

STUDIES ON SYNTHESIS, CHARACTERIZATION AND APPLICATION OF SILVER NANOPARTICLES USING *MIMOSA PUDICA* LEAVES

SAMUEL AKASH RAJ. R¹, DIVYA. S¹, SINDHU. S², KASINATHAN. K³ AND ARUMUGAM P^{2*}

¹Department of Biotechnology, St. Joseph's College of Engineering, Jeppiaar Nagar, Chennai, ²Armats Biotek Pvt Ltd., Maduvankarai, Guindy, Chennai 600 032, ³Biocontrol and Microbial Metabolites Lab, University of Madras, Guindy, Chennai 600025.
Email: armatsbiotek@gmail.com

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ABSTRACT

Objective: The present study is to synthesize nanoparticles using the leaf extracts of *Mimosa pudica* and screen for its α -amylase inhibiting potential as well as antibacterial properties.

Methods: Silver nanoparticles were synthesized using extract of *Mimosa pudica* leaves under various conditions such as sunlight, UV and room temperature. The synthesized nanoparticles were further characterized using UV, FTIR, XRD and SEM analyses. The inhibitory effect of the synthesized nanoparticles on bacterial pathogens and α -amylase activity was also studied.

Results: Exposure of reaction mixture to sunlight produced maximum amount of nanoparticles, comparatively. The particles showed a dose-dependent increase in percentage inhibitory activity on α -amylase enzyme. Moreover, the nanoparticles showed significant inhibitory effect on *Bacillus subtilis* and *Klebsiella pneumoniae*.

Conclusion: Thus the silver nanoparticles synthesized using *M. pudica* leaves prove to be a significant source of therapeutic agents, however further mechanistic studies are necessary.

Keywords: *Mimosa pudica*, α -amylase inhibition, Antimicrobial, Silver nanoparticle, SEM, FTIR.

INTRODUCTION

Nano, a scientific term used for determining the size of the particle [1]. Nanotechnology, a concept in the field of science and technology, in recent years, has also been likely to grow based on their demand, like other technologies. Nanoparticles are usually a cluster of atoms ranging between 1-100nm in size and they exhibit new properties based on their size, distribution and morphology [2]. Many materials are synthesized in nano size for various applications including medicine, mechanical, Biomedical electronics [3, 4]. Metals are commonly used for synthesis of nanoparticles by chemical and biological methods.

The chemical method usually involves use of chemicals for synthesis of nanoparticles which makes them certainly unsuitable against any application as it contains toxic compounds. Some chemical methods cannot avoid the use of chemicals, therefore use of noble metals like silver are into practice for synthesis of nanoparticles. An alternative, eco-friendly and advantageous approach to chemical method is the biological method. Synthesis of nanoparticles by biological method is through microbes like *Aspergillus flavus* [5], *Phoma exigua* [6], *Pseudomonas* spp. [7] and plant sources such as *Chenopodium album* [8], *Acalypha indica* [9], *Diopyros kaki* [10], *Cynodon dactylon* [11], *Glycyrrhiza glabra* [12] *Nigella sativa*, etc. By modifying the shape and reducing the size to up to 100nm, it is possible to increase the properties of the source material against various applications [13].

Researchers in the field of nanotechnology are finding that metal nanoparticles have all kinds of previously unexpected benefits. They are usually prepared from noble metals, that is, silver, gold, platinum and palladium while silver nanoparticles (AgNPs) being most exploited [14], because of its wider range of application in medicine, electronics, energy saving, environment, textile, cosmetics, biomedical, etc.

Though biological method is commonly adopted for the synthesis of silver nanoparticles, use of plant extracts is widely studied due to its advantages over others. A number of investigations had emphasized the antimicrobial effect of nanoparticles synthesized from plants and was found helpful in treatment against bacterial pathogens.

Among different plants, the leaves of *Mimosa pudica* had shown to exhibit various medicinal properties such as anti-diabetic [15], anti-allergic [16], anti-inflammatory, antibacterial, antioxidant and

anticancer activity [17]. However, silver nanoparticles synthesized from *M. pudica* have been assayed for antiparasitic effect. Hence, the present study was deliberately aimed with a simple and an effective approach of synthesizing silver nanoparticles using leaves of *Mimosa pudica* as a reducing agent and to study its inhibitory action against bacterial pathogens such as *Bacillus subtilis* and *Klebsiella pneumoniae*, and to evaluate its amylase inhibitory potential.

