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BIOLOGICAL SYNTHESIS OF SILVER NANOPARTICLES OF *ADIANTUM CAPILLUS-VENERIS* L. AND THEIR EVALUATION OF ANTIBACTERIAL ACTIVITY AGAINST HUMAN PATHOGENIC BACTERIA

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Keywords:

Adiantum capillus-veneris, Scanning electron microscopy, Nanoparticles, antibacterial activity.

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ABSTRACT: To develop a novel approach for the biological synthesis of silver nanoparticles using water extract of whole plant parts of *Adiantum capillus-veneris* L. which has been proven to be active against human pathogenic bacteria such as Streptococcus pyogenes, Staphylococcus aereus, Escherichia coli and Klebsiella pneumonia. Characterization of nanoparticles was determined by using Ultra Violet-Visible (UV-Vis) spectrometry, Scanning Electron Microscopy (SEM), Energy Dispersive X-ray (EDX), X-ray Diffraction (XRD). The synthesis of nanoparticles was confirmed by the change of colour pale green to reddish brown. Further, a peak between 200nm to 2500nm wavelength was obtained on UV-VIS spectrometer which confirmed the biosynthesis of silver nanoparticles. SEM showed the formation of Silver nanoparticles with an average size of 25.71 nm-37.96 nm. X-ray diffraction analysis showed that the particles were crystalline in nature with cubic structure of the bulk silver with the broad peaks 38^0, 64^0, 67^0. It concluded that present study of *A. capillus-veneris* can be good source for biological synthesis of silver nanoparticles which shows a good antibacterial activity of human pathogenic bacteria. The outcome of the study will be the development of new anticancer drugs from the synthesized nano particle of the *Adiantum capillus-veneris* for biomedical and other industrial applications.

INTRODUCTION: The development of green processes for the synthesis of nanoparticles is evolving into an important branch of nanotechnology [1, 2]. The field of nanotechnology is one of the most active areas of research in modern materials science. Nanoparticles exhibit completely novel and improved properties based on specific characteristics such as size, distribution and morphology. New application of nanoparticles and nanomaterials are emerging rapidly [3, 4].

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Nanotechnology involves tinkering work at atomic levels, tweaking and controlling substances 1, 00, 000 times smaller than a strand of human hair, to make useful materials and devices. It involves technology at the scale of one-billionth of a meter. The term 'NANO' is derived from Greek word "Dwarf" [5]. Nanotechnology is foreseen to significantly influence science, economy and everyday life in 21st century and it may become one of the driving forces to the next industrial revolution. Nanoparticles are being viewed as fundamental building blocks of nanotechnology [6].

Nanoparticles are synthesized by reduction of heavy metals in the solutions of plant extract [7], thermal decomposition of silver compounds [8], microwave assisted synthesis [9, 10] laser mediated synthesis [11] and biological reduction method [12].

Drug development of the most preferred way for synthesis of nanoparticles as it offers one step, ecofriendly way of synthesis of nanoparticles. Synthesis of silver nanoparticles by biological method using bacteria, fungi, algae, enzymes and plant extracts has more advantages due to their environment benign process and ability of large scale production over physical and chemical methods [13].

An eco-biological approach to the synthesis of silver nanoparticles is an eco-friendly and cost effective method as compared to the other chemical and physical methods. Several antibiotics use silver compounds such as metallic silver, silver nitrate, silver sulfadizine for treatment of burns, wounds and several bacterial infections [14]. Since nanoparticles of noble metals such as silver, gold, and platinum are widely applied to human contacting areas, there is a growing need to develop environment friendly processes for the synthesis of nanoparticles, that do not use toxic chemicals [15, 16]. Among the above forms, silver nanoparticles play a significant role in the field of biology and medicine[17, 18].

The present studies describe an eco-friendly approach for the synthesis of silver nanoparticles, from plants using biological synthesis. To our best knowledge with all the possible referencing, we state that it is the first study that uses Pteridophytes as plant source for synthesis of silver nanoparticles. *Adiantum capillus-veneris* belongs to the family Adiantaceae; it is also called as 'Maiden hair fern or five finger fern'.

