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EXTRACTION OF SILVER NANOPARTICLES FROM THE LEAF EXTRACTS OF CLERODENDRUM INERME

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This paper describes the first report on the synthesis of silver nanoparticles using extracts of a medicinal leaf *Clerodendrum Inerme*. Nanoparticles were synthesized from three different leaf conditions – fresh leaves, sun-dried leaves, and hot-air oven dried leaves. Atomic force microscopy (AFM) analysis of the nanoparticles revealed differences in sizes for the nanoparticles synthesized from different leaf conditions. Nanoparticles synthesized using fresh leaves possessed the smallest sizes. It is anticipated that optimization of the current synthesis method would yield highly mono-dispersed silver nanoparticles that have great potential in treating skin ailments.

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1. Introduction

Among the various inorganic metal nanoparticles, silver nanoparticles have received substantial attention for various reasons – silver is an effective antimicrobial agent, exhibits low toxicity [1, 2], silver nanoparticles have diverse in vitro and in vivo applications [3, 4]. Although there are many routes [5, 6] available for the synthesis of silver nanoparticles, bioinspired synthesis such as use of plant sources offers several advantages such as cost-effectiveness, eco-friendliness and the elimination of high pressure, energy, temperature, and toxic chemicals necessary in the traditional synthesis methods [7].

Several plants have been utilized for the production of silver nanoparticles [8-10]. In this work, leaf extracts of *Clerodendrum Inerme* were used for the generation of silver nanoparticles. Numerous studies on *Clerodendrum Inerme* [11-13] have been conducted in different traditional medical systems, for example, in Thai traditional medicine it is used to treat skin diseases [14], the tincture or decoction from these leaves is used in the traditional Indian medicine for the treatment of remittent and intermittent fevers, while the juice of leaves and roots is used as an alternative to treat scrofulous and venereal diseases [15]. Anti-inflammatory [16], anti-fungal [17], anti-microbial [18] and several other useful properties of *Clerodendrum Inerme* have also been reported. In order to exploit all the above mentioned benefits of bioinspired synthesis along with those provided by nanotechnology, silver nanoparticles were generated from the leaf extracts of *Clerodendrum Inerme* using a previously published method used by Parashar et al. [10]. Nanoparticles were synthesized from three different leaf conditions – fresh leaves, sun-dried leaves, and hot-air oven dried leaves. The AFM and UV-Vis spectroscopy results demonstrated the formation of silver nanoparticles from *Clerodendrum Inerme* extracts. Future studies will involve the applications of the silver nanoparticles generated from the leaf extracts of *Clerodendrum Inerme*.

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2. Materials and Methods

2.1 Preparation of the Extract

Leaf samples were collected from the campus of VIT University and were corroborated by Dr. V. Palanichamy, Assistant Professor, Plant Biotechnology Division, SBST, VIT University. A sample set of the leaves and pulverized leaf samples have been stored for future reference. Three different types of leaf samples were used in the current experiments – sample one: fresh leaves, sample two: leaves dried under sunlight, and sample three: leaves dried in a hot-air oven (Servewell Instruments Pvt. Ltd., Bangalore, India) at ~ 90-100 °C for three hours. The procedures adapted for all the above-mentioned samples was the same. In the case of sample one, 25 grams of the leaves were thoroughly washed three times in distilled water for 15 min, air dried, cut into fine pieces, and were boiled in a Erlenmeyer flask with 100 mL of sterile distilled water for 5 min and were finally filtered to get the leaf extract. For samples two and three, 25 grams of the leaves were dried, pulverized, and the procedure used for sample one was followed.

2.2 Synthesis of Silver Nanoparticles

A 50 mL leaf extract was added into the aqueous solution of 1 mM silver nitrate ($AgNO_3$, MW 169.87, SISCO Research Laboratories Pvt. Ltd., Mumbai, India) in a 1:1 ratio. Three sample solutions (fresh leaves, sun dried, and hot-air oven dried leaves) and the silver nitrate solution were centrifuged at 1200 rpm for 15 minutes. The supernatant of the samples were then used for the UV-Vis spectrophotometric analysis and visualization of the silver nanoparticles by Atomic Force Microscope. All three samples of *Clerodendrum inerme* were prepared in triplicate.

2.3 Spectrophotometric Analysis of the Samples

An Ultrospec 1100 Pro UV/Visible Spectrophotometer (Amersham Biosciences) was used for the spectrophotometric analysis. The reduction of pure silver ions was monitored by measuring the UV-Vis spectrum of the colloidal solution obtained after 10 min of adding 300 µL of sample solution to 3 mL of deionized water. A graph of wavelength on X-axis and absorbance on Y-axis was plotted.

2.4 AFM analysis of Silver Nanoparticles

The silver nanoparticles extracted by the above protocol were visualized with an Atomic Force Microscope (AFM). A thin film of the sample was prepared on a glass slide by dropping 100 µL of the sample on the slide, and was allowed to dry for 5 min. The slides were then scanned with the AFM (Nanosurf® AG, Switzerland, Product: BT02089, v1.3R0). Nanosurf® Easyscan-2 software was used for the AFM analysis.

3. Results and Discussion

The current study was undertaken to exploit the *hitherto* un-utilized plant sources in the development of silver nanoparticles. The plant *Clerodendrum inerme* (Fig. 1) belonging to the family *Verbenaceae* was selected and used in this first ever report on the synthesis of silver nanoparticles from its leaf extracts.

