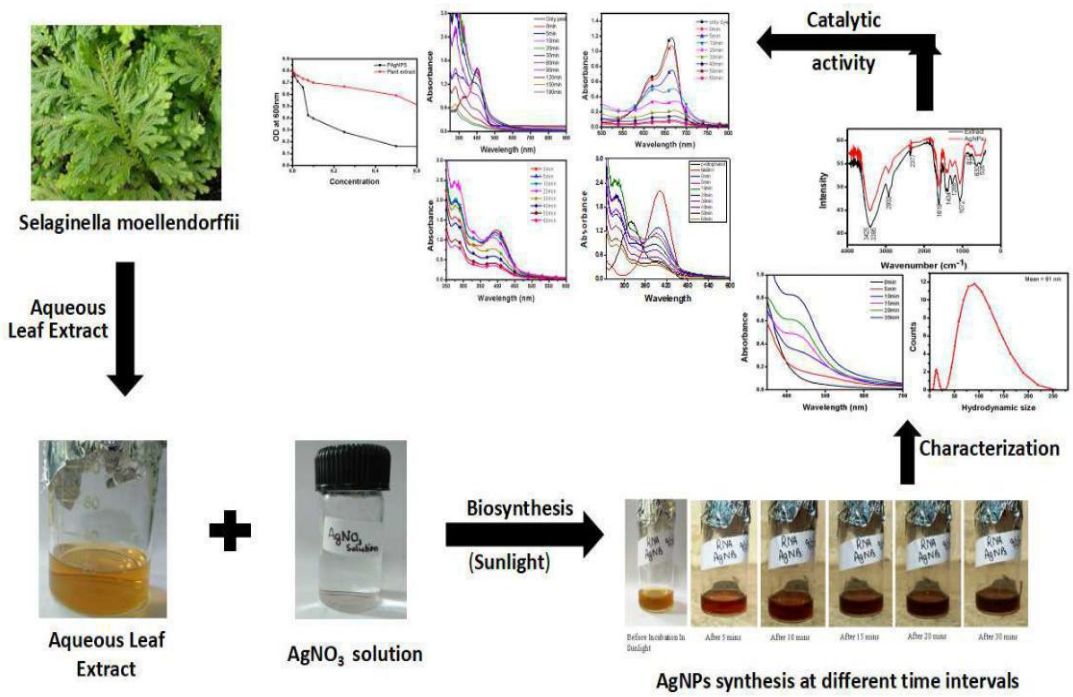
ABSTRACT

Nanotechnology is presently a developing field that provides various novel ways to form the nanoparticles also by exploring the biological sources. Different fractional extracts were extracted from the plant leaves. The present study focuses on different fractions, aqueous, methanolic, pet-ether and chloroform extract preparation. Aqueous extract is used for the AgNPs biosynthesis using the plant aqueous extract as reducing, capping and as stabilizing agent. Ag^{+} ions when exposed to *Selaginella moellendorffii* plant leaf extract were reduced and resulted in a colour change, indicating the formation of AgNPs. The AgNPs synthesis was confirmed by UV-Vis Spectrophotometer, FTIR, DLS and Zeta Potential. The absorption peak was observed at 420 nm. From the DLS study we came to know that the hydrodynamic size of the synthesized AgNPs is 91 nm in counts and the stability of the nanoparticles in the solution, whereas the FTIR analysis shows that the functional groups like N-H, O-H, C-H, C-N, and C-X are present in the aqueous plant extract which undergoes shift in the peak position after the formation of AgNPs. Finally, Qualitative analysis was done to confirm the presence of various phytochemicals and it was analyzed that the chemical compounds like saponins, tannins, phenolic compounds, alkaloids, steroids and glycosides are present in the plant extract. Various catalytic activities were observed for *Selaginella moellendorffii* as it showed 90.3% degradation of pesticide, 73.7% degradation of p-nitro phenol, 97.2% degradation of Rhodamine B dye and 94.1% degradation of Methylene Blue dye which was seen with the PAgNPs at different time intervals. The antibacterial activity of PAgNPs and aqueous extract was seen with MIC method against gram negative bacteria. Thus the IC_{50} for PAgNPs and plant extract is 3.3 and 5.1 µg/ ml respectively.

Graphical Abstract



OBJECTIVES

Following are the specific objectives of this work:

- To synthesize and characterize *Selaginella moellendorffii* plant in different fractions of solvents.
- To analyze different fraction of the solvents qualitatively to determine its photochemical composition.
- Green synthesis of Silver nanoparticles using plant extracts and their characterization.
- Study of its anti-microbial activity.
- Degradation of pesticides and various industrial dyes and nitro compounds to study its catalytic activity.

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CHAPTER 1 INTRODUCTION

In the modern material science the area of the research that is most active is the nanotechnology field. Nanotechnology is termed as the fabrication, characterization, exploration and the application of the nanosized (1-100nm) materials for the science development. It deals with the study of the minute structures and the prefix “nano” is a Greek word which means “dwarf”.

By controlling the size of the materials , the properties of the materials can be engineered by the nanotechnology as nanotechnology has the ability to engineer them and it has been spread to a number of areas that includes biomedical services , food , cosmetics , catalysis , drug gene delivery , environmental health , health care , photo-electrochemical applications , optics etc .

In the field of the medicine, treatment of the water, catalysis and solar energy the nanomaterials they have been a solution to many of the environmental and to the technological challenges in this field[1]. Therefore, the increasing nanomaterials demand should be accompanied by the method of the green synthesis in order to reduce the harmful wastes.

The biosynthesis of the nanoparticles is an eco-friendly, cost-effective, reliable and an important aspect of the green chemistry approach. Microbes and the plants with the good surface area and size are also supportive in the biosynthesis of the nanoparticles and they are in fact efficient fabricators of the nanoparticles that confer a large range of biological properties such as antimicrobial, anticancer, anti-biofuelin, anti-oxidant, anti-parasitic, anti-malarial, etc[2,3].

The“Nanoparticle” is a term that is used to describe the particles that are having a size of 1-100nm, atleast for the one dimension. Nanoparticles of the ideal size can be used as building blocks in the nanotechnology[4]. The nano-structured materials, nanosized particles, nanoscale particles, nano-objects, nanomaterials, nanosized materials are the various terms that are used to describe the nanoparticles[5]. Because of the diverse chemical nature of the metals, polymers, silicates, biomolecules, organics, metal oxides, non-oxide ceramics and carbon they are the most common materials from which the nanoparticles they can be made and these materials they are present in different forms such as in the form of the platelets, spheres , tubes and in the cylindrical form and show various different properties such as biological , physical and chemical properties as compared to their bulk materials[4]. Because of their various applications in the technology and their captivating properties the nanomaterials they are in a great demand by the scientists. The nanoparticles as compared to their bulk materials exhibit high mechanical strength and lower melting point due to the crystal defects[6].

