Research Article





Green Synthesis and Characterization of Silver Nanoparticle Using Mimosa pudica Leaves for Anti-Diabetic Activity

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ABSTRACT

The present investigation was carried out for the synthesis of Silver Nanoparticles of *Mimosa pudica* leaf extract by standard Facile green synthesis for Anti diabetic activity. The Characteristics of Silver Nanoparticles of *Mimosa pudica* were studied using X-Ray Diffraction, Scanning Electron Microscope, Energy dispersive X-ray spectroscopy techniques. The crystalline nature of particles was confirmed by the peaks in the X-Ray Diffraction pattern. The size and shape of particles was calculated from Scanning Electron Microscope and the size measured between 20 and 32nm. Energy dispersive X-ray spectrum of nanoparticles was confirmed the presence of elemental silver. The In vitro Antidiabetic activity was carried out by α -amylase inhibition assay. The result showed that 800µg/ml concentration of drug was found to inhibit 50% of α -amylase which was the same as that of the standard Acarbose. Hence the drug has potent antidiabetic activity.

Keywords: Anti-diabetic activity, α-Amylase inhibition assay, Mimosa pudica, Silver Nano particles, Standard Acarbose.

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INTRODUCTION

Ithough some herbal medicines have promising potential and were widely used, many of them remain untested and not scientifically proved. Hence, the present study was to screen the phytochemical and analyze the antidiabetic activity of *Mimosa pudica* extract tagged with *silver nanoparticles*.

Mimosa pudica belongs to the family Mimosaceae is also known as Touch me not plant. It is a stout straggling prostate shrubby plant with the compound leaves sensitive to touch, spinous stipules and globose pinkish flower heads and grows as weed¹.



Figure 1: Mimosa pudica Plant

Thottalvadi Chooranam (*Mimosa pudica*) is a Siddha herbal preparation has been studied extensively in recent years for its anti-diabetic in animal models².

Mimosa pudica was used for its anti-hyperglycemic³, antidiarrheal⁴, anti-convulsant⁵ and cytotoxic properties ⁶. Ethno pharmacologically, the root of this plant has been used for snakebite by traditional and tribal healers⁷⁻¹¹; stems used against scorpion sting¹².

It also has anti-hepatotoxic, antioxidant and wound healing activities. It has been used in the treatment of urogenital disorders, sinus and wounds¹³. It is very useful in treating diarrhea, amoebic dysentery, bleeding piles and urinary infections¹⁴.

Two well-known movements are observed in *Mimosa pudica*. one is the very rapid movement of the leaves when it is stimulated by touch, heat etc., and the other is the very slow, periodical movement of the leaves called nyctinastic movement which is controlled by a biological clock¹⁵. The movement is caused by a rapid loss of pressure in strategically situated cells that cause the leaves to droop right before one's eyes.

Roots of *Mimosa pudica* was reported to contain alkaloids, glycosides, flavonoids and tannins¹⁶. Seeds contains d-xylose and d-glucuronic acid. It contains Mimosine¹⁷⁻¹⁸ which is a toxic alkaloid. The plant was reported to contain tubuline and a new class phytohormone turgorines is found to be active in the plant. The periodic leaf movement factors are the derivatives of 4- α -(b-D-glucopyranosyl-6-sulphate) gallic acid.

Phytochemical studies on *Mimosa pudica* have revealed the presence of alkaloids, fatty acids, non-protein amino acid^{19,20}, flavonoids, C-glycosides, sterols, terpenoids, and



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tannins²¹. It has antiviral properties, aphrodisiac properties, antimicrobial properties, diuretic effect etc^{22,23}.

Hence, the present study was aimed with an effective approach of synthesizing silver nanoparticles using leaves of *Mimosa pudica* as a reducing agent and to study the characterization of silver nanoparticles of *Mimosa pudica* leaf extract and its inhibitory effect against α -amylase.

Nano, a scientific term used for determining the size of the particle²⁴. 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 ²⁵. Many materials are synthesized in Nano size for various applications including medicine, mechanical, biomedical electronics²⁶⁻²⁷.

