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Green synthesis and characterization of silver nanoparticles using ethanolic extract of Mimosa Pudica linn leaves

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Green synthesis of metal nanoparticles using plant extract is an eco-friendly and cost-effective method for synthesizing metal nanoparticles. In this present work silver nanoparticles have been synthesized using ethanolic extract of Mimosa Pudica Linn plant (MPL) leaves. Green synthesized silver nanoparticles (AgNps) have been characterized by UV-Vis spectrometer, Fourier Transform Infra-Red ((FTIR) spectra, dynamic light scattering (DLS), Scanning Electron Microscopy (SEM) and X-ray diffraction (XRD) methods. UV-Vis spectrum of synthesized silver nanoparticles from Mimosa pudica Linn leaves extract shows a characteristic absorption peak at 421 nm. FTIR analysis reveals that presence of silver nanoparticles the presence of some biomolecules in extracts that act as reducing and capping agent for green synthesis of silver nanoparticles. DLS analysis showed AgNPs are drifted widely from 58.6 to 157.7 nm, with an average particle size of 104.7 nm. The particles are found to be polydisperse and slightly agglomerated due to the presence of phytochemicals present in the plant extract. Scanning electron microscope showed silver nanoparticles are spherical shaped. The XRD pattern revealed the presence of crystalline, dominantly spherical silver nanoparticles in the sample having size ranging from 42 to 50 nm. The XRD peaks 38.08°, 44.22°, 64.42°, and 77.32° for leaves extract and 38.1°, 44.3°, 64.5°, 77.5°, and 81.33° for callus extract can be assigned the plane of silver crystals (111), (200), (220), and (311), respectively, and shows face-centered, cubic and crystalline nature of the silver nanoparticles. The green synthesized silver nanoparticles show significant antibacterial activity.

Keywords: Antibacterial, FTIR, Mimosa pudica Linn, Particle size, SEM, Silver nanoparticles, UV-Vis, XRD

The process of synthesizing nanoparticlesof variable sizes in the range of 1-100 nm, shapes, and chemicalcompositions with controlled dispersity for human benefits is known as nanotechnology[1]. Nowadays, metal nanoparticles synthesis has become an emerging area of research due to its potential application in various fields of science and engineering[2]. Metal nanoparticles have important role in field of high compassion biomolecular detection, medicine, biosensors and catalysis[3-6]. In addition to that, Silver nanoparticles have been proven to be a strong antibacterial effect along with the anti-fungal, anti-angiogenesis and anti-inflammatory activities[7].

Even, numerous physicochemical techniques such as chemical reduction, photochemical reduction, lithography, sonication, sputtering and sol gel are available for the preparation of silver nanoparticles, though, they include of hazardous chemicals which are harmfulfor sustaining the environment and also highly expensive[8-10]. However, recent issues on environmental sustainability led to explorations of concept of green synthesis for metal nanoparticles production using various biological entities[11].

Green chemistry isoneof the most profound implications on the wet chemical synthesis of metal nanoparticles, sinceit promotes the reactions without toxic chemicals such as solvents, reducing substance and stabilizers[12,13]. Nowadays, green synthesis of silver nanoparticles using plant extracts, micro-organism and proteins becomes the most popular due to their biocompatibility[12]. Many research works have been reported that silver nanoparticles are synthesized using plant extracts having bioactive reducing agents such as alkaloids, terpenoids, tannins, flavonoids and phenolic acids[14,15].

Mimosa pudica Linn (MPL) is one of the non-agricultural, eco-friendly and ecologically important plants in south India. Many research work have reported that Mimosa pudica Linn leaves extract possesses antidiabetic, antioxidant, antiviral, anti-inflammatory, anticancer, antipyretic, hypolipidemic, hepato-protective, and gastro protective activities[16-18]. Indeed, previous studies reported the phytochemicals such as alkaloid, flavonoids, tannin and phenolic acids present in Mimosa pudicaLinn[16-18]. Although, many high potential plants have been used as sources for synthesizing, metal nanoparticles are still largely unexplored[19]. Hence, in this present work silver nanoparticles particles (AgNPs) are synthesized by silver nitrate from its aqueous solution using ethanolic extract of MPL leaves extract as a reducing agent. AgNP sprepared by using Mimosa pudica Linn (MPL) leaves extract was characterized by UV-Vis spectrometer, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction XRD, dynamic lightscattering (DLS) and Scanning electron microscopy SEM analysis. Antibacterial activities of synthesized silver nanoparticles have also been analyzed against both Gram-positive and Gram-negative species of bacteria.