The nanoparticles they can be classified according to their dimensionality, composition, agglomeration and uniformity[7]. They are classified into three types according to the dimensionality – one, two and three-dimension nanoparticles and

into two types on their basis of synthesis and their source of origin: Natural and Engineered Nanoparticles [8,9] . From the previous studies it was reported that the nanoparticles they are present in the nature and there they can be synthesized naturally by the process of bio-degradation and bio mineralization process and they can also be generated under in-vivo conditions naturally by the processes like soil erosion , weathering of rocks , volcanic eruptions and forest fires etc and are also produced by the various activities by the humans like fuel combustion , automobiles exhaust and industrial effluents etc and they are known as incidental nanoparticles[10]. Chemically the nanoparticles can be synthesized by various conventional methods. The Engineered nanoparticles they are classified into three types: Carbon based Organic and Inorganic nanoparticles.

The biological synthesis route for the various metal nanoparticles by the plants or their extracts is more eco-friendly and it allows the controlled nanoparticles synthesis with well defined shape and their size[11] .

Because of the high fraction of the surface atoms and high surface area the nanomaterials of noble metals like Ag, Au and the platinum have many applications in the magnetic, electronics, information storage, optoelectronics and the antibacterial properties[12,13].

Silver Nanoparticles (AgNPs) are the most important members of metal nanoparticles. Silver (Ag) it is a white lustrous element and the Silver chloride and $AgNO_3$ are the metallic salts of the silver and they are soluble in water but the metallic silver it is insoluble in water[14]. Various properties like catalytic activity, non-linear optical behaviour, electrical conductivity, antibacterial effects and chemical stability are displayed by the metallic silver[15]. Due to the easy silver nanoparticles synthesis and its chemical modifications they are highly unique in the nanoscale system and the AgNPs they are widely used in the areas of medicine, electronics and material science etc for the development of the new technologies[16].

The silver nanoparticles synthesis can be done by two approaches: Top-down (Physical method) and Bottom-up (Chemical method and via green synthesis) approach[17].

The nanoparticles produced by the physical processes have low thermal stability and have short shelf life as a result of the short shelf life of the nanoparticles that are produced makes the addition of the capping agents indispensable[18]. The Chemical processes for the production of the silver nanoparticles require the use of the explosive solvents, multiple purifications, use of the high precised equipments, high consumption for the processes such as micro fluidization and the harmful effects of the by-products formed during this process[19]. Therefore the chemical and physical methods for the silver nanoparticles synthesis are toxic as well as they are very expensive so there is an increasing demand for the silver nanoparticles synthesis by green synthesis method as it avails a feasible alternative[20] .

The biological methods for AgNPs synthesis proved to be a boon to the nanotechnology field. The nanoparticles that were produced by this method have a larger stability and shelf life as natural capping it takes place and this method is a single step, cost - effective and it causes easy downstream processing of the nanoparticles produced. For the synthesis of the nanoparticles the plants they are considered as the green nano -factories[21]. The main advantage of using the plants and their extracts for biosynthesis of AgNPs is because of their ease of availability; they are non toxic and safe and consist of a large number of metabolites that contribute to the Ag^{+} ions reduction[22].

Therefore, increasing interest in minimization of cost, waste and the time etc for the development of simple and environment-friendly methods for the AgNPs biosynthesis has led to the introduction of the photo biological approach[23]. The Green Nanotechnology is also referred to as the photo biological approach that uses the plants and the plant extracts as reducing and capping agent for AgNPs synthesis. In this context, the AgNPs they are biosynthesized using the *Selaginella moellendorffii* plant species extract[24].

Pteridophytes are considered as one of the oldest group of the plants that are present on the earth and they consist of a large group of vascular cryptograms. They have made the group fascinating because of their position between the lower cryptograms and the higher vascular plants[25].

They are present in the Coastal and the Himalayan regions of India and they prefer moist and shady habitats with the moderate temperature conditions[26].

Out of 1,200 species of the Pteridophytes that are present in India , about 170 species have been found to be used for as medicine , food , oil , dye , bio-fertilizer , flavour , fibre , and for the production of the bio-gas etc [27].

As per the folk medicine, they have been known to the man since more than 2000years and they are also mentioned in the ancient literature. One of the most important factors for the success of the Pteridophytes is that they are not infected by the microbial pathogens.

As compared to the Angiosperms the Pteridophytes they have found less application in the medicine. Since the ancient times for the treatment of the various human ailments the tribal communities and the ethnic groups throughout the world are using the stems, leaves, spores, pinnae, fronds, rhizome of the Pteridophytes in different ways. Various chemical compounds like flavonoids, alkaloids, glycosides, steroids, phenolic compounds, terpenoids and sesquitorpens etc are present in the Pteridophytes besides the sugar, amino acids and the proteins and because of their presence they are used as potential components in various industries and have many medicinal values[28,29].

Nanotechnology is widely being used term these days to label a vast variety of research activities around the world. It is the fabrication, exploration of the properties of the materials at the nanoscale[30]. Generally, the particles that are having the size ranging between 1-100nm are referred to as nanoparticles[31]. The Noble Laureate Richard Feynman, emphasized the nanotechnology idea in his famous lecture that was held at California Institute of Technology on 29th December. In 1960, in one of his articles that he published titled, "There is a plenty of room at the bottom" he discussed the nanomaterials idea. Norio Taniguchi first defined nanotechnology term in 1970[32,33]. In the various areas such as mechanics, space industries, optics, electronics, biomedical sciences, optoelectronic devices etc the nanotechnology is thus gaining the importance[34]. In the nanotechnology field the most common nanoproduct is the silver nanoparticles which are not toxic to the human health when present in low concentration and they have been significantly used in the areas of textiles, medical ingredients, clothing, packaging of food and household usage etc[35]. Green synthesis by the biological methods plays important role in silver nanoparticles synthesis[11]. The silver nanoparticles synthesis by the use of the plants and their extracts by the biological methods is the most environmental-friendly method and alternative to available chemical and the physical methods for nanoparticles synthesis[13,36].

Roy et al., 2013 synthesized AgNPs by making use of the extract of the fruit of Vitis vinifera as a reducing agent[37]. The compounds like flavanoids, terpenoids, phenolic compounds and the polysaccharides that were present in extract of fruit to them reduction was attributed[38] . The TEM (Transmission Electron Microscope) investigated the size and lattice image of ynthesized silver nanoparticles. The synthesized AgNPs were having average size of 18-20nm and were spherical and had a weak crystalline structure and they also showed significant antimicrobial activity against Bacillus subtilis (gram positive) and E. coli (gram negative)[39] .