MATERIALS AND METHODS

Preparation of plant extract

The leaves of *Mimosa pudica* were washed several times with de-ionized water and extracted by boiling and homogenization methods. Briefly, 20g of thoroughly washed leaves were homogenized (using a sterile mortar and pestle) and boiled with 100ml of de-ionized water each. The homogenized and boiled suspensions were filtered through Whatman No 1 filter paper and the filtrate was collected and stored at 4°C for further use.

Optimization of synthesis of silver nanoparticles

Silver nanoparticles were synthesized using the homogenized and boiled extracts by treating leaf extracts with 1mM silver nitrate solution in varying ratio such as 1:10, 3:10 and 5:10 (v/v). The reaction mixture was then exposed to different conditions like sunlight irradiation, UV irradiation and room temperature. The colour change of the solution was checked periodically and the conical flasks were incubated at room temperature for 24 h. After incubation, the solution was centrifuged at 10,000rpm for 15 min and the pellets were suspended in 0.1ml of toluene water and air dried.

Characterization of silver nanoparticles

The silver nanoparticles thus synthesized were subjected to various characterization techniques such as UV-Vis spectra, SEM, FTIR and XRD.

Antimicrobial and amylase inhibiting activity of silver nanoparticles synthesized using *M. pudica* leaf extract

Antimicrobial activity - Well diffusion assay

The prepared nutrient agar was poured on to sterile Petri plates and 17 h growing cultures of *Bacillus subtilis* and *Klebsiella pneumoniae*

were swabbed on to the agar plates. Wells of 8mm diameter were cut on the agar plates and loaded with silver nanoparticle solution and a standard antibiotic (chloramphenicol). The plates were incubated overnight at room temperature and the zone of inhibition was measured [18].

α -amylase inhibition assay

A total of 500 μ l of silver nanoparticles (10mg/ml) and standard drug (Metformin) were added to 500 μ l of 0.2mM phosphate buffer (pH 6.9) containing α -amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After incubation, 500 μ l of starch solution (1% in 0.2mM sodium phosphate buffer; pH 6.9) was added to each tube and re-incubated at 25°C for 10 min.

The reaction was stopped by adding 1ml of 3, 5 dinitrosalicylic acid and heating in a boiling water bath for 5 min. The reaction mixture was then cooled to room temperature, diluted with 10 ml distilled water and the absorbance was measured at 540 nm.

RESULTS AND DISCUSSION

The synthesis of silver nanoparticles using *M. pudica* leaf extract was found to be significant in Boiled extract exposed to sunlight irradiation method compared to other methods. The colour of the reaction medium gradually changed to dark brown because of the surface plasmon resonance (Fig. 1).

Characterization of silver nanoparticles

UV-Visible spectroscopy

An absorption peak between 430-460nm confirms the presence of silver nanoparticles. A similar pattern was observed by [19], where the synthesis of silver nanoparticles was done using ethanol extract of *Andrachnea chordifolia*, by sunlight irradiation.

XRD analysis

XRD analysis showed four distinct diffraction peaks at 37 and 44 that can be indexed at the (220), (240) crystalline planes of cubic Ag. This analysis revealed that nanoparticles were orthorhombic crystals. The high peaks in the spectrum indicate the active silver composition with the indexing (Fig. 2). Thus, the XRD analysis confirmed the crystalline nature of the silver nanoparticles.

FTIR analysis

FTIR measurement was carried out to identify possible bio molecules of *M. Pudica* leaf extract responsible for the formation and stabilization of nanoparticles (Fig. 3). The linkage at 3413 cm^{-1} and 1560 cm^{-1} confirms the N-H stretching and deformation, the carbonyl stretch was confirmed by the peak formation at 1653 cm^{-1} . The coupled C-O stretch and the O-H deformation were confirmed by the peaks at 1405 cm^{-1} and 1045 cm^{-1} .

The overall observation confirms the presence of protein in samples of silver nanoparticles along with amino acids for its stability and to protect it from further changes. The silver nanoparticles that are obtained through biological process were more stable when compared to chemical reduction method. The silver nanoparticles are more biocompatible as they are synthesized from the natural sources and also have the stability for the further bio control studies and the medicinal application [20].