Experimental Section

Preparation of plant leaves extract

The fresh leaves of Mimosa pudica Linn (MPL) plant were collected from Trichy district in Tamil Nadu-India. The leaves were thoroughly washed with de-ionized water for four - five times to remove surface contaminant and it is air dried at room temperature. About 20 of the dried leaves were finely powdered with mortar and pestle and extracted with 250 mL of ethanol using Soxhlet apparatus. The leaves extract was stored at room temperature and used for green synthesis silver nanoparticles and further experimental analysis[16,19-20].

Phytochemical screening of plant extract

Ethanolic extract of MPL leaves was subjected preliminary phytochemical screening test such as tannins, saponins, flavonoids, alkaloids and terpenoids as per the standard procedure given in the literature[16,17].

Synthesis of silver nanoparticles

In-order to synthesis silver nanoparticles, about 10 mL of ethanolic extract of MPL leaves was added into flask containing 50 mL of 1mM aqueous silver nitrate solution with constant magnetic stirring at room temperature. The colour of the mixture changed from green to dark brown with the black suspended mixture within five min at room temperature. Then silver nanoparticles were separated from the suspended mixture by centrifugation[19-23].

Characterization of silver nanoparticles

UV-Visible absorption spectral of the above synthesized silver nanoparticles (AgNps) were measured using Spectrophotometer (model Shimadzu, UV-1601)[19,24]. Fourier transform infrared (FTIR) spectra for MPL leaves extract and silver nanoparticles were examined in the IR region 4000 to 400 cm^{-1} using Shimaduz 84005 FT-IRS spectrometer[19,23,25]. The particle size distribution of silver nanoparticles was monitored periodically by dynamic light scattering(DLS) method and nanoparticle size was measured using particle size analyzer (Beckman DelsaNano C series, USA)[26]. Surface morphology of the AgNps were analyzed by Scanning electron microscope (SEM) (JOEL JSM 6360) machine[23]. Crystalline nature of silver nanoparticles was determined using X-ray diffraction spectroscopy (Philips PAN analytical) equipped with Cu Kα (wavelength 1.5406 Å nm) as radiation source at a current of 30 mA, with an application of 45kV tension. The sample was examined at a temperature of 25°C in the 2θ ranges between 10° and 80°. Debye-Scherrer equation is used to calculate average particle size of the silver nanoparticles synthesized using MPL leaves extract. The Debye-Scherrer equation is

$$D = \frac{k\lambda}{\beta cos\theta}$$

where D is the size of AgNPs, λ is wavelength of the X-ray source (0.1541 nm) used in X-ray diffractometer. β is the FWHM value (full width at half maximum) of the diffraction peak and K is the Scherrer constant with a value from 0.9 to1, and θ is the Bragg angle[23]. As well as the antibacterial activity of green synthesized AgNPs has been investigated against multidrug resistant human pathogens.

Analysis for antimicrobial activity

The antimicrobial activity of AgNps prepared by using Mimosa pudica Linn (MPL) leaves extract was evaluated against *Escherichia coli* and *Bacillus subtilis* by disc diffusion method. The discs were prepared by Whatmann No.1 filter paper and kept on Mueller-Hinton agar plates. The sample of biosynthesized AgNps was placed on the disc using micropipette. The plates were placed in incubator overnight at 37°C and of inhibition zone of the microorganism were measured.