In the literatures, many health benefits associated to occurrence of different classes of bioactive secondary metabolites have been reported in this plant species. These classes include, among others, flavonoids and polyphenol which possessed antimicrobial, antioxidant and cytotoxic activities [19, 20]. *Adiantum capillus–veneris* is an important natural source of compounds from both classes. Because of the high level content of flavonoids and phenols presented in it, the biological properties attributed to this species, including anti-inflammatory, anti-infective and anti-tumors may originate from the these components [21] and the probable functional mechanism were antimicrobial and antioxidant effects [22, 21, 23].

MATERIALS AND METHODS:

Collection of Plant Material:

The whole plant of *Adiantum capillus-veneris* was collected from the fields, at the foothills of Valparai, Western Ghats of Coimbatore district, of southern India. The samples of plants were identified self and binomially by Botanical Survey of India (Southern part Coimbatore, Tamilnadu, India) and voucher specimens were deposited at the Herbarium Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamilnadu, India.

Preparation of the Extract:

The whole plant of *Adiantum capillus-veneris* was used to make an aqueous extract. The plant materials weighing 20g were thoroughly washed in distilled water and filtered through Whatmann No.1 filter paper (Pore size 25 µm). The filtrate was further filtered through 0.6 µm sized filters.

Synthesis of silver Nanoparticles:

1mM aqueous solution of Silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 10 ml of plant extract was added into 90ml of aqueous solution of 1mM silver nitrate for reduction into Ag^+ ions and kept at room temperature for 10-12 hours.

Characterization of silver nanoparticles:

UV-VIS Spectra Analysis:

The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 10-12 hours. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-2450 (Shimadzu).

XRD Measurement:

The silver nanoparticle solution obtained was purified by repeated centrifugation at 5000 rpm for 30 minutes followed by redispersion of the pellet of silver nanoparticles in to 10 ml of deionised water. After freeze drying of the purified silver particles, the structure and composition were analyzed by XRD and SEM. The dried mixture of silver nanoparticles was collected for the determination of the formation of Ag nanoparticles by an X' Pert

Pro X-ray diffractometer (PAN analytical BV, The Netherlands) operated at a voltage of 40 kV and a current of 30 mA with Cu K α radiation in a configuration.

Sem analysis of silver nanoparticles:
Scanning Electron Microscope (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the films on the SEM grid were allowed to dry by putting (placing) them under a mercury lamp for 5 minutes.

EDX Measurements:
Energy dispersive X-ray analysis, the whole plant extracts of *Adiantum capillus-veneris* were dried and drop coated on to carbon film and performed on JEOL-MODEL 6390 SEM instrument equipped with a Thermo EDX attachments.

Antibacterial assay:
The effectiveness of silver nanoparticles in antibacterial assays were done on human pathogenic microorganisms using two positive and negative bacteria such as *Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumonia* by disc diffusion method. Fresh overnight culture of inoculums (100 mL) of each culture was spread on to Muller Hinton Agar (MHA) plates. Sterile paper disc of 5 mm diameter containing 10 mg/l silver nanoparticles and standard antibiotic erythromycin discs were placed in each plate as control. The plates were incubated at 37^{o}C for overnight (24 h) and the inhibition zones around the discs were measured [24].

Statistical analysis:
All analyses were carried out in triplicate and the data were reported as mean±SD. Where there was significance of the difference between means was determined by Duncan's multiple range test ($P<0.05$) using ANOVA.

RESULTS:

Synthesis of silver nanoparticles:
Synthesis of silver nanoparticles from silver nitrate is one of the most widely used methods for the synthesis of silver colloids. During the biosynthesis using the extract the colour of the reaction medium changed rapidly from light greenish to dark yellowish brown (**Fig**. **1a**, **b**) due to Surface Plasmon Resonance. This occurs due to the collective oscillations of the conduction electrons confined to metallic nanoparticles.

This results in strong light scattering, by an electric field at a wavelength where resonance occurs resulting in appearance of strong absorbance bands. It is well known that silver nanoparticles exhibit a yellowish-brown colour in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles. Reduction of silver ions to silver nanoparticles could be followed by a colour change and UV-Vis spectroscopy.