Jha et al., 2010 synthesized the AgNPs by making use of the biotechnological method by using the extract of the leaf of the Cycas[40]. The AgNPs synthesized were characterized by various techniques. The synthesized silver nanoparticles had fcc unit cell structure was analysed by X-Ray studies. The extract of the leaf of the cycas contains phytochemicals like the glutathiones ,ascorbates , polyphenols and metallothioneins were analysed by the phytochemical analysisand therefore these are responsible for the production of the nanoparticles[41] . The plants that were exposed to the metal stress in them the glutathione played an important role and the metallothioneins also played an important role as it has the capacity to bind to the both heavy metals and the xenobiotics[42]. They show antioxidant action because of higher tendency of the phenolic compounds to chelate the metals[43]. Therefore the plants that having higher phenolic comments (e.g Pinus species) are the best for nanoparticles synthesis[44] .

Bankar et al., 2010 reported use of extract of the peel of the banana (Musa paradisiaca) for the synthesis of silver nanoparticles . The banana peels were washed with distilled water and were boiled for 30minutes at 90 ^{o}C temperature[45].

Therefore it is known that reduction process of metals is affected by incubation temperature. Therefore the increase in temperature leads to the formation of the smaller nanoparticles as the reactants they are consumed rapidly. The synthesized silver nanoparticles showed significant antifungal activity against C.albicans and C.lipolytica and also showed significant antibacterial activity against E.coli , Klebsiella sp., E.aerogenes , and Shigella sp[46].

Vilchis - Nestor et al., 2008 synthesized the silver and the gold nanoparticles by using the extract of the green tea (Camellia sinensis)[47]. They also reported the initial concenterations of the extract of the tea and the metal ions as the controlling factors and also investigated the size change , change of the optical property and the morphology of the nanoparticles[46] . Therefore as the C.sinensis extract amount increased , the size of the nanoparticles produced were bigger and were spherical . They believed that for the silver and the gold nanoparticles production and stabalization the phenolic acid type biomolecules that were present in extract of C.sinensis they were responsible[48].

Banerjee et al., 2014 synthesized the AgNPs by making use of the extracts of the three Indian medicinal plants , M.balbisiana (Banana) , A.indica (neem) , and O.tenuiflorum (black tulsi)[49]. The characterization of the AgNPs synthesized was done by various techniques such as the UV-vis spectrophotometer, DLS, Transmission Electron Microscopy (TEM) , Energy dispersive spectroscopy (EDS) and Scanning Electron Microscope (SEM) . The nature of the capping agents in each of the extracts was analysed by the FTIR analysis . The antimicrobial activity of nanoparticles was also carried against E.coli and Bacillus sp and they showe significant antimicrobial activity

 Furthermore ,they also carried out the toxicity evaluation on the seeds of the Vigna radiata (Moong bean) and Cicer arietinum (Chick pea) by the silver nanoparticles containing the solutions and the results they showed that the seeds they exhibited better rates of germination after the treatment with the silver nanoparticles solution[2] .

Awwad et al., 2013 used the extract of the Carob leaf for Ag^{+} ions reduction to Ag^{0} nanoparticles from the silver nitrate solution within 2minutes of the reaction time [51]. The AgNPs were characterized by various techniques such as the UV-vis spectrophotometer, XRD , FTIR, SEM, and AAS. Therefore it was also reported that by varying the concenteration of the silver nitrate the extract of the leaf the average size of the synthesised AgNPs can be controlled to 5-40nm . The antibacterial activity was carried out for the biosynthesised silver nanoparticles against E.coli bacteria and the nanoparticles were found to be highly effective.

S. S Panda et al., 2014 studied the phytochemical analysis and investigated the antimicrobial property of the pteridophyte plants such as *Thelypteris interrupta* , *Salvinia minima* Baker and *Marsilea minuta* L. and these three plants are commonly

found in Odisha , India[4]. The extraction of the whole plants of the three pteridophytes was extracted using the two of the solvent such as the methanol and the chloroform. Therefore then they investigated the antimicrobial activity of the three pteridophytes against the eighteen different pathogens of bacteria such as *S.aureus* , *S.citreu* , , *E. coli* , *Pseudomonas* sp. , *P. aeruginosa* , *S.typhi* , , *S. paratyphii* , *S. paratyphii* B , *Citrobacter fruendii* , *Klebsiella* , *Chromobacter* , *Vibrio cholera* , *Shigella sonnie* , *Enterobacter*, *S. boydii* , *Providenci* , , *P. mirabilis* , *P. vulgaris* and four of the fungal pathogens such as *the A. flavus* , *Candida albicans* , *A. niger and the Rhizopus* sp . and the results showed that methanol extract of the *Thelypteris interrupta showed the* highest antibacterial activity (28mm) against the *S.citreus* whereas the methanol extract of *Salvinia minima showed the* maximum antifungal activity (22mm) against the *A. flavus*[52]. The phytochemical analysis was carried out and it showed that methanol extract it showed the presence of various phytochemicals such as the alkaloids, tannins , steroids , anthroquinone, , and the terpenoids , as compared to the chloroform extract . Therefore , large number of phytochemicals were present in the *Thelypteris interrupta* among the three of the pteridophytes studied[53] .

K N DUBAL et al., 2013 carried out the investigation to understand the bioactive compounds that were present in the rhizome of T. coadunata[11] .The rhizome of the T.coadunata was extracted using the methanol through the soxhlet extraction .The study revealed that methanol extract of rhizome of the T.coadunata showed presence of 16 bioactive compounds and the presence of these 16 different bioactive compounds justified the use of the rhizome of T.coadunata in the treatment of various human ailments and it may also help in the protection against various incurable diseases .

Pradeep Parihar et al., 2006 investigated the antibacterial activity of Athyrium pectinatum (Wall.) Presl. against the bacteria such as E.coli , A.tumefaciens , S. arizonae , S.typhii and S.aureus[54] . The aqueous and alcoholic extracts of roots , rhizomes and leaves of Athyrium pectinatum was extracted and the obtained alcoholic and the aqueous extracts were found to be effective against the bacteria . It was observed that the roots and the rhizome extracts they inhibited the growth of the microorganisms whereas the leaves extract did not show any inhibition except Salmonella arizonae and it was also observed that the antibiotic the root extract showed the maximum inhibition against the Agrobacterium tumefaciens than the antibiotic alone [55].The leaves and the roots extract did not show any inhibition against E.coli and the root and the rhizome extracts against Salmonella arizonae .The aqueous extract of the root antibiotic has shown higher inhibition against Staphylococcous aureus than the antibiotic alone and therefore the rhizome extracts were found to be more effective than the antibiotic alone[56].

