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Green synthesis of silver nanoparticle using *Selaginella tenera* leaf extract and its biological activity

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Abstract
Synthesis of metal oxide nanoparticles is an expanding area of research due to their potential applications in the development of novel technologies. An ecofriendly based synthesized nanoparticles has become an important branch of nanotechnology. in the present work, we have developed the synthesis of silver Nanoparticles (AgNPs) using aqueous leaf extract of *Selaginella tenera* act as a reducing agent/ capping agent. Formations of green synthesized silver nanoparticles were characterized by UV-visible spectroscopy (U-Vis), X- ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), Scanning electron microscope (SEM) analysis. Further, the green synthesized silver nanoparticlesare stabilized by plant metabolities, and their significant antioxidant activity of 1, 1-diphenyl-2-picrylhydrazyl (DPPH)free radicals were effectively. Furthermore, the green synthesized silver nanoparticles showed significant bactericidal effect against four pathogenic bacterial strains namely *Escherichia coli, Pseudomonas aeruginosa, Staphylococcushaemolyticus* and *Staphylococcus aureus* shows zone of inhibition by agar well diffusion method. Finally, the investigation suggests that green synthesized silver nanoparticles arehaving potential applications for various industrial fields and also helpful to the scientific community.

Keywords: green synthesis, *Selaginella tenera*, silvernanoparticles, characterization, antibacterial activity, antioxidant activity

Introduction

The concept of nanotechnology was first presented by Richard Feynman through his famous lecture, entitled "There's a plenty of room at the bottom" at the American Institute of Technology. The word nanotechnology was introduced by Prof. Norio Taniguchi of Tokyoscience University. The nanotechnology is emerging as a cutting-edge technology involving many academic disciplines such as physics, material science, chemistry, biology, and medicine (Raveendran *et al*., 2003; Thakkar *et al*., 2010; Willard *et al*., 2004) [21, 24,]. The prefix "nano" in the term nanotechnology is derived from a Greek word *nano*, which means "dwarf". It relates, to any engineered matter that is one billionth (10−9 m) in size or at least one of its dimensions, and is considered nanometer (nm) or approximately, it is the length of three atoms next to one another. In contrast, the DNA molecule is 2.5 nm wide, a protein is about 50 nm in length, a flu virus is about 100 nm and a human hair is about 10,000 nm thick Nanoparticles (NPs) are interesting nanoscale systems because of the ease with which they can be produced in different shapes. This infinitesimal particle is the answer to what science dreams to achieve in the near future (Martin, 1994; Raj Preeth *et al.*, 2019) [13, 19].

Activities involving the synthesis of nanoparticles are the present area of interest due to their unique biomedical, agricultural, environmental, and physiochemical areas (Abbasi *et al*., 2016; Rai *et al.*, 2016; Rao & Gan, 2015) [1, 18, 20]. These unique properties are due to the quantum confinement effect, tuning of electronic energy levels through size effect andincrease in the surface area to mass ratio of the nanoparticles which causes dominance in the behaviour of atoms on the surface of the particle than those in the interior of the particles. The unusual physicochemical properties of Nanoparticles are attributed to their small size (< 100 nm); surface structure – surface reactivity, groups, and coatings; chemical composition purity, crystallinity, and electronic properties; solubility surface morphology, the effect of adsorbed chemicals; shape and aggregation (LaVan *et al.*, 2002; Raj Preeth *et al*., 2019) [9, 19].

The metal oxide Nanoparticles such as gold, silver, platinum, aluminium, zinc, carbon, titanium, palladium, iron, and copper have gained colossal consideration in recent times due to their indispensable and technological importance (Rai *et al*., 2016) [18].

Selaginella tenera is belongs to family selaginellaceae (Pteridophytes). It is well known for Chinese herbal medicines. Plants have been a source of medicine and a major resource for health care since ancient times, with some traditional herbal medicines having been in use for more than 2,000 years (Zou *et al*., 2020) [26]. Next toflowering plants the dominant vegetation on the earth is formed by the non-flowering vascularplants called Pteridophytes. There are about 1200 species of Pteridophytes in India. The medicinal use of *Selaginella* in India goes back to the period of 'Ramayana' (Zou *et al*., 2020) [26].

