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Ajithadas Aruna
College of Pharmacy, Madurai Medical College, Madurai-20, Tamil Nadu, India.
Email: nandhinibpharm@gmail.com

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Synthesis and Characterization of Silver Nanoparticles of Insulin Plant (*costus pictus* D. Don) Leaves

Ajithadas Aruna*, Ramraj Nandhini, Venkatachalam Karthikeyan, Pandi Bose

College of Pharmacy, Madurai Medical College, Madurai-20, Tamil Nadu, India.

Abstract

Objectives: Cost effective and eco-friendly technique for green synthesis of silver nanoparticles from the methanolic extract of *Costus pictus* leaf and characterization of synthesized silver nanoparticles was carried out in this study.

Methods: Synthesis of silver nanoparticles of Insulin plant (*Costus pictus*) leaves was done by using 1mM $AgNO_3$ solution and incubates 5hr at room temperature. Characterization of synthesized nanoparticles was done by UV–Vis absorption spectroscopy, SEM, polydispersity index and zeta value.

Results: The biological synthesis of silver nanoparticles using *Costus pictus* extract was shown to be rapid and produced particles of fairly uniform size and shape. As the methanolic leaf extracts of *Costus pictus* D. Don were mixed with the aqueous solution of silver nitrate, it changed into brown colour due to the excitation of surface Plasmon vibrations, which indicated the formation of methanolic extract of *Costus pictus* silver nanoparticles (MECPAgNPs). UV-Visible spectroscopy analysis of nanoparticles showed the broadening of the peak indicated the particles are poly dispersed. The surface Plasmon band in the silver nanoparticles in the solution remains close to 420nm. Throughout the reaction period indicating the particles are dispersed in the aqueous solution of silver nitrate, with no evidence for aggregation. The average particle size (z-average) was found to be 132.6nm, its polydispersity index was 0.248 and zeta values were measured and it was found to be -25.1mV with the peak area of 100% intensity. This indicates that the formed silver nanoparticle is stable. A SEM images showed that the silver nanoparticles formed were spherical in shape, with an average size of around 100nm. SEM showed uniformly distributed silver nanoparticles on the surface of the cells was observed.

Conclusion: The Biomedical application of silver nanoparticles can be rendered more effective by using biologically synthesized nanoparticles that are found to be exceptionally stable, and also minimize toxicity and cost.

Keywords: Silver nanocrystals, Costus pictus, SEM, UV-Vis, green synthesis.

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INTRODUCTION

Nanotechnology is applied widely to offer targeted drug therapy, diagnostics, tissue regeneration, cell culture, biosensors and other tools in the field of molecular biology. To minimize drug degradation and loss, to prevent harmful side-effects and to increase drug bioavailability and the fraction of the drug accumulated in the required zone [1, 2]. The use of plant products for the synthesis of nanoparticles adds a new dimension for modern nanobio-technology [3]. Phytotherapeutics needs a scientific approach to deliver the components in a sustained manner to increase patient compliance and avoid repeated administration. This can be achieved by designing Novel Drug Delivery System (NDDS) for herbal constituents. NDDS not only reduce the repeated administration to overcome non-compliance, but also helps to increase the therapeutic value by reducing toxicity and increasing the bio-availability. One such novel approach is nanotechnology. Nano-sized drug delivery system of herbal drugs has a potential future for enhancing the activity and overcoming the problems associated with plant medicines which essential to treat more chronic diseases like asthma, diabetes, cancer and others [4-6]. Green synthesis of nanoparticles is an emerging branch of nanotechnology. Biosynthesis of nanoparticles using plant extracts is the favourite method of green, exploited to a vast extent because the plants are widely distributed, easily available, advancement over physical and chemical methods, safe to handle and with a range of metabolites and compatibility for pharmaceutical and biomedical applications as they do not use toxic chemicals in the synthesis protocols [7-11]. Metallic nanoparticles are emerging as new carriers which provide way to site-specific targeting and drug delivery by these nanoparticles. Silver (Ag) a noble metal, has potential applications in medicine due to its unique properties such as good conductivity, chemically stable, catalytic activity, surface enhanced Raman scattering and antimicrobial activity, Increases the oral bio-availabilty and to overcome the poorly water soluble herbal medicines[12-14]. There are various methods for silver nanoparticles preparation, for example; sol-gel process, chemical precipitation, reverse micelle method, hydrothermal method, microwave, chemical vapor deposition and biological methods, etc.[15] However; biological methods are preferred for being eco-friendly, cost effective, and don't involve the use of toxic chemicals.