A John De Britto et al., 2012 analyzed the phytochemical constituents of the five different medicinal ferns Pteris biaurita , Lygodium flexiuosam , Hemionitis arifolia , Actinopteris radiate and Adiantum latifolium[49]. The whole plant material of all the

plants were extracted with the Chloroform , benzene ,petroleum ether , methanol , and with the distilled water using a soxhlet extractor for 8 hours at a temperature of 50-60 ^{o}C . The phytochemical analysis was done for all the extracts according to the standard procedures and the results showed that twenty of the extracts out of the twenty five showed the presence of the flavanoids[57]. The presence of the phenolic compounds was shown by the methanol extract of all the ferns. The presence of the terpinoids and the catechins was shown by only five extracts[58]. The occurrence of the reducing and the non-reducing sugars was shown by the ten extracts and the presence of the alkaloids was shown by the fifteen extracts[59]. The tannins and the saponins are present in the eight extracts and the steroids were present in all the extracts. The presence of the amino acids and the anthroquinones was shown by only three extracts. Therefore from this investigation it was concluded that these five medicinal ferns contained more of the bioactive principles and they can be used as bio-control agents[60].

D.Herin Sheeba Gracelin et al., 2013 reported the phytochemical study of methanol extract of five ferns belonging to the Pteridaceae family such as the P.argyreae , *P. vittata* , *P.confusa* , *P. biaurita and P. multiaurita* and the results they revealed that methanol extract of the *P.biaurita* showed the10 phytochemical tests as positive and the extract of the *P. vittata* showed the 9 phytochemical tests as positive and therefore the methanol extract of the *P. argyreae and the P. confusa* showed 7 phytochemical tests as positive and the 5 phytochemical tests were shown positive by the extract of *P. multiaurita and then the* quantitative analysis was performed in which secondary metabolites such as tannins ,alkaloids, saponins , phenolic compounds, and the flavonoids were tested for all the extracts of the five ferns[61,5] . Therefore as a result the methanol extract of the *P. biaurita* showed the presence of the large amount of the phytochemical compounds as compared to the other solvent extracts [62,63].

- **Plant Material Collection:**

The *Selaginella moellendorffii* plant was collected from Kasauli (H.P) as it was easily available and was cost effective. The leaves were washed with the distilled water and were then dried at room temperature.

- **Preparation of Leaf Extract:**
  - **Plant water extract:**

About 3gms of the plant leaves were weighed and were transferred to 250ml beaker to which 150ml distilled water was added and was then boiled for an hour. The extract was filtered using Whatman No. 1 filter paper and a clear solution was obtained and was then refrigerated at 4 ^{o}C for further experiments. Sterile conditions were maintained throughout the experiment against contamination.

  - **Different fractions using soxhlet apparatus:**

10grams of plant sample was layed down inside thimble that was fixed in the main chamber of the soxhlet. Then 150ml of solvent (Chloroform, Petroleum Ether, Methanol) was poured into the distillation flask. The solvent were heated to reflux and the vapours moved up to the distillation arm and was collected in chamber collecting thimble of solid. The condenser cools the solvent vapour and drops it back to chamber containing the solid material. During every cycle, the part of non-volatile compound was dissolved in solvent. After large number of cycles desired compound was concentrated in the distillation flask and then it was stored for the further experimental use.

- **Silver Nanoparticles Synthesis**:

0.01 M Silver Nitrate ($AgNO_3$) was prepared by dissolving $AgNO_3$ in distilled water by covering it with foil paper to avoid its reduction. To 1ml of distilled water, $AgNO_3$ solution was taken in a beaker and to it 4ml of aqueous plant extract was added and it was then kept in the sunlight for green synthesis. As a result, the change in colour from light yellowish to yellowish brown to reddish brown mixture was monitored periodically for 1 hour and it showed the silver nitrate complete reduction.

- **MIC (Minimal Inhibitory Concentration) Method:**

MIC is the least concentration of antimicrobial agent that inhibits the growth of microorganisms. The importance of this test is for the confirmation or for the determination of the resistance of the microorganisms to an antimicrobial agent and also to monitor the activity of antimicrobial agents. The bacterial *E.coli* strain (Gram negative) was cultured in nutrient broth medium and was screened against the

pteridophyte aqueous extract and the biosynthesized AgNPs, to find out the minimum concentration of the aqueous plant extract and the biosynthesized AgNPs, which inhibits the growth of bacteria. As a result of antibacterial activity it was found that the both plant aqueous extract and the biosynthesized AgNPs showed the significant activity against *E.coli* bacteria.

- **Collection of Catalyst:**

The industrial dyes Methylene Blue and Rhodamine B were gifted from some industrialist to check the effect of adsorption. Pesticide (Paradol) was collected from an farms that use pesticides for fields and p- nitrophenol was collected from organic labs, Thapar institute of Engineering and Technology, Patiala.

- **Characterization**

The biosynthesized particles were characterized with various instruments. The silver nanoparticles production was confirmed with UV- spectrophotometer. The optical property of AgNPs was observed by monitoring the absorbance between 200-1100nm wavelength. Further, the chemical composition of the AgNPs that were synthesized was studied by using FTIR spectrometer. Samples were air dried and characterized in the range 4000-400cm^{-} using KBr pellets. To confirm the size of the synthesized AgNPs Dynamic Light Scattering (DLS) was performed.

- **Qualitative Analysis**

Qualitative phytochemical analysis of aqueous and alcoholic extracts of the plant was conducted by following standard procedures.

- **Test for Alkaloids:**

  - Wagner's Test (lodine- Potassium-Iodide Solution): In distilled water 1% of iodine and 2% of potassium iodide solution were prepared.and in a test tube 10ml of alcoholic extract was taken and to it 1.5% v/v of HCl was added and few drops of Wagner's reagents were then added. The formation of brown coloured or yellow coloured precipitates indicated that the alkaloids are present.

  - Meyer's Test (Potassium Mercuric Iodide): About 2% of $HgCl_2$ and 5% of KI was prepared in distilled water and both were mixed and were then diluted to 100ml to prepare working solution. To 1 ml of acidic aqueous extract, few drops of the reagent were added. The white coloured or pale precipitates formation indicated that the alkaloids are present.

- **Test for Flavonoids:**

To 0.5% of alcoholic extract in a test tube 5-10 drops of dilute HCl and small piece of Zn or Mg was added and then it was boiled for few minutes.The reddish pink or brown colour formation indicated the presence of flavanoids..