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*²⁸, *Phoma exigua*²⁹, *Pseudomonas* spp³⁰ and plant sources such as *Chenopodium album*³¹, *Acalypha indica*³², *Diopyros kaki*³³, *Cynodon dactylon*³⁴, Glycyrrhiza glabra³⁵, *Nigella satia*, etc. By modifying the shape and reducing the size up to 100nm, it is possible to increase the properties of the source material against various applications³⁶.

In Ayurvedic and Unani medicine, *Mimosa pudica* root is used to treat bilious fevers, piles, jaundice, leprosy, dysentery, vaginal and uterine complaints, inflammations, burning sensation, fatigue, asthma, leucoderma and blood diseases. In Western medicine, Mimosa root is used for treating insomnia, irritability, premenstrual syndrome (PMS), menorrhagia, hemorrhoids, skin wounds, and diarrhea. It is also used to treat whooping cough and fevers in children, and there is some evidence to suggest that Mimosa is effective in relieving the symptoms of rheumatoid arthritis³⁷.

MATERIALS AND METHODS

Collection of plant materials

The leaves of the plant *Mimosa pudica* were collected from Siruseri (Sipcot), Navalur, Chennai on May, 2017.

The plant material was identified and authenticated by Professor Dr. J. Jayaraman, Ph.D. Director, Plant Anatomy Research Center, West Tambaram, Chennai. A voucher specimen was submitted at C. L. Baid Metha College of Pharmacy, Chennai-97.

Phytochemical screening studies

The chemical tests for various phyto constituents like alkaloids, flavonoids, saponins, phenols, terpenoids, anthraquinone, proteins and amino acids, carbohydrates and glycosides etc. were carried out and the results were recorded.

Biosynthesis of Silver Nanoparticles of Mimosa pudica:

10 grams of finely powdered leaves were mixed with 100 ml of deionized water and boiled for 30 minutes, cooled and filtered through Whatmann filter paper no.1. The extract was used freshly within an hour. 40 mL of Mimosa pudica leaf aqueous broth was added to 60 ml of 1 mM aqueous AgNO₃ solution and the solution was placed in an orbital shaker at room temperature, for reduction of Ag+ to SNPs. The bio-reduction of the silver ions in the solution was monitored periodically by measuring the UV-Vis spectroscopy of the solutions. The reaction is rapid if the brown color appears within 10 minutes and this reaction will confirm the formation of SNPs. The different concentration of AgNO₃ solution was used to get maximum SNPs. The SNPs obtained from the solution were purified by repeated centrifugation at 2000 rpm for 10 minutes followed by the dispersion of the pellet thrice in deionized water to remove the water-soluble biomolecules such as proteins and secondary metabolites. The water-suspended NPs were kept under vacuum for 24 hours to dry the NPs³⁸.

Characterization of Silver Nanoparticles of *Mimosa pudica*:

The UV absorbance of the synthesized NPs was measured in CYBERLAB UV-100 spectrophotometer operated at a resolution of 1 nm. The synthesized NPs were dried, powdered and used for X-ray diffraction (XRD) analysis. Scanning electron microscopy (SEM) images were acquired by ICON ANALYTICAL, QUANTA 200. X-ray diffraction analysis (SPECTRIS TECHNOLOGIESPANalytical, X' Pert PRO) was performed by preparing a thin film of powdered SNPs. To study the average particle size distribution and stability of nanoparticles, an ICON ANALYTICAL, Genesis XM4 instrument was used³⁹.