Results and Discussion

Synthesis of silver nanoparticles and UV absorption study

Phytochemical analysis of Mimosa pudica Linn (MPL) *leaves extracts* as per the standard procedures given in the literature confirmed the presence of tannins, saponins, flavonoids, alkaloids, terpenoids and phenolic acid like phytochemicals in the extract. In this present work silver nanoparticles were prepared by reduction of silver metalion from its aqueous solution using the extract of MPL plant leaves. Green colour of MPL leaves extract is changed into dark brown colour suspension solution within 5 min by the addition of aqueous solution of silver nitrate. Which indicates reduction of silver ion in aqueous solution has been accelerated by phytochemical substance present in the MPL plant leaves extract[19,24]. Reduction of silver metal ions to AgNPs was analyzed using UV-Visible spectrophotometer. Figure 1 shows UV-vis spectrum of silver nanoparticles synthesized using MPL leaves extracts. UV-Vis spectrum shows an absorbance maximum peak at 421 nm which confirms the formation of AgNPs[27].

FTIR spectral analysis of silver nanoparticles

FTIR spectrum of *MPL leaves extracts* and silver nanoparticles are given away in the Fig. 1a. In the FTIR spectrum of MPL leaves extract shows a broad band at 3476.87 cm^{-1} may be corresponding to stretching vibration of phenolic -OH group[19,25,27]. The peaks at 2922.86 cm^{-1} represents stretching -C-H vibration stretching of alkanes[25]. The peak observed at 2725.49 cm^{-1} may be due to the presence of -C=O stretching mode of aldehydes[23,25,27]. The N-H bend vibration of primary amines and C-H bend of alkanes appear at 1654.37 $cm^{-1}$and 1326.97 cm^{-1} respectively[19,27]. The FTIR spectrum of MPL leaves extracts exhibits several absorption peaks at different locations including at 3476 cm^{-1}, ~2725 cm^{-1}, ~1654 cm^{-1}, and ~1053cm^{-1}, which are associated with the several oxygen and nitrogen-comprising functional groups[25-28]. Most of the peaks also exist in the FTIR spectrum of AgNPs synthesized by using MPL leaves extracts with few marginal shifts. The above mentioned FTIR spectral peaks of MPL leaves extracts also appear in FTIR spectra of AgNPs (Figure 1b)with marginally shift in absorption peak and exist at ~3443 cm^{-1}, ~2850 cm^{-1}, 1631 cm^{-1} and 1033 cm^{-1}., which suggests that silver nanoparticles were surrounded by the phytochemicals present in the MPL leaves extracts[28]. Hence the present study reveals that MPL leave extract able to actas both reducing agent and stabilizing agent in green synthesis of silver nanoparticles[23,28].

Particle size analysis

Particle size of AgNps nanoparticles synthesized by using MPL leaves extracts was analyzed by dynamic light scattering (DLS) method using particle size analyzer. Figure 2 and Table 1 show that particle size of green synthesized AgNps particles are found to be in the ranges of 58.6 to 157.7 nmwith an average particle size of 104.7 nm. The Polydispersity index (PDI) is found to be 0.723, whichimplies high the nanoparticles are not monodispersed but slightly agglomerated[26,29,33]. This indicates, phytochemicals present in MPL leaves extract act as legends to effectively stabilize the silver nanoparticles by forming coordination bond with it[26,29].

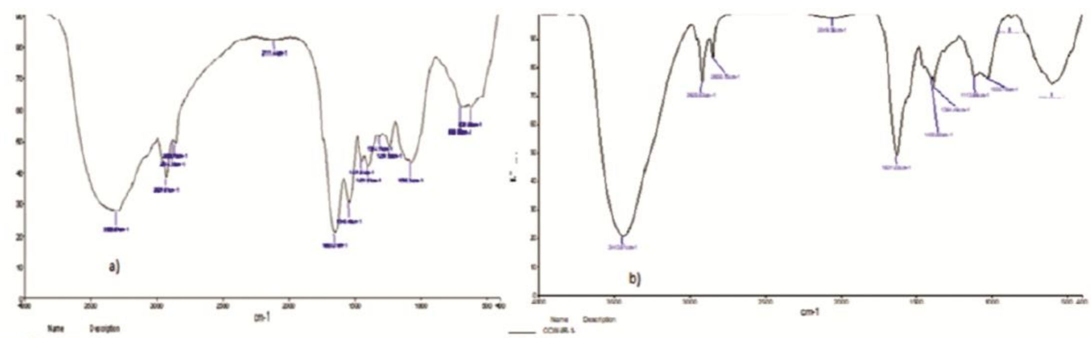

Fig. 1 — FTIR spectrum of a) Mimosa pudica Linn leaves extract and b) AgNPs synthesized using Mimosa pudica Linn leaves extract.