❖ **Identification of tannins and saponins:**

◆ **Tannins:**

Ferric chloride test:

In a test tube 1.2 ml of aqueous solution was taken and to it few drops of 1% solution of lead acetate was added. The formation of yellow coloured or red coloured precipitates indicated that the tannins are present.

◆ **Saponins:**

5.0 ml of an aqueous extract was taken in a test tube and to it sodium bicarbonate drop was added and was shaked and kept for 3 minutes.The formation of froth indicated that the saponins are present.

❖ **Identification of phenols:**

◆ **Phenols:**

Ferric Chloride test:

1% of alcoholic solution was added in a test tube and was diluted and to it few drops of 10% aqueous ferric chloride solution was added. The appearance of blue/green colour indicated that the phenolic compounds are present.

Lead acetate test:

1% of alcoholic solution was added in a test tube and it was diluted to 5 ml and to it few drops of 1% aqueous solution of lead acetate was added. The yellow coloured precipitates were formed which indicated the presence of phenolic compounds.

❖ **Identification of glycosides:**

◆ **Glycosides**

Small amount of alcoholic extract was dissolved in 1 ml of distilled water and to it aqueous NaOH solution was added. The appearance of yellow colour indicated that the glycosides are present.

❖ **Identification of steroids:**

◆ **Salkowski reaction:**

2 ml of chloroform extract was added to test tube and to it 1 ml of concentrated H_2SO_4 was added along the sides of test tube. Red colour appearance in chloroform layer indicated that the steroids are present.

Applications of Pteridophyte plant Extract and the biosynthesized AgNPs

- **Antibacterial Activity**

The antibacterial activity of aqueous extract and biosynthesised were studied by MIC (Minimal Inhibitory Concentration method) against gram negative *E. coli.* Luria broth was prepared by autoclaving and mother Culture of *E.coli* was prepared. The biosynthesized AgNPs and the plant aqueous extract, was dispersed in the autoclaved de-ionized water. The aqueous dispersions of the AgNPs and aqueous extract in various concentrations (0.01 , 0.025 , 0.050 , 0.075 , 0.1 , 0.25 , 0.50 , 0.75 , 1)ml/5ml of media were prepared. The Working culture of *E.coli* was added to the test tubes containing the medium and different concentrations of AgNPs and aqueous extracts and kept for incubation at $37^{o}C$ for 12hrs. The absorbance was measured in UV spectrophotometer at 600nm.

- **Adsorption of Dyes**

The Industrial dyes that were used for adsorption are Methylene Blue and Rhodamine B. Stock Solutions of both the dyes (Methylene Blue (0.01M) and Rhodamine B (0.01M)) were prepared. Then for each dye 2% of AgNPs were taken and to it 10ul of the stock solution of dye was added after every 5minutes .The reading was taken at UV-vis spectrophotometer after every .For Methylene Blue dye the peak was observed at 660nm and for Rhodamine B the peak was observed at 550nm.

- **Adsorption of Pesticides**

The pesticide used for the adsorption is Methyl parathion (Paradol) .The stock solution (0.01M) was prepared. Then 2.5% of PAgNPs were taken and to it 10ul of stock solution was added after every 5 minutes. The absorbance was measured at 400nm in UV-vis spectrophotometer after different time intervals.

- **Adsorption of p-nitrophenol**

Stock solution of Para-nitrophenol (0.0001M) and $NaBH_4$ (0.1M) was prepared. Then to 3ml water, 30ul of p-nitrophenol and 200ul of $NaBH_4$ was added. To it 20ul of AgNPs were added and absorbance was measured at 400nm in UV-vis spectrophotometer after every 5min.

- **Visual Screening (PAgNPs):**

The addition of the *Selaginella moellendorffii* plant leaf extract to the $AgNO_3$ solution resulted in the colour change of the solution from light yellowish to reddish brown indicating the production of the particles. The change in the colour from light yellowish to reddish brown is due to the excitation of surface plasmon vibrations with the AgNPs.

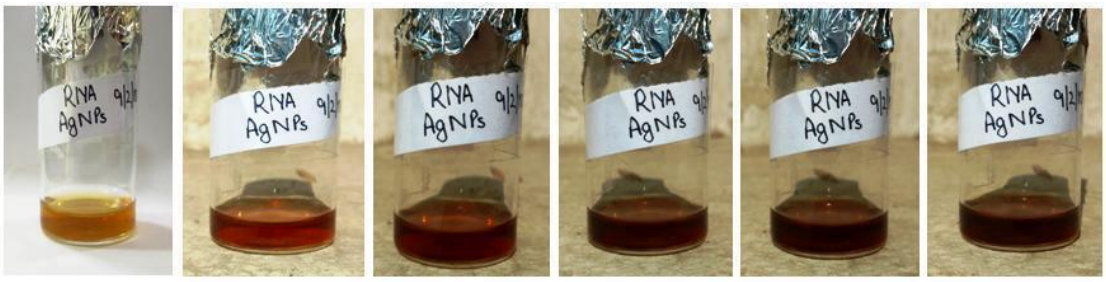

Fig1: Visual analysis of formation of AgNPs at different time.

- **Measurement of Absorbance:**

The UV-vis analysis was performed to investigate optical properties of the PAgNPs. . The UV-vis spectra were recorded after every 5 min of the time intervals of 0min, 5 min, 10 min, 15 min, 20 min, 25 min and 30min from the initiation of the reaction. The AgNPs synthesized has absorption maxima in the range 400- 475nm due to Surface Plasmon Resonance. Therefore it was observed that the bio-reduction of Ag^+ ions into the PAgNPs started just at the start of reaction and also it was observed that the formation of the PAgNPs occurred rapidly within the first 15 minutes only.

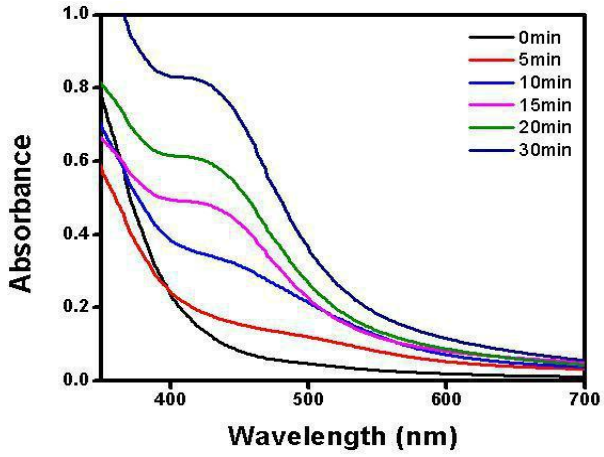

Fig2: UV-vis spectra of the biosynthesized PAgNPs.