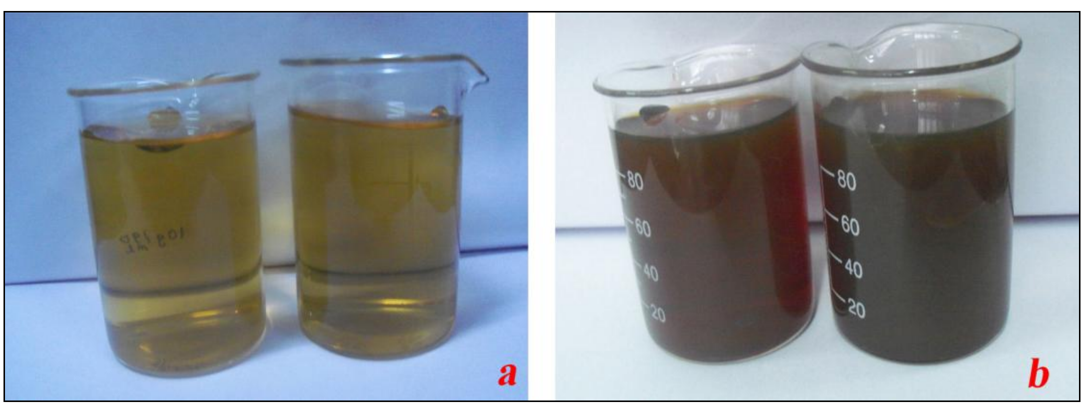

FIG. 1: PHOTOGRAPHS SHOWING COLOR CHANGES AFTER ADDING $AgNo_3$ BEFORE REACTION (a) AND AFTER REACTION TIME OF 10-12 H (b)

UV-Vis spectra analysis: The silver nanoparticles were characterized by UV-Vis spectroscopy, one of the most widely used techniques for structural characterization of silver nanoparticles. The absorption spectrum (**Fig**. **2**) of the yellowish-brown silver nanoparticles solution prepared with the proposed method showed a surface Plasmon absorption band with a maximum of 200-2500 nm, indicating the presence of spherical Ag nanoparticles.

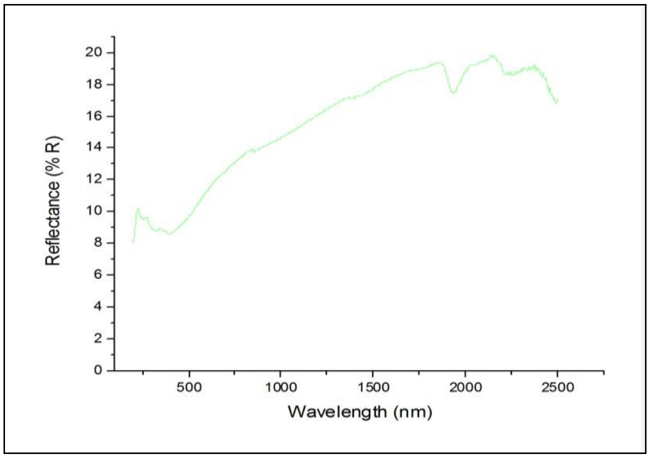

FIG. 2: SHOWING THE UV-VIS ABSORPTION SPECTRA OF AQUEOUS SILVER NINTRATE WITH WHOLE PLANT PARTS OF *ADIANTUM CAPILLUS-VENERIS*.

Scanning Electron Microscope and EDX studies:

SEM technique was employed to visualize the size and shape of silver nanoparticles. In (**Fig**. **3**), SEM images were obtained from synthesis of silver nanoparticles of *Adiantum capillus-veneris* whole plant extract. The formation of silver nanoparticles as well as their morphological dimensions in the SEM study demonstrated that the average size was from 25.71 nm-37.96 nm with interring particle distance. The shape of the silver nanoparticles proved to be spherical shaped. Analysis through EDX spectrometers confirmed the presence of elemental silver signal of the silver nanoparticles (**Fig**. **4**).