Table 1 — XRD results of silver nano particles

Pos. [°2θ]	Height [cts]	FWHM Left [°2θ]	β radian	d-spacing [Å]	Rel. Int. [%]	h k l Identifield from peak	Crystalline size D
38.0898	295.23	0.5669	0.0099	2.36064	100.00	(111)	148.16
44.2202	70.22	1.2114	0.0211	2.04656	23.78	(200)	070.92
64.4244	109.19	0.6056	0.0106	1.44506	36.99	(230)	154.59
77.3298	107.84	0.6870	0.0120	1.23294	36.53	(311)	147.98

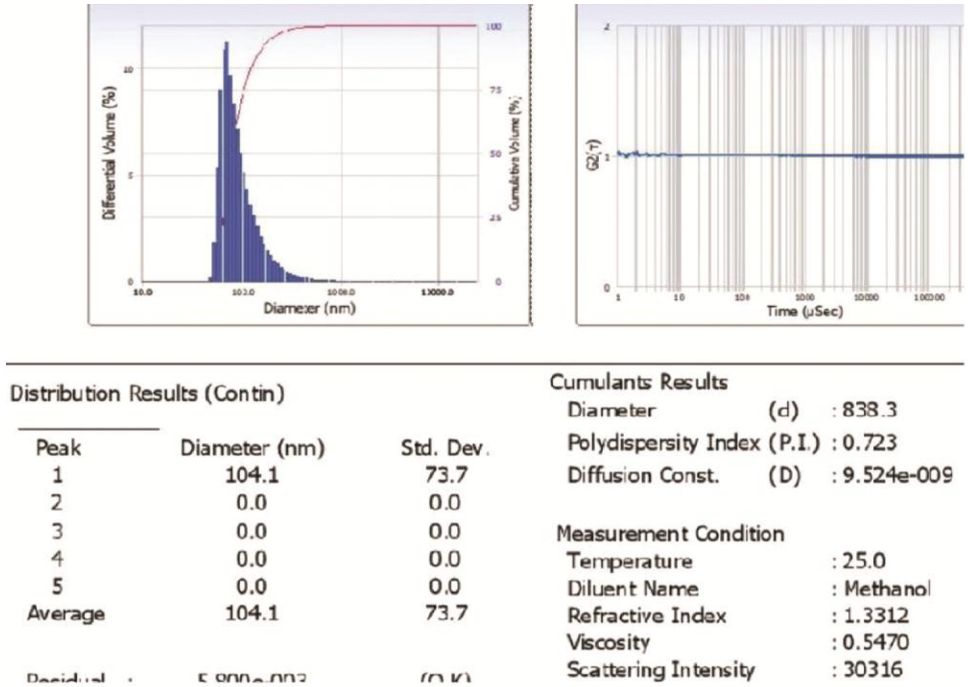

Fig. 2 — Particle size distribution of green synthesized AgNPs using Mimosa pudica Linn leaves extract on the basis of volume and number.

SEM morphological analysis

SEM morphological analysis of the silver nanoparticles is shown in Figure 3. The SEM image indicates the silver nanoparticles are found to be spherical and crystalline nature with particle size in the range of 200 nm. SEM analysis also indicates ununiform distribution of silver nanoparticles. The large size silver nanoparticles may be due to the aggregation of small particles[28,30,31].