- **FTIR (Fourier Transform Infrared Spectroscopy):**

For the FTIR analysis,reaction mixture was centrifuged at 15000 rpm for 15minutes after the reduction of $AgNO_3$ by aqueous leaf extract. After centrifugation the pellet that was obtained was re -dispersed in the distilled water and finally then the samples they were dried and grinded with KBr pellets and the analysis was performed. The peak in the FTIR was observed from 4000-400cm^{-1}.

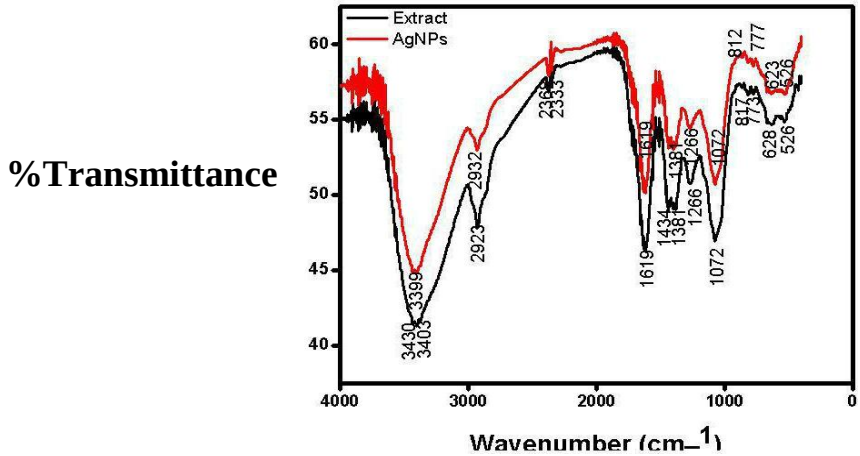

Fig3: FTIR pattern of biosynthesized PAgNPs in comparison to plant extract.

Functional Groups (cm^{-1})	*(N-H primary and secondary amines,amides bend)*	*(N-H primary and secondary amines ,amides stretch)*	*(O-H alcohols phenols H-bonded)*	*(O-H Alcohols, Phenols Carboxylic Acids)*	*(C-H Alkanes Stretch)*	*(C=C Alkene)*	*(C-O Alcohols,Esters,Ethers, Carboxylic acids, anhydrides)*	*(C-N Amines)*	*(S-O Sulfones,Sulfonyl chloride ,sulfates ,sulfonamides)*	*(C-H Alkene Out of plane bend)*	*(C-H Aromatics Out of plane bend)*	*(C-X Fluoride)*	*(C-X Bromide, iodide)*	*(C-X Chloride)*
Plant Aqueous extract	*1619*	*3430, 3403*	*3430, 3403*	*3430, 3403, 2923*	*2923*	*1619*	*1266, 1072*	*1381, 1266*	*1266*	*817, 773*	*817, 773*	*1381, 1266, 1072*	*628, 526*	*773, 628*
AgNPs	*1619*	*3399*	*3399*	*3399, 2932*	*2932*	*1619*	*1266, 1072*	*1266, 1072*	*1266*	*812, 777*	*812, 777*	*1381, 1266, 1072*	*623, 526*	*777, 623*

Table1: Comparative table of FTIR showing the changes in the peak positions after AgNPs synthesis.

- **Dynamic Light Scattering and Zeta Potential:**

DLS technique is used to determine the size distribution and Zeta potential to measure the stability of particles synthesised after centrifugation. The size of the particles according to the figure in the colloidal solution is defined as following. The

hydrodynamic size of AgNPs is measured as 91nm in counts. Whereas, Zeta was calculated as -16.75mV.

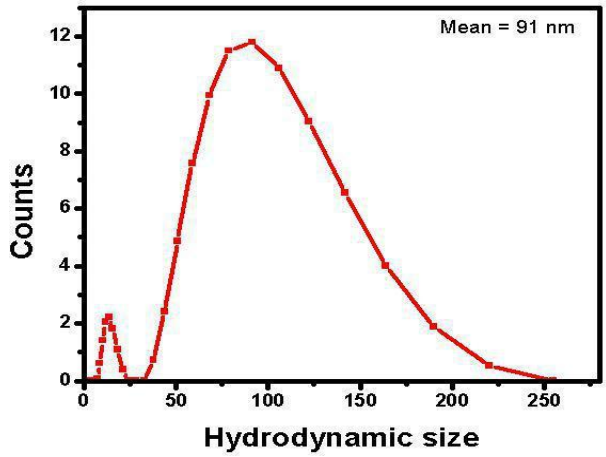

Fig4: Hydrodynamic mean of biosynthesized PAgNPs.

- **Antibacterial activity:**

The IC_{50} value for PAgNPs and pteridophyte plant extract is 3.3 and 5.1µg/ ml. Thus PAgNPs shows higher antibacterial values than plant extract. The growth of E. coli is maximum inhibited in PAgNPs whereas plant extract shows less inhibition.

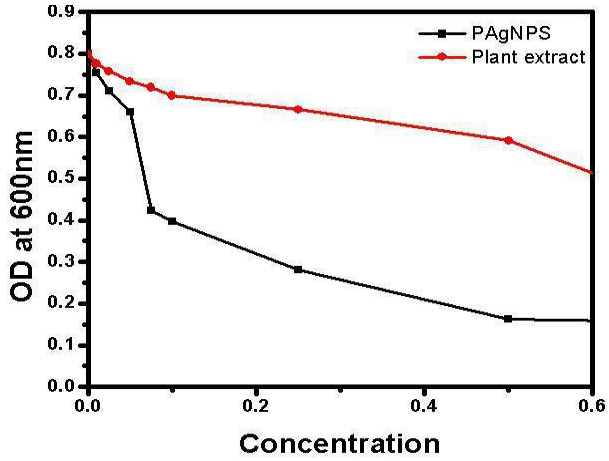

Fig5: Antibacterial effect of biosynthesized PAgNPs and aqueous leaf extract on growth inhibition of *E.coli.*

- **Qualitative Phytochemical Analysis:**

In qualitative analysis, the aqueous and the alcoholic extracts of *Selaginella moellendorffii* plant exhibited positive results for the phytochemical tests. Phytochemical compounds such as the tannins, saponins, alkaloids, phenols, steroids and glycosides were screened in the alcoholic and the aqueous extract of the plant and the flavanoids were absent in the plant extract. Among these phytochemical compounds tannins, saponins, phenolic compounds and alkaloids are important

secondary metabolites and are responsible principles for the medicinal value of the *Selaginella moellendorffii* plant.