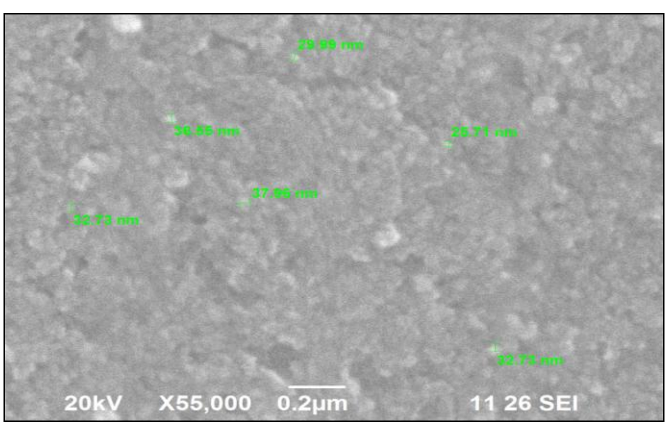

FIG. 3: SCANNING ELECTRON MICROSCOPIC IMAGES OF SILVER NANOPARTICLES FORMED *ADIANTUM CAPILLUS-VENERIS*

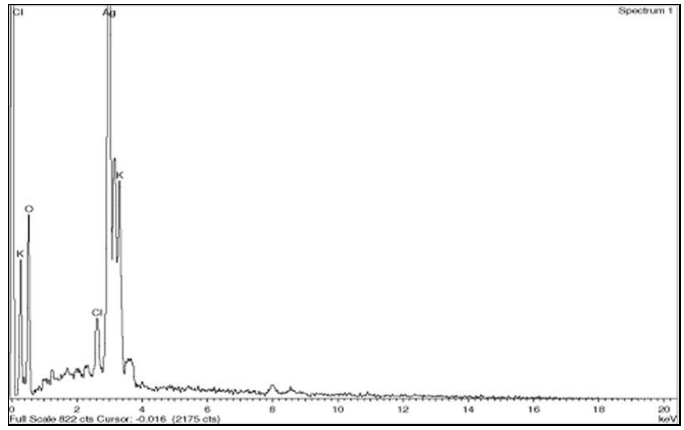

FIG. 4: EDX SPECTRA RECORDED FROM A FILM, AFTER FORMATION OF SILVER NANOPARTICLES WITH DIFFERENT X-RAY EMISSION PEAKS LABELED.

XRD studies:

Figure showed the XRD Conforming the existence of silver colloids in the sample. The Braggs reflections were observed in XRD pattern at 2 Θ = 38^0, 64^0, 67^0. Hence the XRD pattern thus clearly illustrated that the silver nanoparticles formed in this present synthesis are crystalline in nature. Peak obtained at around 38^0 indicate that the prepared samples are crystalline in nature. The crystalline size (D) was calculated using the Scherer's formula from the fill width maximum (FWHM)

$$D = kd / \beta \cos \Theta$$

Where, kd constant = 1.47897×10^{-10}

Where D is the average crystallite domain size perpendicular to the reflecting planes. λ is the X ray wave length. β is the full width at half maximum and θ is the diffraction angle.

The crystalline size was found to be around 18.76 nm to 33.74 nm.

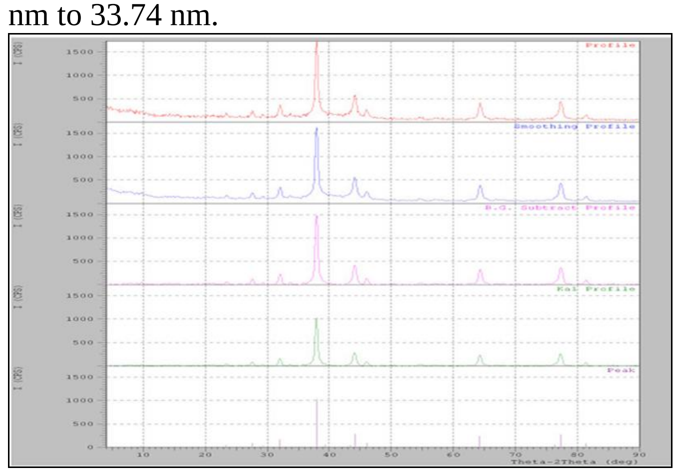

FIG. 5: XRD PATTERN OF SILVER NANOPARTICLES FORMED AFTER REACTION OF PLANT EXTRACTS *ADIANTUM CAPILLUS-VENERIS*

Antibacterial activity:

The antibacterial activity of silver nanoparticles is reported to a large extent. The silver nanoparticles obtained from *Adiantum capillus-veneris* also showed antibacterial activity against four strains of laboratory human pathogenic bacteria viz. *Streptococcus pyogenes, staphylococcus aureus, Escherichia coli* and *Klebsiella pneumonia*. The water extract of *Adiantum capillus-veneris* silver nanoparticles showed very high inhibitory activity against *staphylococcus aureus,* followed by *Streptococcus pyogenes, Escherichia coli* and *Klebsiella pneumonia* than standard drugs. The measurement of zone of inhibition is summarized in **Table 1**. From the table, it is evident that the nanoparticles synthesized are good candidate their usage as antibacterial drug.