XRD analysis

XRD analysis of the silver nanoparticles was done to determine crystalline nature of the AgNps. Figure 4 shows X-ray diffractograms pattern of silver nanoparticle. The XRD clearly showsfour welldefined high intensity peakswith 2θ values at 38.08° (111), 44.22° (200), 64.42 (220) and 77.32°(311) representing face centered cubic (FCC) structure silver nanoparticles compared with the standard powder diffraction card of Joint Committee on Powder Diffraction Standards (JCPDS), silver file No. 04–0783. Table 1 shows Miller indices values calculated from the experiential 2θ values corresponding to each peak in XRD pattern of silver nanoparticles.The average particle size calculated by using Debye-Scherer equation[32]. The average particle size estimated was approximately 130.60 nm which is in good agreement with the average particle size of the silver nanoparticles determined by DLS method.

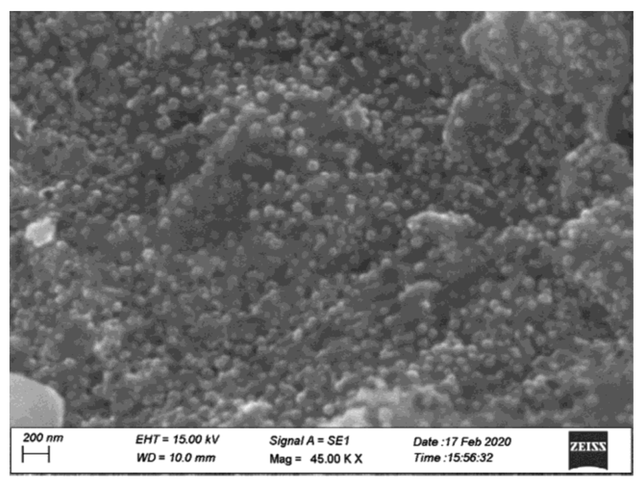

Fig. 3 — SEM image of AgNPs synthesized using Mimosa pudica Linn leaves extract.

Antimicrobial activity of the silver nanoparticles

Antibacterial activities of green synthesizesilver nanoparticles were analyzed by using disc diffusionmethod against most common diseases causing and multi-drug resistant pathogen (*Escherichia coli*, *Candida abicans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Aspergillus niger)*. Four wells with 6-mm diameter were made on LB agar plate using gel puncture to inculcate 25, 50, 75 and 100 µg/mL of synthesized sliver nanoparticles and then the plates were incubated at 35ºC overnight[33]. Figure 5 reveals the

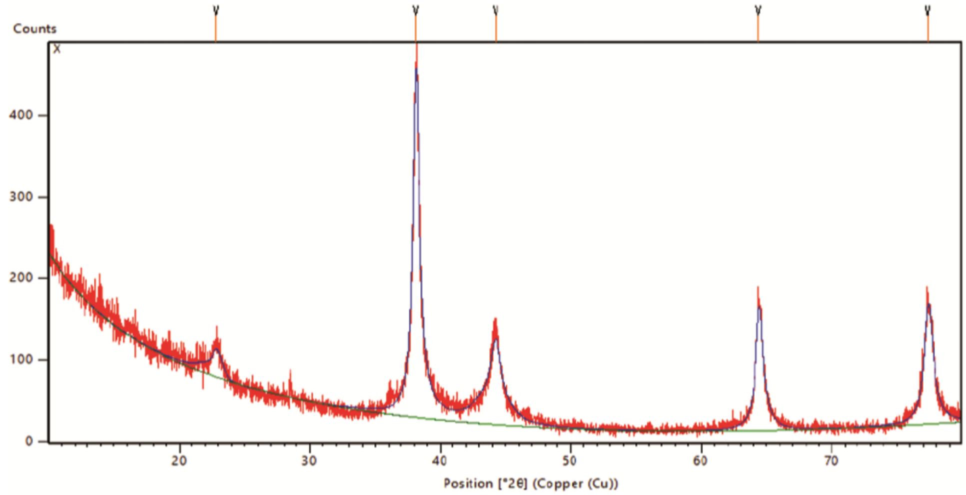

Fig. 4 — XRD-Pattern of AgNPs synthesized using Mimosa pudica Linn leaves extract.