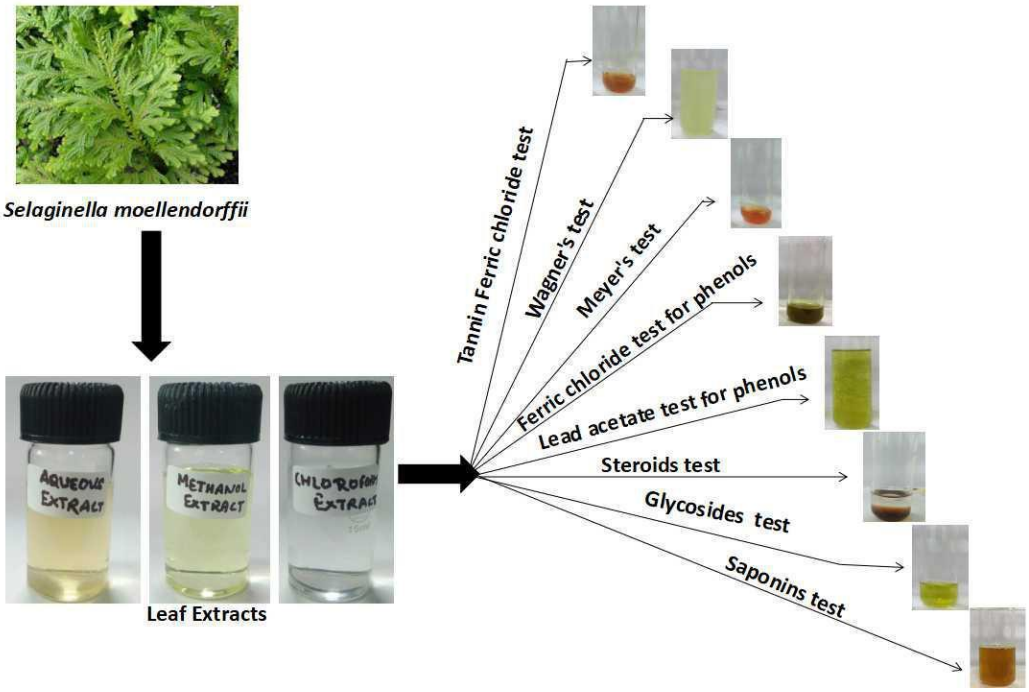

Fig6: Schematic diagram of the qualitative analysis of plant extract

Test Performed	Pteridophyte Plant Extract
For Alkaloids - Meyer's Test - Wagner's Test	+ +
Flavonoids Test	-
Tannin Ferric Chloride Test	+
Saponins Test	+
For Phenols - Ferric Chloride Test - Lead Acetate Test	+ +
Steroids Test	+
Glycosides Test	+

Table2: Qualitative analysis of *Selaginella moellendorffii plant extract*

- **Adsorption of Dyes:**

The plot of relative intensity with wavelength reveals the adsorption of cationic dyes in around 60 mins. Thus, the result proves that after 60 mins, strong reducing agent silver nanoparticles with plant extract degrades the dye and disappearance of blue color in methylene blue and pink color in rhodamine.

Methylene Blue Dye degradation:

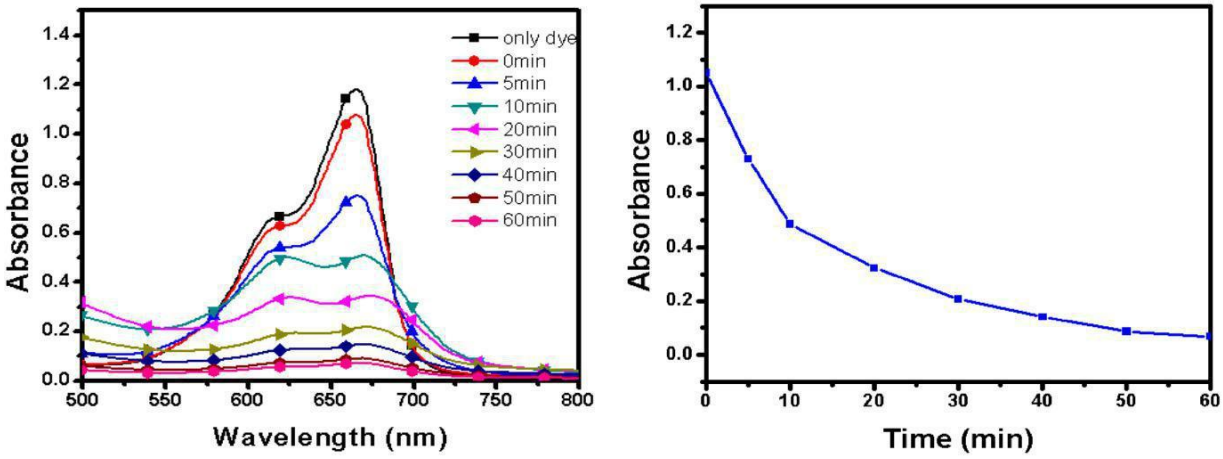

Fig7: (A) Adsorption spectra of Cationic dye from 500-800 nm showing degradation of MB and **(B)**Adsorption spectra of MB at λ max 600 nm showing 94.1% degradation at different time intervals.

Rhodamine B Dye degradation:

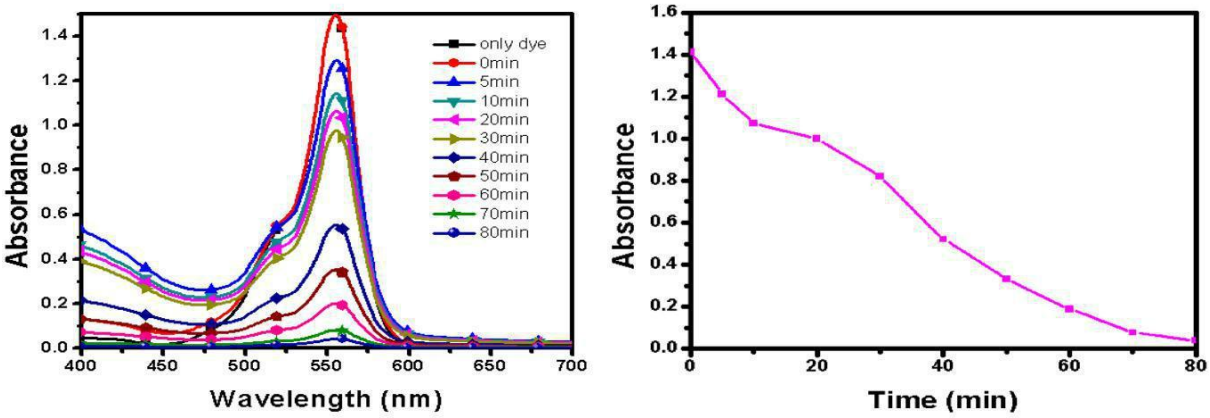

Fig8: (A) Adsorption spectra of Cationic dye from 400-700 nm showing degradation of RB and **(B)** Adsorption spectra of RB at λ max 550nm showing 97.2% degradation at different time intervals.