According to the principles of traditional Chinese medicine, *Selaginella* has sweet, spicy, bitter and cold properties, and is associated with the Liver, Lung and Stomach meridians. Its main functions are to clear heat, reduce toxicity, and drain damp heat. Among the conditions *Selaginella* is used for are coughs, sore throats, and jaundice. *Selaginella* is also used to treat cancer of the liver and cirrhosis of the liver (Lin *et al.*, 1994) [11]. More recent research has shown that *Selaginella* may be effective against both acute and chronic hepatitis. *Selaginella* may also be used externally to help stop bleeding and promote wound healing. The typical dose of *Selaginella* is between 15 and 30 grams. Larger doses are considered if *Selaginella* is being used to treat cirrhosis or other liver disorders. *Selaginella* is available in a variety of forms.

Especially, in Asian markets and herbal shops. Prepared *Selaginella* can be found in pill, powder and tablet forms.

Selaginella should be taken with caution by patients diagnosed with cold deficiency. Taking large amounts of *Selaginella* may result in loss of appetite and abdominal discomfort.

There are no known drug interactions associated with *Selaginella doederleinii*, a popular anticancer herb, may contain an as yet unidentified substancethat contributes to reversible bone marrow suppression. These results suggest that *Selaginella tamariscina* could be a candidate chemopreventive agent against gastric cancer (Duraiswamy, 2010) [4].

Most of the information available on the medicinally important species of *Selaginella* is from other countries and there is very little information about species of *Selaginella* from India. In '*Ayurvedic Pharmacopoeia of India'* out of hundred drugs, only one is of the fern (*AdiantumlunulatumBurm.*) (Lee *et al.*, 2008) [10]. In contrast, several value added natural medicines are introduced in the market from the developed countries. As mentioned above, numerous bioactive compounds, particularly biflavonoids with various bioactivities, particularly cytotoxic activity, have been reported in various species of *Selaginella* from other countries (Pan *et al*., 2001) [15].

In the present work, we have described (Kharissova *et al*., 2013) [8] the synthesis of silver nanoparticles (AgNPs)using aqueous leaf extract of *Selaginella tenera*acts as reducing agent. Formations of silver nanoparticles were characterized by UV- visible spectroscopy, X- ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), Scanning electron microscope (SEM) analysis. Furthermore, the green synthesized silver nanoparticles (AgNPs)are screening for various biological properties such as antioxidant and antibacterial activity in *invitro* condition (Khalil *et al.*, 2014) [7].

Materials and methods

1. Materials

Selaginella tenera were collected from Western Ghats of Karnataka, India. The pathogenic bacterial strains (National Chemical Laboratory-NCL) were purchased from Pune, India. All chemicals were analytical grade without further purification.

2. Preparation of extract

The fresh leaves of *Selaginella tenera* were thoroughly washed under tap water to remove the adhered dust particles present on the surface and then rinsed with doubled distilled water (Pourmortazavi *et al*., 2015; Prathna *et al*., 2011) [16, 17]. The cleaned leaves were completely dried at room temperature on a blotting paper. The dried leaves (10g) were chopped into small pieces and mixed with 100 mL of water in a 250 mL flask and the contents were boiled for 30 min at 60°C. The contents were cooled to room temperature and filtered through Whatman No. 1 filter paper. The clear leaf extract of *Selaginella tenera* thus obtained was used for synthesis of silver nanoparticles

3. Synthesis of silver nanoparticles

In a typical experiment, 10 mL of the leaf extract of *Selaginella tenera*was mixed with 90 mL of 1 mM silver nitrate solution and then mixing uniformly using magnetic stirrer at 1000 rpm for 10 min homogenously, and then kept in dark condition for 24 h. After incubation, a homogenous mixture change from yellow to brown designates the formation of colloidal Silver NPs shows in Further, the formations of silver nanoparticles were confirmed by characterization and also investigated the antibacterial and antioxidant activities.