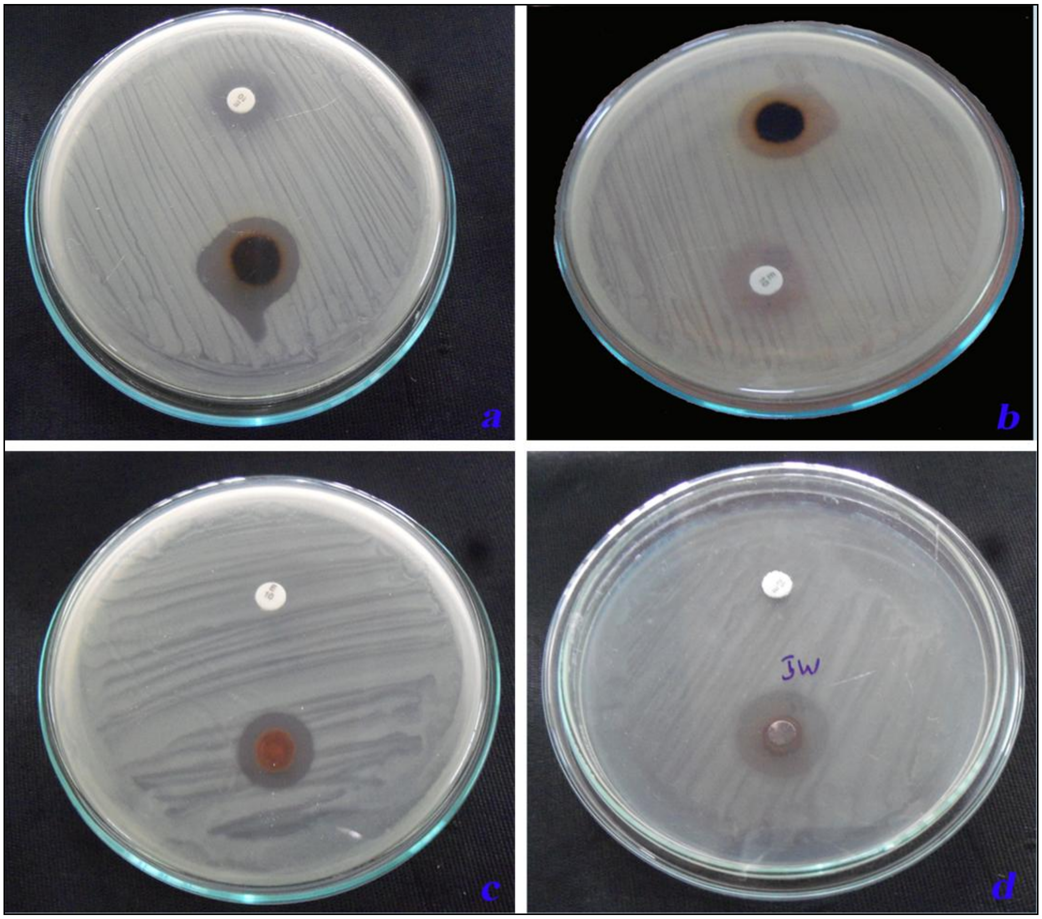

FIG. 6: PHOTOGRAPHS OF ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES AGAINST a) *STREPTOCOCCUS PYOGENES*, b) *STAPHYLOCOCCUS AUREUS*, c) *ESCHERICHIA COLI*, d) *KLEBSIELLA PNEMONIAE*

TABLE 1: ANTI-BACTERIAL ACTIVITY OF *ADIANTUM CAPILLUS-VENERIS* AGAINST PATHOGENIC BACTERIA

S. No	Pathogenic bacteria	Zone of inhibition (mm)	
		Silver nanoparticles	Erythromycin*
1	*Streptococcus pyogenes*	25.3 ± 1.24^{ab}	0.7 ± 0.0^{a}
2	*Staphylococcus aureous*	27.6 ± 1.24^{a}	0.6 ± 0.0^{ab}
3	*Escherichia coli*	17.6 ± 1.2^{c}	0.0
4	*Klebsiella pneumonia*	14.6 ± 1.2^{d}	0.0

*Standard antibiotic

Each value represents the mean ± SD, n=3 mean followed by same letter(s) in each column are not significantly different at p< 0.001 according to Duncan's multiple range tests.