Costus pictus D. Don is commonly known as spiral ginger, belonging to the family Costaceae. It is a magical cure of diabetes. Its leaf helps to build up insulin in the human body. So, it is commonly known as Insulin plant [16, 17]. Many *in-vivo* and *in-vitro* studies of this plant are carried out which showed it is having very potent antidiabetic effect [18-24]. Consumption of this leaves is believed to lower blood sugar level. Diabetes patients are advised to chew two leaves per day in the morning and evening. After one week the patient should take one leaf in the morning and evening. This dosage should be continued for a month. It is turning out to be munching dietary supplement for diabetes [25, 26].

Though being widely used, no formulation containing this plant is available in the market. The patient feels difficulty in chewing the leaves for a month. In order to overcome this and to enhance the oral bio-availability and pharmacological activity, protection from toxicity, physio-chemical degradation, improved tissue macrophages, to avoid repeated administration of the dose and to achieve the selective or targeted drug delivery towards a specific tissue or organ an attempt is made in this study to design and standardize a nano-formulation of *Costus pictus.*

MATERIALS AND METHODS

Chemical reagents: Methanol, Silver nitrate ($AgNO_3$), Double distilled water.

Instruments Required: Centrifuge (REMI), Magnetic stirrer with hot plate, Lyophilizer (Lyodel- Delvac Pumps Pvt. Ltd, USA), Shimadzu UV-Visible spectrophotometer (Model 1800), Scanning Electron Microscopy (Hitachi X650, Tokyo, Japan)

All the glass wares were washed with dilute nitric oxide followed by double distilled water and dried in hot air oven.

Procedure:

Collection of the leaves: Healthy plant leaves of *Costus pictus* D. Don were collected and cleaned properly in running tap water.

Leaf drying and pulverizing: The leaves were collected and shade dried. It was powdered in a mixer. The powder was sieved in a No.60 sieve and kept in a well closed container in a dry place.

Preparation of methanolic leaf extracts of *Costus pictus* D. Don: About 500g of the dried powdered leaf of *Costus pictus* D. Don was defatted with 1.5L petroleum ether (60-80°C) by maceration. The solvent was removed by filtration and the marc was dried. To the dried marc 1.5L of methanol was added and the extraction was performed by triple maceration (72hr process). It was then filtered and the combined filtrate was evaporated to a cohesive mass using rota vapour.

Preparation of stock solution 2mg/20ml: 2mg of the methanolic extract was weighed and diluted to 20ml with methanol. It was stored at 4˚C until further use **(Fig.1).**

Preparation of 1mM silver nitrate aqueous solution (AgNO3): An accurately weighed 0.017g of silver nitrate was dissolved with 100ml of double distilled water and stored in amber colour bottle until further use **(Fig.2).**

Figure 1: Stock solution of MECP 2mg/ 20ml

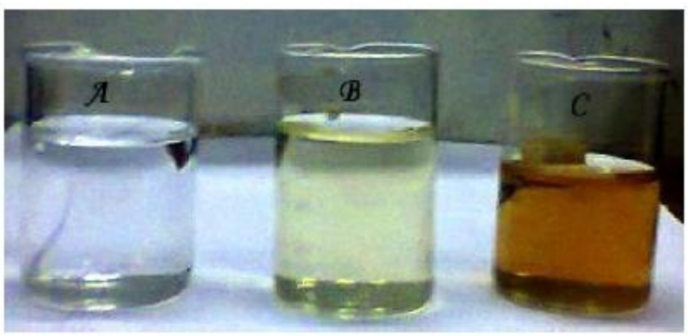

Figure 2: Aqueous solutions A) 1Mm AgNO3 B) 1Mm AgNO3 with MECP after zero minutes C) 1Mm AgNO3 with MECP after 5hrs