3. Characterization

The green synthesized AgNps was monitored by UV–Vis spectra in a wavelength range of 200– 800 nm at a resolution of 1 nm using Thermo scientific Spectrophotometer (Model Evolution-220).The X-ray diffractometer employing the Shimadzu-7000 X-ray diffractometer with mono chromatized Cu Kα radiation was used for the phase identification and characterization of crystalline metallic AgNPs. The FTIR spectrum was obtained with Shimadzu8400S spectrophotometer using potassium bromide pellets at a resolution of 4 cm1 in the diffuse reflectance mode. Scanning electron microscopy (Hitachi tabletop, Model TM 3000) was used for morphological characterization and the size distributions of AgNPs (Naika *et al*., 2015) [14].

4. Antioxidant activity

Antioxidant activity of silver NPs was carried out by free radical scavenging activity by DPPH assay using modified method (Arya & Yadav, 2011; Gupta & Sharma, 1899) [5]. The 1,1-Diphenyl-2-picrylhydrazyl (oxidized form) was a stable free radical with purple color, which can donate an electron to DPPH radical decays, and the change in absorbance at 517 nm was followed which can be measured spectrophotometrically with respectively antioxidant activity. Briefly, the different concentration of green synthesized silver NPs (1, 2, 3, 4, 5 mg/mL) is diluted with 50% of methanol was added in to 1 ml of 0.1mM of DPPH solution in 95% methanol. After, the given mixture was incubated at 37°C for 30 min, followed by 50 % methanol used as blank, and then measuring the absorbance at 517 nm.

Radical scavenging assay was calculated by using the formulae:

$$\text{\% of in hibition } \frac{Ac - At}{Ac} \times 100 - - - - - [1]$$

Whereas 'Ac' is the control is the absorbance of the control reaction and 'At' is test is the absorbance ofthe extract reaction. IC_{50} value was calculated using the formula

$$IC_{50} = \frac{\Sigma C}{\Sigma I} \times 50 - - - - - [2]$$

Where 'ΣC' is the sum of nanoparticles concentrations used to test and 'ΣI' isthe sum of percentage of inhibition at different concentrations.

5. Antibacterial activity of silver nanoparticles

In the present study, *invitro* antibacterial activities were carried out by the using of agar well diffusion method (Banerjee *et al*., 2014; Sasikala *et al*., 2015; Veerasamy *et al*., 2011) [2, 22, 25]. Antibacterial activity of green synthesized silver NPs against pathogenic bacterial strains such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus haemolyticus* and *Staphylococcus aureus.* Nutrient agar

(NA) media (Hi media laboratories Pvt.Ltd, Mumbai, India) was used for antibacterial activity. Preparation of Nutrient agar plates were prepared by using Nutrient agar media (3.7% w/v) is dissolved in 1L of distilled water,and then subject to the sterilized by autoclaved (15 lbs pressure (121ºC) for 15 min). After sterilization, the nutrient agar medium was poured into sterile petri-dishes and allowed to solidify then after,100 µl of 24h mature broth culture of individual pathogenic bacterial strains in nutrient brothwhile spreading all over the surface of agar plates using sterilized L-shaped glass rod.Previously prepared silver nanoparticles were dispersed in double distilled water at different concentrations (200,400 and 600 µg/mL) for bacteria were placed aseptically on sensitivity plates with appropriate controls.

The tests were conducted with given concentration with three replicates. Negative control was prepared using respective solvent. Ciprofloxacin (5 µg/µL) was used as positive control. All the plates were then incubated for 36 h at 37 °C respectively. The sensitivity was recorded by measuring the clear zone of growth inhibition of agar surface around the well in millimeter.The experiments were carried out in triplicates with each compound and the average values were calculated for determining the bactericidal activity.