In vitro anti-diabetic activity

α - Amylase inhibition assay

 α -amylase was dissolved in phosphate buffer saline (PBS, 0.02 mol/L, pH 6.8) at a concentration of 0.1 mg/mL. Various concentrations of sample solutions (0.25 mL) were mixed with α -amylase solution (0.25 mL) and incubated at 37°C for 5 minutes. Then the reaction was initiated by adding 0.5 mL 1.0% (w/v) starch substrate solution to the incubation medium. After incubation at 37°C for 3 minutes, the reaction was stopped by adding 0.5 mL DNS reagent (1% Dinitrosalicylic acid, 0.05% Na₂SO₃ and 1% NaOH solution) to the reaction mixture and boiling at 100°C for 5 minutes. After cooling to room temperature, the



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absorbance (Abs) at 540 nm was recorded by a spectrophotometer. The inhibition percentage was calculated by the following equation:

Where,

Abs1=sample and Abs2 = control⁴⁰.

The SNPs were synthesized using *Mimosa pudica* leaf extract by the method described earlier. On mixing the leaf aqueous extract with silver nitrate solution, the color of the mixture was found to be changed into reddish brown from yellowish color. The color change indicated the reduction of silver nitrate into silver ions that was resulted in the formation of SNPs. The depicts the absorbance spectrum of reaction mixture containing aqueous silver solution (1Mm) and *Mimosa pudica* broth (prepared from 10 g plant powder). The absorption spectrum obtained from the synthesized SNPs within 10 minutes was to examine the Surface Plasmon resonance (SPR). The peak which obtained at the bio-reduction of silver ions was

found to be 427nm. On adding the after mentioned plant broth to AgNO₃ solution, the solution changed from greenish yellow to brown color, the reason employed for the spectra under the wavelength of 200 to 800nm. The final color turns into deep brownish with passage of time. The intensity of the absorbance was found to increase as the reaction proceeded further. The strong and narrow diffraction peaks indicates that the product has well crystallized. The XRD peaks at 32.06°, 46.25°, 67.52° and 76.79° can be indexed to the (100), (200), (220), and (311) Bragg's reflections of cubic structure of silver respectively. The broadening of Bragg's peaks indicates the formation of nanoparticles. SNPs with controllable size and uniform shape can be easily obtained in the simple aqueous reduction method. The mean size of SNPs was calculated using the Debye-Scherrer's equation by determining the width of the (1 1 1) Bragg's reflection. The crystalline size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Scherrer formula.

RESULTS AND DISCUSSION

Phytochemical screening studies

S.NO	Phytochemicals	Water	Methanol	Ethanol	Acetone	Ethyl acetate	Chloroform	Petroleum ether
1	Alkaloids	-	-	-	-	-	-	-
2	Cardiac Glycosides	-	+	+	+	-	-	-
3	Carbohydrates	+	+	+	+	+	+	+
4	Flavonoids	-	+	+	+	-	-	-
5	Phenols	-	+	+	+	-	-	-
6	Phlobatannins	-	-	-	-	-	-	-
7	Proteins	-	-	-	-	-	-	-
8	Saponins	-	-	-	-	-	-	+
9	Sterols	-	-	-	-	-	-	-
10	Tannins	+	+	-	-	-	-	-
11	Terpenoids	-	+	+	+	+	+	-
12	Quinones	-	-	-	-	-	-	-
13	Oxalates	-	-	-	-	-	-	-

Table 1: Observations of Phytochemical screening studies.

"+" ve indicates the presence of the constituent; "-" ve indicates the absence of the constituent

Characterization of silver nanoparticles

SEM Spectrum Analysis

The SEM micrograph of *Mimosa pudica* – AgNPs image clearly indicated the presence of spherical particles with size ranges between 20 and 32 nm.

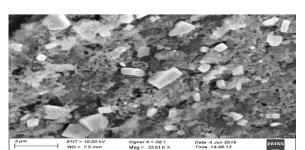


Figure 2: SEM images of Mimosa pudica – AgNPs



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EDX Spectrum Analysis

The energy dispersive X-ray spectroscopy (EDX) analysis of *Mimosa pudica* AgNPs confirmed the presence of elemental silver as the major constituent.

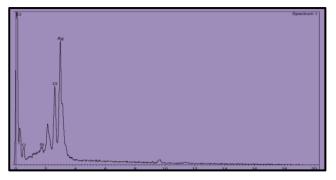


Figure 3: EDX spectrum of Mimosa pudica – AgNP

XRD Analysis

The X-Ray Diffraction studies of *Mimosa pudica* – AgNPs clearly interpreted the structure of the synthesized nanoparticles.