- **Pesticide Degradation:**

The reaction was performed according to time dependent batch. At different time intervals the absorbance was measured at UV- Vis spectrophotometer at 400nm. To 2% (0.01M) solution around 100µl PAgNPs were added and absorbance was measured at different time till saturation.

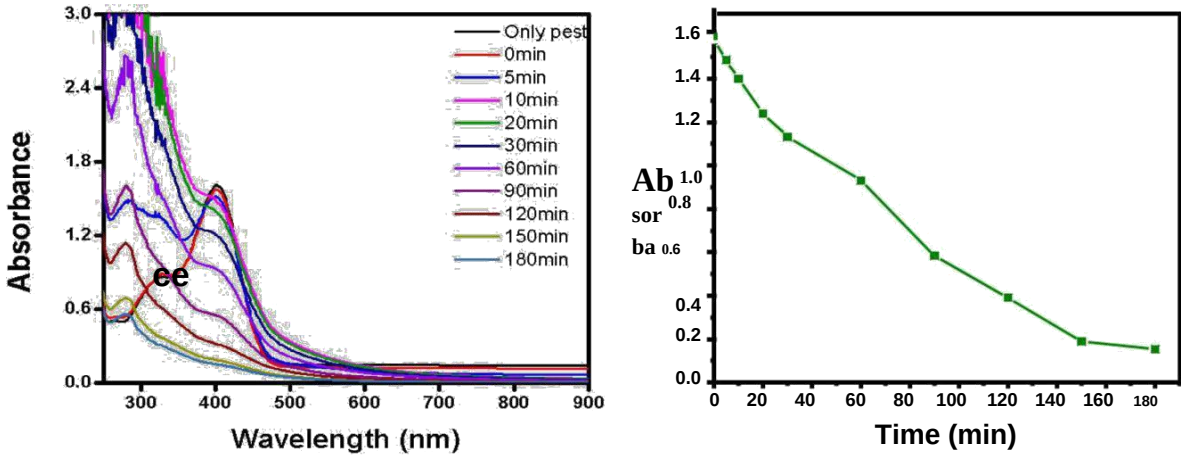

Fig9: (A) Adsorption spectra from 300-900 nm showing degradation of pesticide and **(B)** Adsorption spectra of pesticide at λ max 400 nm showing 90.3% degradation at different time intervals.

● **Adsorption of p-nitrophenol:**

Degradation of NP was done using NaBH4 with PAgNP as a catalyst. The catalytic degradation was measured using UV- vis spectrophotometer. After the addition of freshly prepared NaBH4 (0.1M) in the NP (0.001M) solution, a sharp peak shift is observed from 317nm to 400nm. The catalytic degradation was monitored by characterisation of the decrease in 400nm peak.

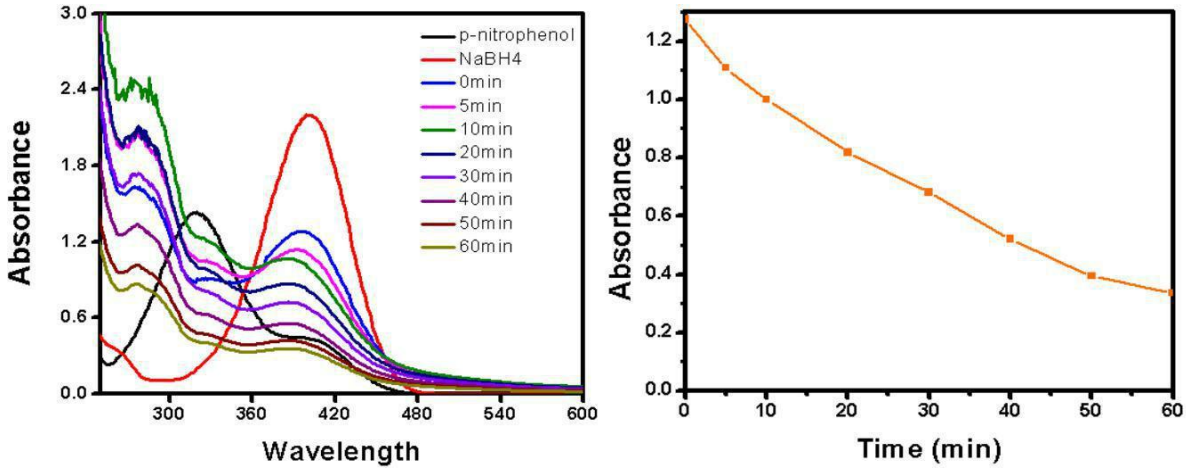

Fig10: (A) Adsorption spectra of p-nitrophenol from 250-600 nm showing degradation of p-nitrophenol and **(B)** Adsorption spectra of p-nitrophenol at λ max 400nm showing 73.7% degradation at different time intervals.

Conclusion

The Silver Nanoparticles (AgNPs) were synthesized successfully from the bio-reduction of $AgNO_3$ solution using the plant leaf extracts of the *Selaginella moellendorffii*. The biosynthesised silver nanoparticles were appropriately characterized using the UV-vis spectroscopy, FTIR analysis, DLS and Zeta Potential. The UV-vis analysis showed that the absorption spectra of the silver nanoparticles synthesized in the reaction media have absorption maxima in the range of 400 - 475 nm because of the surface plasmon resonance of the silver nanoparticles. From the DLS study we came to know about the hydrodynamic size that was 91nm in counts and the stability of the nanoparticles in the solution, whereas the FTIR analysis shows that the functional groups like N-H, O-H, C-H, C-N, and C-X are present in the aqueous plant extract which undergoes shift in the peak position after the formation of AgNPs. The qualitative analysis was also performed that showed the phytochemical constituents such as the alkaloids, tannins, saponins, phenolic compounds, steroids and glycosides are present and the flavonoids were found to be absent in the *Selaginella moellendorffii* plant species and which may possess various medical and pharmaceutical values. Therefore, plant species also possessed some catalytic activities as it shows 90.3% degradation of pesticide, 73.7% degradation of p-nitro phenol, 97.2% degradation of Rhodamine B dye and 94.1% degradation of Methylene Blue dye which was seen with the PAgNPs at different time intervals. The synthesized silver nanoparticles and the aqueous leaf extract showed the significant antibacterial activity against *E.coli*. These pteridophyte plants are pocket friendly and easily available in the hilly areas. Thus these species reduces pesticides and industrial dyes to high extent, they can be used in purification of impure water.

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