Result and discussion

1. Powder X-ray diffraction studies (PXRD)

The PXRD pattern reveals that the biosynthesized silver Nanoparticles (Ag NPs) prepared by using leaf extract of *Selaginella tenera*. All the diffraction patterns (111), (200), (220) and (311) were well indexed to a colloidal Ag NPs with JCPDS Card No: 04-0783. Four intense and sharp peaks at 2θ = 38.16°, 44.29°, 64.62° and 77.50° can be indexed to the Bragg's reflection of silver respectively.

The PXRD pattern thus clearly indicated that (Fig.1) the AgNPs organized by the reduction of Ag^{+} ions by the aqueous extract of Selllagenella were crystalline in nature. The size range of the synthesized AgNPs was 5–20 nm with cubic type.

The average crystallite size (D) was estimated from the line broadening in PXRD using Debye Scherrer’s formula [91-92] is found in the range 5-20nm.

$$D = \frac{K\lambda}{\beta\cos\theta} \qquad (1)$$

Where D is the average crystallite size in Å, K is the shape factor (0.9), λ is the wavelength of X-ray (1.5406 Å) Cu Ka radiation, θ is the Bragg angle, and β is the full width at half maximum (FWHM) (Chandra Babu *et al*., 2013) [3].

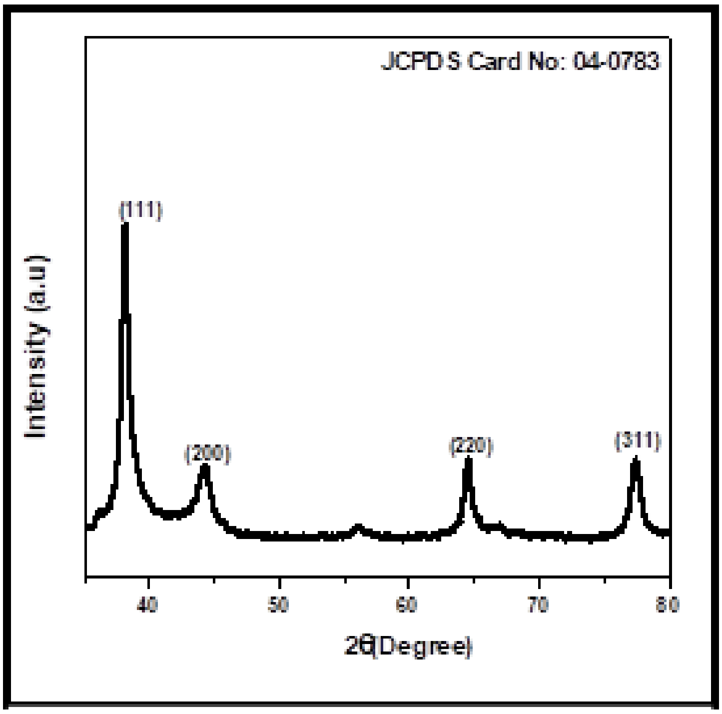

Fig 1: PXRD pattern of green synthesized silver Nanoparticles

2. Ultraviolet–visible spectroscopy (UV-Vis) studies

The UV–Visible spectrum of the Synthesized Silver nanoparticles dispersed in deionized water. The UV–Vis spectrum of silver nano particles giving a plasmon resonance. The surface plasmon absorption, metal oxide to collective oscillation of the free conduction band electrons which is excited by the incident electromagnetic radiation. This type of resonance, the wavelength of the incident light far exceeds the particle diameter. Surface Plasmon absorption band with a maximum at 360-380 nm indicates the formation of silver nanoparticles (Holt & Bard, 2005; Sasikala *et al*., 2015) [6, 22].