Synthesis of methanolic leaf extracts of *Costus pictus* D. Don silver nanoparticles (MECPAgNPs) [27-29]

5ml of the methanolic leaf extract of *Costus pictus* D. Don was taken in the conical flask separately and placed on a magnetic stirrer with hot plate. To this 50ml of 1mM AgNO3 solution was added drop wise with constant stirring 120rpm at 50-60˚C. The colour change of the solution was checked periodically. The colour change of the medium from colourless to brown after 5h **(Figs.3.1 to 3.6)** was observed which indicated the formation of silver nanoparticles. It showed that aqueous silver ions could be reduced by the methanolic extract of *Costus pictus* D. Don to generate extremely stable silver nanoparticles.

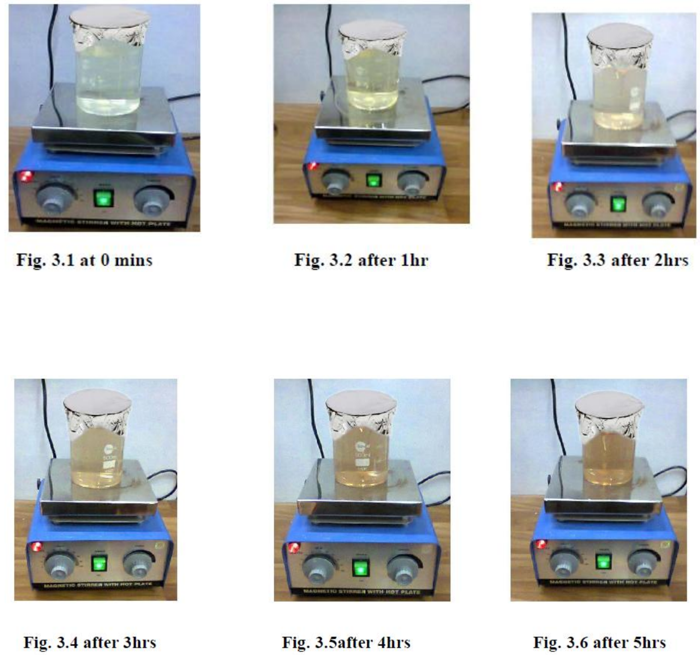

Figure 3: The colour change of the medium from colourless to brown colour after 5hrs

Seperation of silver nanoparticles: The synthesized silver nanoparticles were separated by centrifugation using a REMI centrifuge at 10,000rpm for 15min. The supernatant liquid was re-suspended in the sterile double distilled water. The process was carried out thrice to get rid of any unco-ordinated bio molecules. After, the desired reaction period, the supernatant liquid was discarded and the pellets were collected and stored at 4˚C for further use.

Lyophilization: The pellet obtained was then lyophilized by using freeze dryer (Lyodel-Delvac Pumps Pvt. Ltd, USA) to enhance the stability of silver nanoparticles. The freshly prepared MECPAgNPs are lyophilized with cryoprotective agent (mannitol). Then it was rapidly cooled down to -50^{0}C for 2hr followed by primary drying at 1.03m bar and secondary drying at 0.001m bar. After lyophilization the synthesized MECPAgNPs was stored at 4˚C for further use **(Fig. 4)**

Figure 4: Synthesized MECPAgNP

Characterization of synthesized MECPAgNPs [30, 31]

The characterization of synthesized MECPAgNPswas carried out by using the following analytical parameters:

Particle size

Zeta potential studies

Polydispersity index

UV-Visible spectral analysis

Morphological studies using SEM

Determination of Particle size and Zeta potential: The mean particle size (z-average), polydispersity index (PI) and zeta potential of MECPAgNPs were determined by dynamic light scattering technique using a zeta size analyzer (Nano ZS 90, Malvern Instruments Ltd., UK). The freeze dried powders were dispersed with water to obtain a proper scattering intensity before measurement.