Fig. 1: It shows the colour change in reaction mixture before and after exposure to sunlight

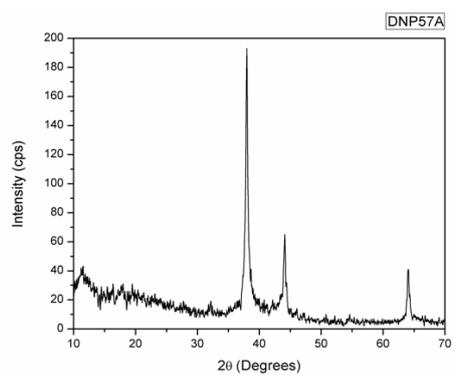


Fig. 2: It shows the XRD pattern of silver nanoparticles synthesized using *M. pudica* leaves

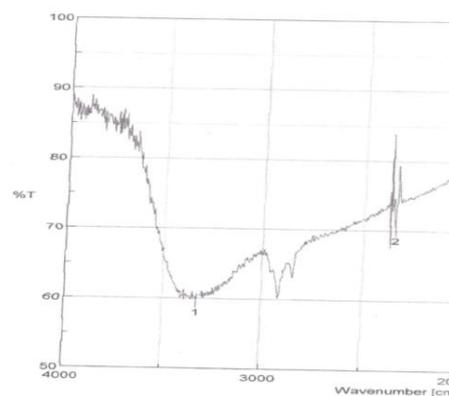


Fig. 3: It shows FTIR image of silver nanoparticles synthesized using *M. pudica* leaves

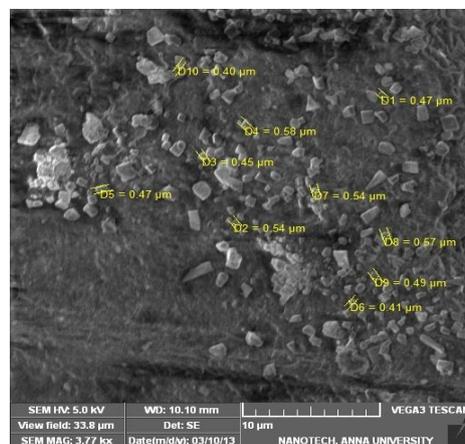


Fig. 4: It shows SEM image of silver nanoparticles synthesized using *M. pudica* leaves

SEM analysis

The results of SEM analysis (Fig. 4) show that the silver nanoparticles synthesized are of uniform size (40-58 μ m) distributed evenly and cubic in shape. Researchers have reported the synthesis of nanoparticles from various plant sources to be mostly spherical in shape and of different sizes [21, 22, 23].

Antimicrobial activity

Antibacterial activity of the synthesized silver nanoparticles was studied for pathogenic bacteria namely *B. Subtilis* and *K. pneumoniae*. The results depict (Table. 1) that silver nanoparticles are efficient giving a zone of inhibition of 16 mm

and 11 mm against *B. subtilis* and *K. pneumoniae*, respectively. In addition, it was also studied that the inhibitory effect was enhanced when nanoparticles were combined with the standard antibiotic. Thus, the silver nanoparticles of *M. Pudica* leaves significantly inhibits the pathogens, however, further investigation is required for understanding its mechanism better.

α -amylase inhibition assay

The inhibitory effect of silver nanoparticles synthesized using *M. pudica* leaves on α -amylase activity was found to be increasing progressively with increasing concentration of nanoparticles (Fig. 6). The inhibitory activity of silver nanoparticles was found to range between 8 and 28%, while that of the standard used was 6 and 34% (Table 2)

Table 1: It shows the antimicrobial effect of silver nanoparticles synthesized using *M. pudica* leaves

S. No.	Organism	Zone Of Inhibition (mm)		
		Nanoparticle	Antibiotic	Antibiotic+ Nanoparticle
1	<i>B. subtilis</i>	16	16	17
2	<i>K. pneumoniae</i>	11	23	25

Table 2: It shows the α -amylase inhibitory effect of silver nanoparticles synthesized using *M. pudica* leaves

S. No.	Concentration(μ g/ml)	Inhibition (%)	
		Standard	Nanoparticles
1	250	6.09	8.17
2	500	10.06	11.73
3	750	19.70	18.55
4	1000	34.42	28.75

CONCLUSION

In conclusion, the bio-reduction of silver ions using leaves of *M. pudica* as reducing agent has been illustrated. From the present study, it is clear that the silver nanoparticles synthesized through the green route using leaves of *Mimosa pudica* can be used effectively in the treating bacterial diseases and diabetes management.

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