3. Fourier Transform Infrared (FTIR) Spectroscopy studies

The FTIR measurements were carried out in order to identify the presence of various functional groups in biomolecules responsible for the bio-reduction of Ag+and capping/stabilization of silver nanoparticles. The observed intense bands were compared with standard values to identify the functional groups. The (Fig. 02) FTIR analysis of this study show different stretches of bonds shown at different peaks; 3405.65—N–H stretch, 2920.06—single aldehyde, 2847.13—C–H; O–H, 1645.60—C≡C, 1642.78—C=C, 1450.72—C=C, 1234.32—C=O and 1031.08—C=O.

The peaks near 3440 cm−1, 2924 cm−1, and 2854 cm−1 assigned to OH stretching and aldehydic C–H stretching, respectively. The weaker band at 1629 cm−1 corresponds to amide I arising due to carbonyl stretch in proteins. The peak at 1041 cm−1 corresponds to C–N stretching vibration of the amine. The peak near 1645.60 cm−1 corresponds to C=C stretching (non conjugated). FTIR spectra of silver nanoparticles exhibited prominent peaks at 2920.06, 2847.13, 1645.60, 1642.78, 1450.72, 1234.32—and 1031.08. The spectra showed sharp and strong absorption band at 1,642.78 cm−1 assigned to the stretching vibration of (NH) C=O group. The band 1,450.72 developed for C–C and C–N stretching; presence of the sharp peak at 2,920.06 cm−1 was assigned to C–H and C–H (methoxy compounds) stretching vibration, respectively (Raveendran *et al.*, 2003; Shaik *et al.*, 2013) [21, 23].

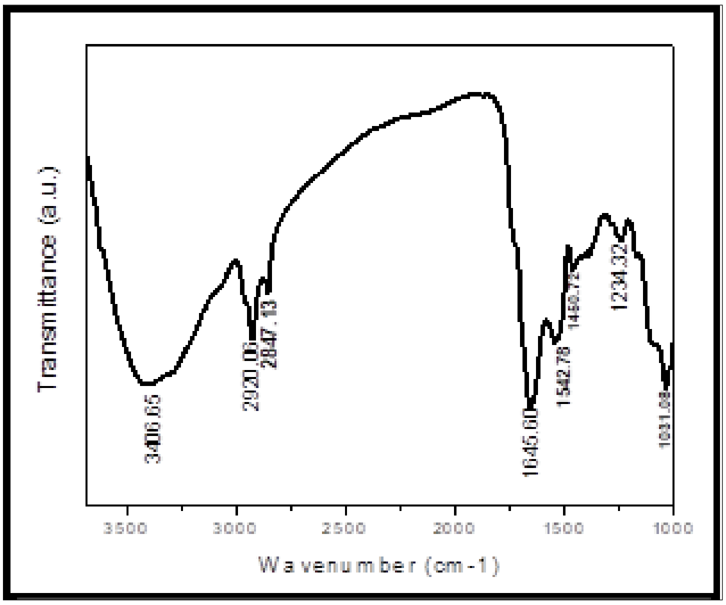

Fig 2: FTIR analysis of green synthesized silver nanoparticles

4. Scanning electron Microscopy (SEM) studies

SEM image (Fig.3) of the samples obtained from the colloidal Ag solutions prepared at room temperature confirms the existence of very small and uniformly spherical nano particles. From the SEM images it can be observed that larger particles of Ag NPs are formed due to aggregation of nano particles which might be induced by the phyto constituents are present in the concentration of plant extracts.