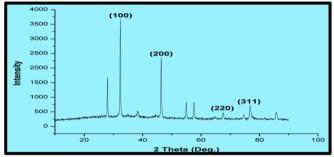


Figure 4: X-ray diffraction pattern of *Mimosa pudica* – AgNPs

In vitro antidiabetic activity

Table 2: *In vitro* antidiabetic activity of *Mimosa pudica* by α – amylase inhibition assay

Concentration (in μg)	MPN (% Inhibition)	Acarbose (% Inhibition)
50	17.53	12.89
100	20.33	22.95
200	33.40	27.30
400	45.75	40.75
800	51.70	53.31
1600	58.60	64.84

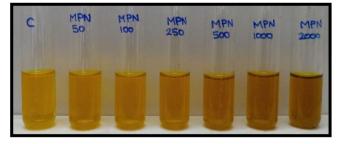
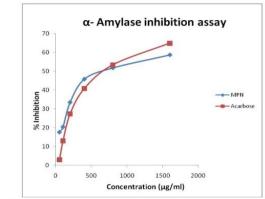


Figure 5: α – amylase assay of synthesized *silver* nanoparticles of Mimosa pudica extract.



Graph 1: *Invitro* antidiabetic activity of *Mimosa* pudica by α – amylase assay.

DISCUSSION

The *Mimosa pudica* leaf was collected, authenticated, pulverized and tested for phytochemical studies. Different solvents like Petroleum ether, Chloroform, Ethyl acetate, Acetone, Ethanol, Methanol and Water were macerated, Phytoconstituents like Cardiac glycosides, Carbohydrates, Flavonoids, Phenols, Tannins and Terpenoids were found in the leaves.

Silver nanoparticles were synthesized using *Mimosa pudica* leaf by standard method, further it was characterized by Scanning Electron Microscope (SEM), X-Ray Diffraction (XRD) and Energy Dispersive X-ray Spectroscopy (EDX). Silver nanoparticles tagged with *Mimosa pudica* was confirmed by above all techniques.

The In vitro antidiabetic activity was carried out for the synthesized silver nanoparticles of *Mimosa pudica* extract by α -amylase inhibition assay. The IC₅₀ value of the given sample (MPN) was 689.6 µg/ml and the standard drug (Acarbose) was 725.96µg/ml. The result showed that 800µg/ml concentration of drug was found to inhibit 50% of

 $\alpha\text{-amylase}$ which was the same as that of the standard Acarbose. This proves that the drug has potent antidiabetic activity.

CONCLUSION

In present days, herbal medicine plays major roles in health care system all over the world. This made us to explore the traditional medicinal plant *Mimosa pudica* leaf for antidiabetic activity by synthesizing with silver nanoparticles.

Review of literature showed the presence of many bioactive components such as flavonoids, glycosides, phenols, tannins, etc. and many pharmacological activities like antidepressant, antimicrobial, anti-hepatotoxic, antiulcer, etc. Thus, the present study was to investigate the phytochemicals, synthesize nanoparticles and characterize the nanoparticles and evaluate anti-diabetic activity.

The preliminary phytochemical screening of *Mimosa pudica* leaf extract showed the presence of bioactive components



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terpenoids, flavonoids, glycosides, alkaloids, phenols and tannins.

Silver nanoparticles were synthesized using *Mimosa pudica* leaves by standard method and characterized by Scanning Electron Microscope (SEM), X-Ray Diffraction (XRD) and Energy Dispersive X-ray Spectroscopy (EDX). These studies confirmed the *Mimosa pudica* silver nanoparticles tagging.

By α -amylase activity, it was proven that Silver nanoparticles tagged with *Mimosa pudica* have potent antidiabetic activity.

Hence, this green synthesis will be further tested for different pharmacological activities and the dose will be standardized for human consumption.

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