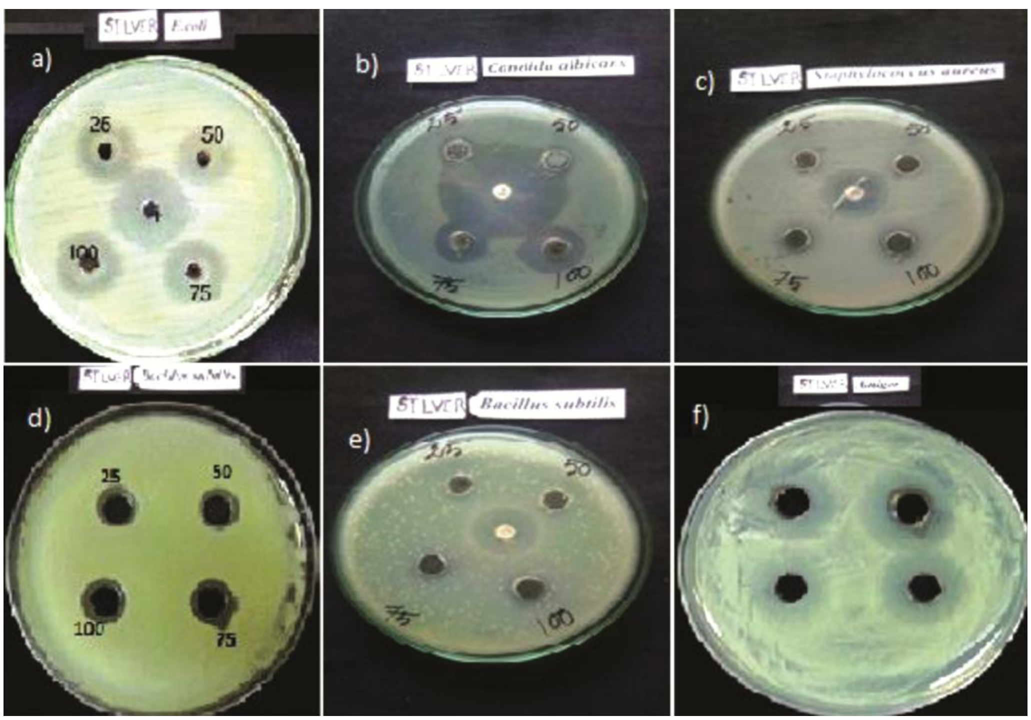

Fig. 5 — Anti-microbial activity of AgNPs against a) *Escherichia coli* b) *Candida albicans* c) *Staphylococcus aureus* d) *Pseudomonas aeruginosa* e) *Bacillus subtilis* and f) *Aspergillus niger*.

clear zone of inhibition around the discs saturated with synthesized AgNPs at 50, 75 and 100 μg/mL. The green synthesized AgNPs using MPL leaves extract exhibited the highest antibacterial activity against drug resistant *Candida abicans* followed by *Escherichia coli* and *Pseudomonas aeruginosa*. The results of this study propose that the green synthesized AgNPs were able to control the multidrug resistant pathogenic bacteria and it could be used in the medical field.

Conclusion

The present study has investigated an eco-friendly and novel approach for biosynthesis of AgNPs using the phytochemicals present in the leaves extract of Mimosa pudica Linn. The primary conformation for the formation of silver nanoparticles in for MPL leave extract medium was change in colour of the solution from green to dark brown and absorption peak found at 451 nm in UV-Vis spectral confirm the presence of silver nanoparticles[27]. The FT-IR spectrum accredited the phytochemicals present in the MPL leaves extract perform dual functions of formation and stabilization of silver nanoparticles[23, 27]. The peaks observed for the synthesized silver nanoparticles have face centered cubic crystalline structure the spherical shape of the synthesized silver nanoparticles is SEM analysis further confirm results of XRD analysis[23]. The present study shows that green synthesized silver nanoparticles have more effective antibacterial activity against the diseases causing pathogens. Thus, it is concluded that the green synthesis of AgNPs using MPL leaves extract is an eco-friendly, cost effective and simple method and this process could be easily scaled up for large scale production of silver nanoparticles and could be used as medicine.

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