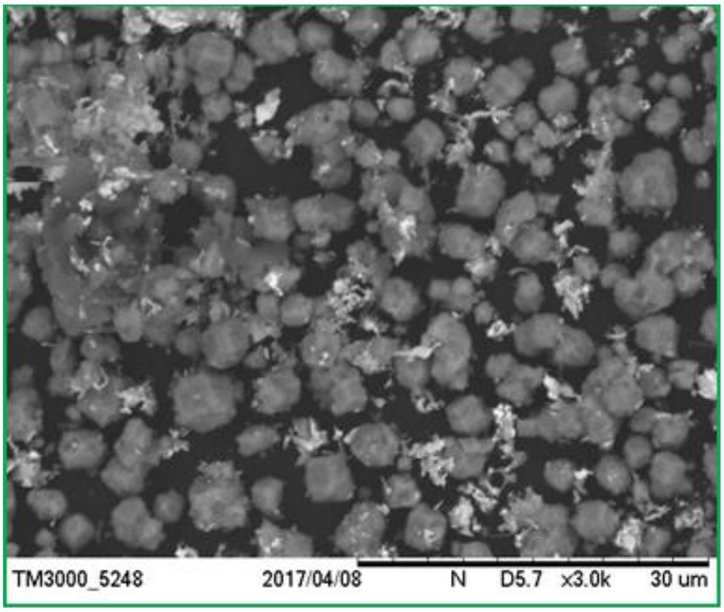

Fig 3: SEM micrograph of green synthesized Silver Nanoparticles

5. Antioxidant activity

Antioxidant activity of Silver NPs by DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity. This activity was increased by increasing the concentration of the sample of Silver NPs. DPPH antioxidant assay is based on the ability of 1,1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The IC_{50} value of the Green synthesized AgNPs is 3.12 mg/ml and its well-known antioxidant as shown in the Fig.04 (Gupta & Sharma, 1899) [5].

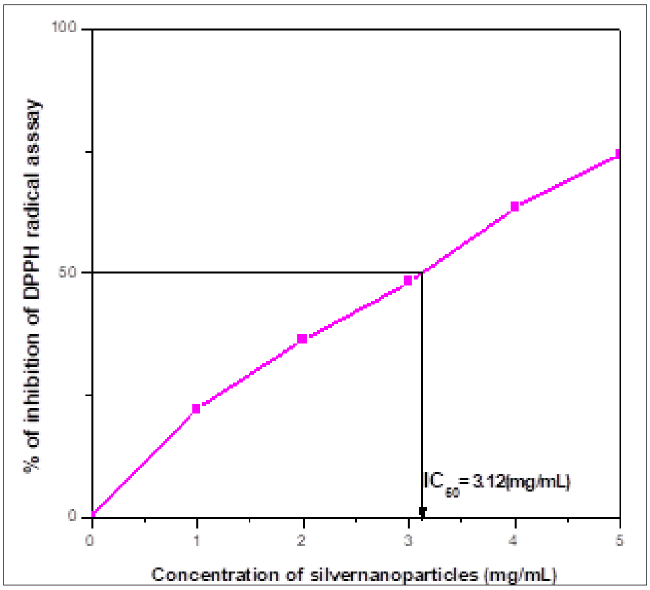

Fig 4: Antioxidant activities of green synthesized silver Nanoparticles

6. Antibacterial activity

Antibacterial activity of AgNPs was screened by agar well diffusion against pathogenic bacterialstrainssuch as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus haemolyticus*and *Staphylococcus aureus*. The inhibition zone of Ag NPs with different concentration (200, 400, and 600µg/well) with respect to the positive control (Ciprofloxacin) were depicted in the Fig.05 and the values are noted in table.1.

Green synthesized silver nanoparticles shows significant antibacterial activity highly moderate in *E.coli*and followed by*P.aeruginosa*, *S.haemolyticus* less moderate in *S.aureus*pathogenic bacterial strains. The antibacterial activity ofAg-NPs is related to the formation of free radicals (Logeswari *et al*., 2013; Rao & Gan, 2015) [12, 20]. The free radicals generated by the Ag-Apoinducer bacterial cell membrane damage and its role of ROS can exist naturally in intracellularand extracellular locations. Under certain conditions, high levels of ROS can increase oxidative stress in cells. Oxidative stress can not only cause damage to the cell membrane, but can also cause damage to the proteins, DNA, and intracellular systems such as the respiratory system. The significant of bactericidal effects indicate that Ag-NPs can form ROS with water, and so bacterial cell membrane, protein structure and intracellular system can be damaged owing to the ROS formed by Ag-NPs.