DISCUSSION: Reduction of silver ion into silver particles during exposure to the plant extract could be followed by colour change. Silver nanoparticles exhibit dark yellowish-brown colour in aqueous solution due to the surface plasma resonance phenomenon. Formation and stability of silver nanoparticles in aqueous colloid solution are conformed using UV-VIS spectral analysis. As the Adiantum capillus-veneris extract with mixed with aqueous solution of silver nitrate, it started to change the colour yellowish brown to reddish brown due to the reduction of silver iron. Similar results were obtained from the extra cellular biological synthesis of silver nanoparticles (Ag NP's) using plant extracts *Lantana camera* for the reduction of aqueous Ag^+ ions. Silver nanoparticles were formed by treating aqueous solution AgNO3 with the plant leaf extracts as reducing against Ag^+ to AgO [25].

Scanning electron microscopic observation of crystal formation size ranges from 24nm-38nm was recorded in the gold nanoparticles from the plant species of *Putranjiva roxburghii* [26]. The energy dispersive x-ray diffraction spectra, it is clear that silver nanoparticles by *Adiantum capillus-veneris* have the weight percentage of silver as 48.41% and 43.47% respectively. Similar phenomenon was recorded by the various researcher in plant species of *Aloe vera*, *Cassia auriculata* and *Ocimum basillicum* [27, 28, 29]. In our investigation of the energy dispersive x-ray diffraction analysis, the specimen is bombarded with an electron beam inside the scanning electron microscope. The bombarding electrons collide with the specimen atoms' own electrons, knocking some of them off in the process. A position vacated by an ejected inner shell electron is eventually occupied by a higher energy electron from an outer shell. To be able to do so, however, the transferring outer electron must give up some of its energy by emitting an X-ray.

In addition to the Braggs peaks representative FCC silver nanoparticles, additional yet unassigned peaks were also observed suggesting that the crystallization of bioorganic phase occurred on the surface of the nanoparticles. Similar result was recorded in Ag_2O obtained major peak at 38^0 [30]. The mechanism of the bactericidal effect of silver and silver nanoparticles remains to be understood. Several studies propose that silver nanoparticles may attach to the surface of the cell membrane disturbing permeability and respiratory function of the cell [31]. It is also possible that silver nanoparticles not only interact with the surface of membrane, but can also penetrate inside the bacteria. It may be observed that silver nanoparticles have comparatively higher anti-bacterial activity against gram negative organism than gram positive, probably due to thinner peptidoglycan layer and presence of porins.

The attachment of either silver ions or nanoparticles to the cell wall caused accumulation of envelope protein precursors, which resulted in dissipation of the proton motive force. Silver nanoparticles also exhibited destabilization of the outer membrane and rupture of the plasma membrane, thereby causing depletion of intracellular ATP [32]. Similarly the silver nanoparticles were obtained from bryophytic plant species of *Anthocerous* showed the antibacterial activity against laboratory pathogens, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeurginosa* and *Bacillus subtilis* [33]. Recent reported were obtained from the extracellular biosynthesis of silver nanoparticles using *Pseudomonas fluorescens* [34].

The antibacterial efficacy of the biogenic silver nanoparticles could be ascribed to the mechanism described above but still remains to clarify the exact effect of the nanoparticles on important cellular metabolism like DNA, RNA and protein synthesis. Therefore, it can be stated that emerging trends in nanotechnology has made possible to address the problems and challenges associated with potential natural products to be developed as antimicrobial drug [35]. During recent times, nanoparticles have gained importance in the field of biomedicine. The most important and distinct property of nanoparticles is that they exhibit larger surface area to volume ratio. Specific surface area is relevant for catalytic reactivity and other related properties such as antimicrobial activity in silver nanoparticles. As specific area of nanoparticles increased, their biological effectiveness can increase due to the increase in surface energy.

CONCLUSION: Here in, reported for the first time the synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by the cell free aqueous extract of *Adiantum capillus-veneris*. These biologically synthesized silver nanoparticles were found highly toxic against different multi drug resistant human pathogenic bacteria. We have described a simple environmentally benign method of synthesis of silver nanoparticles from a novel primitive plant source. The silver nanoparticles can be used in various fields of application such as medicine, agriculture, foods, paint industry, pharmaceutical industry. Future therapeutic directions of silver nanoparticles could be anticancer, anti-inflammatory agent, antiviral drug, and anti-platelet agent.

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