UV-Visible spectroscopy [32]: The formation and completion of silver nanoparticles was characterized by UV-Visible spectroscopy by using Shimadzu UV-Visible spectrophotometer, Model 1800. The bio-reduction of the Ag^+ ions in solution was monitored by periodical sampling of aliquots and the UV-Visible spectra of these aliquots were monitored as a function of time of reaction in 200-600nm range operated at a resolution of 1nm. Distilled water was used as a blank.

Morphological studies of synthesized MECPAgNPs by using Scanning Electron Microscopy (SEM): Morphological evaluation of the MECPAgNPs was carried out by using scanning electron microscope (SEM) (Hitachi X650, Tokyo, Japan). SEM gave high-resolution images on the surface of the sample.

RESULTS AND DISCUSSION

Synthesis of Methanolic Leaf extracts of *Costus pictus* D. Don silver nanoparticles (MECPAgNPs): There was a visible color change after the substrate was added to the plant extract. Initially the plant extract was colourless. Upon adding the silver salt, it turned brown. After 5hr, no significant colour change was observed. Increased concentrations of silver nitrate resulted in a brown solution of nanosilver indicating the completion of reaction. Reduction of silver ions into silver nanoparticles using methanolic leaf extract of *Costus pictus* D. Don was evidenced by visual change of colour from colourless to brown colour which indicated the formation of silver nanoparticles due to the excitation of surface Plasmon vibration in silver nanoparticles [33].

Determination of Particle size and Zeta potential: Particle size, size distribution and zeta potential were important characterizations of the silver nanoparticles because they govern the other characterizations, such as saturation solubility and dissolution velocity, physical stability, or even biological performances [34].

Particle size measurements: Mean particle size diameter and polydispersity indices were all measured in solutions directly after synthesis, using photon correlation spectroscopy (PCS). The size of the colloidal silver nanoparticles, their granulometric distribution has been recorded, expressed against the particles number and their occupied volume [35].

The average particle size (z-average) is found to be **132.6 nm**. Particle size analysis showed the presence of nanoparticles with polydispersity indices PDI value **0.248** with intercept **0.643.** It is presented in the **Table 1 & Fig. 5.**

Parameter	Value	Peak No	Peak size (d.nm)	Peak intensity (%)	Peak width(d.nm)
Z-Average (d.nm)	132.6	Peak 1	141.8	100.0	102.5
PDI	0.248	Peak 2	0.000	0.000	0.000
Intercept	0.643	Peak 3	0.000	0.000	0.000

Table 1: Mean Particle Size Diameter and Poly dispersity Index (PDI) of Bio-synthesized MECPAgNPs

Zeta Potential measurement: A zeta potential was used to determine the surface potential of the silver nanoparticles. Zeta potential is an essential characterization of stability in aqueous silver nanoparticles. A minimum of +30mV zeta potential is required for the indication of stable silver nanoparticles. For the obtained nanoparticles, zeta values were measured and found to be -25.1mV with a peak area of 100% intensity. These values provide full stabilization of the nanoparticles, which may be the main reason in producing particle sizes with a narrow size distribution index **(Fig. 6).**

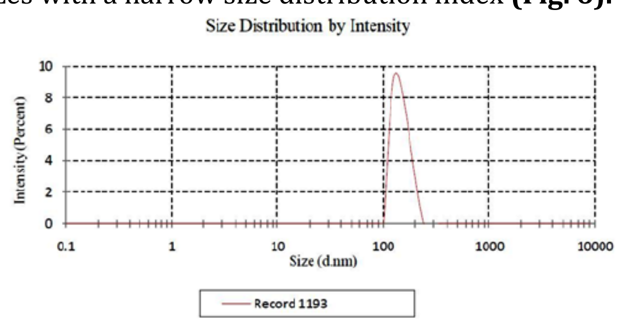

Figure 5: Percentage intensity of particle size distribution of biosynthesized MECPAgNPs