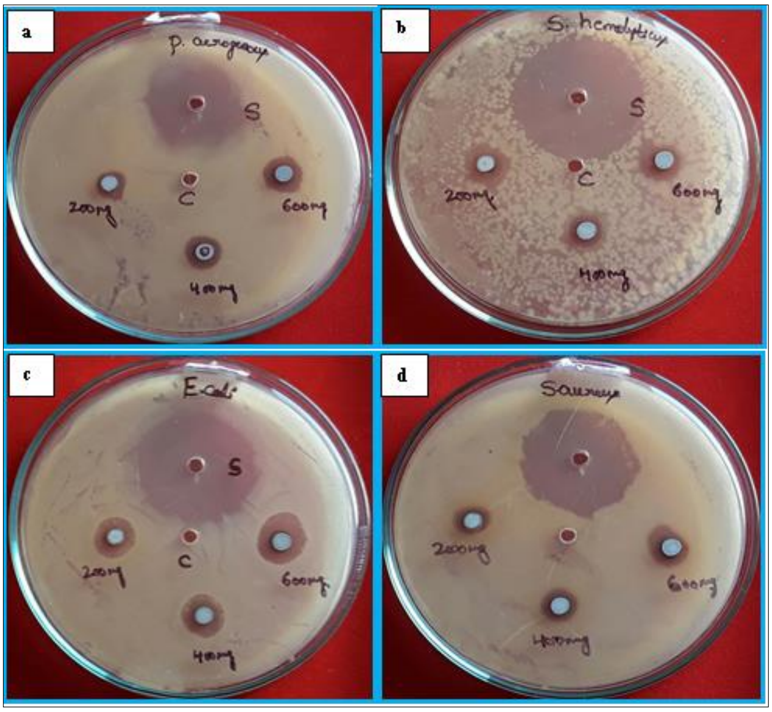

Fig 5: Antibacterial activity of silver nanoparticles against bacterial strains (a) *P.aeroginosa* (b) *S.haemolyticus* (c) *E.coli* (d) *S.aureus*

Table 1: Antibacterial activity of silver nanoparticles on pathogenic bacterial strains

Treatment	*P.aerogenosa* (Mean±SE)	*S. hemolyticus* (Mean±SE)	*E.coli* (Mean±SE)	*S.aureus* (Mean±SE)
Standard(5µg/µL)	10.33±0.67	13.33±0.67	11.67±1.33**	12.67±1.33**
(200µg/µL)	1.33±0.67	1.33±0.33**	1.67±0.33**	1.33±0.33**
(400µg/µL)	2.67±0.33**	2.33±0.33**	2.67±0.33**	2.33±0.33**
(600µg/µL)	3.67±0.33**	3.67±0.33**	4.67±0.33**	3.33±0.33**

Values are the mean ± SE of inhibition zone in mm. *Symbols represent statistical significance, *P < 0.05, **P < 0.01 as compared with the control.

Conclusion

In the present investigation, a facial approach for biosynthesis of AgNPs from leaves extract of *Sellaginella tenera* acts as reducing agent. The PXRD pattern shows that the Ag NPs are cubic in structure, and the average crystallite size as 10-20 nm. The UV-Visible absorption spectrum shows the Surface Plasmon absorption band with a maximum at 360-380 nm indicates the formation of silver nanoparticles. FTIR measurements were carried out in order to identify the presence of various functional groups in biomolecules responsible for the bio reduction of Ag+ and capping/stabilization of silver nanoparticles. From SEM micrographs, the particles show uniformly spherical like shape and high dependant on concentration of fuel. The synthesized AgNPs have shown more significant antioxidant and antibacterial activities *invitro* conditions. Thus, it is concluded that the phytosynthesis of AgNPs using *Sellaginella tenera* leaves extract is a cost effective, simple and ecofriendly method that excludes the hazards arising out of the use of harmful reducing/capping agents. Moreover, this process could be easily scaled up for the industrial applications to increase the yield of the nanopartilces significantly, which undoubtedly would establish its commercial viability in medicine.

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