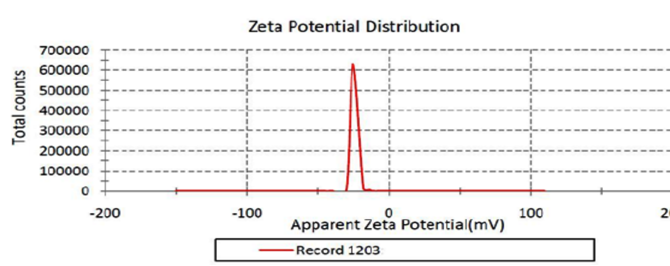

Figure 6: Zeta potential distribution of bio-synthesized MECPAgNPs

UV-Visible Spectroscopy: The UV-Vis Spectroscopy was the preliminary technique for the characterization of the silver nanoparticles. The reduction of the pure Ag^{+} ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours (complete colour change) following the dilution of a small aliquot of the sample in distilled water. The UV-Vis spectral analysis was conducted using Shimadzu UV-Vis spectrophotometer, Model 1800 range between 200 and 600 nm. The reduction of silver ions in the aqueous solution of nanoparticles in the solution could be correlated with the respective UV-Vis Spectra of the colloidal solution which exhibited a strong absorption at 420nmas shown in **Fig. 7.** A typical peak was obtained due to the presence of surface Plasmon resonance silver nanoparticles [36].

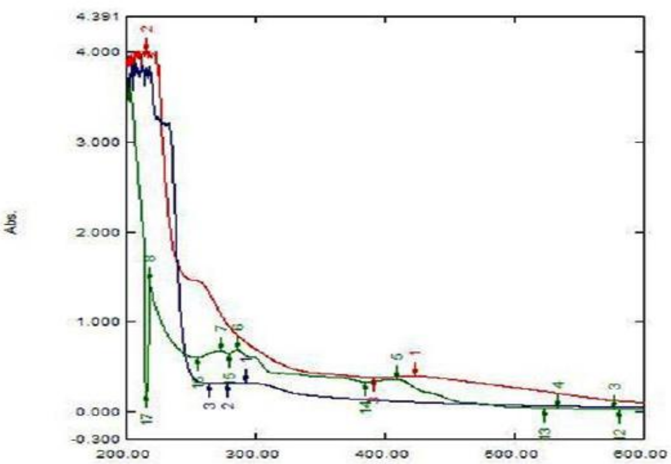

Figure 7: UV-Vis spectra of MECPAgNPs

Surface Plasmon resonance: It is the collective oscillation of electrons in a solid or liquid stimulated by incident light. The resonance condition is established when the frequency of light photons matches the natural frequency of surface electrons oscillating against the restoring force of positive nuclei. SPR in nanometer-sized structures is called localized surface plasmon resonance. SPR is the basis of standard tools for measuring adsorption of material onto planar metal (typically gold and silver) surfaces or onto the surface of metal nanoparticles. It is the fundamental principle behind many color-based biosensor applications and different lab-on-a-chip sensors [37].

Morphological studies of silver nanoparticles by using Scanning Electron Microscopy (SEM): A SEM employed to analyze the morphology and size details of the silver nanoparticles that were formed. From (**Fig. 8)** it was showed that the silver nanoparticles formed were spherical in shape, with an average size of around 100nm and uniformly distributed silver nanoparticles on the surface of the cells was observed.

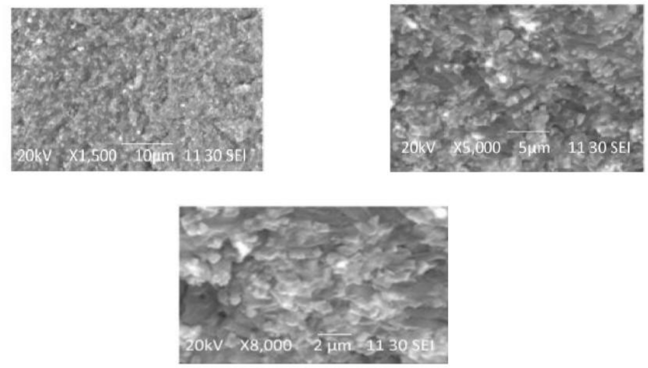

Figure 8: SEM images of MECPAgNPs

CONCLUSION

Green synthesis of *Costus pictus* leaves silver nanoparticles are found to be exceptionally stable, and also minimize toxicity and cost. It may be better therapy for treating diabetes instead of other formulations.

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