Fig. 1 Photograph of Clerodendrum inerme plant

3.1 UV-Vis Spectrophotometric Analysis of Leaf Extract Samples

After the extraction of silver nanoparticles, UV-Vis spectrophotometric measurements were performed for all three samples. The reduction of silver ions in the aqueous solution of silver complex during the reaction with the ingredients of the leaf extracts revealed that silver nanoparticles in the solution could be correlated with the respective UV-Vis spectra. Using spectrophotometric analysis, the colloidal solution of each sample studied exhibited a strong absorption between 200 and 400 nm [see Figs. 2, 3 and 4].

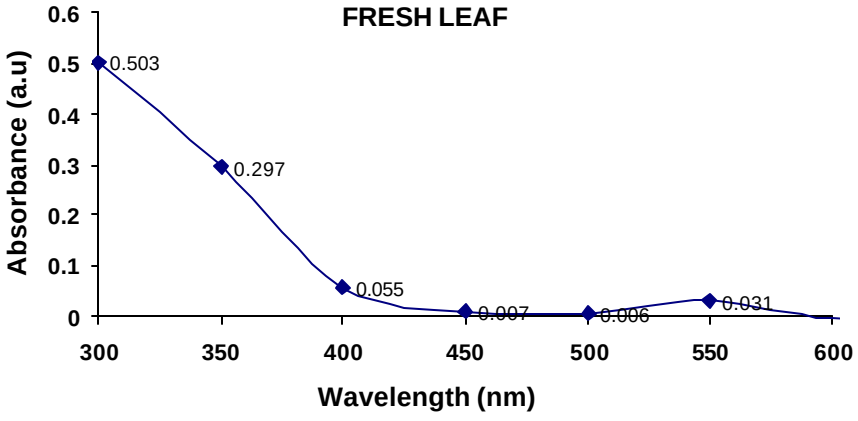


Fig. 2 UV-Vis absorption spectra recorded as a function of time of reaction of 1:1 solution of silver ions by Clerodendrum inerme fresh leaves extract after 10 minutes reaction kinetics.

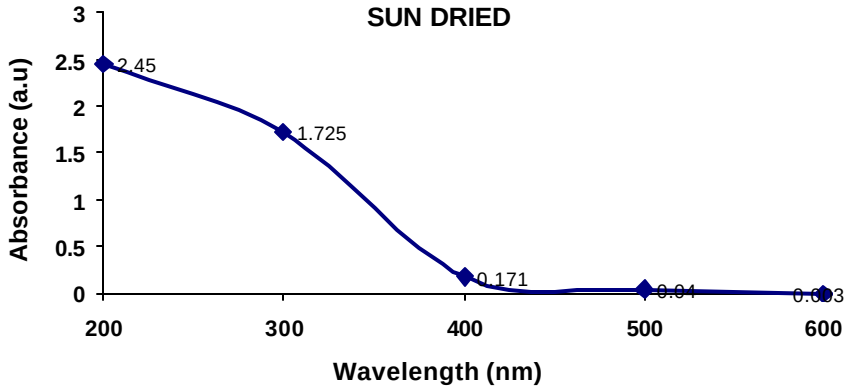


Fig. 3 UV-Vis absorption spectra recorded as a function of time of reaction of 1:1 solution of silver ions by Clerodendrum inerme leaves dried under sunlight after 10 minutes reaction kinetics.

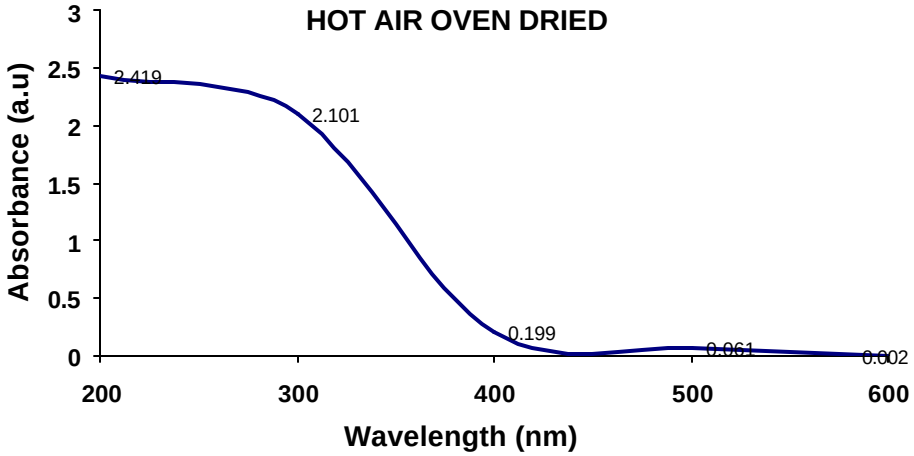
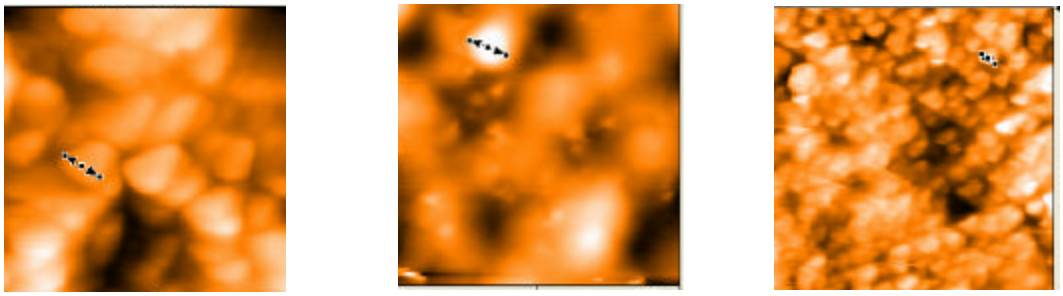


Fig. 4 UV-Vis absorption spectra recorded as a function of time of reaction of 1:1 solution of silver ions by Clerodendrum inerme leaves dried in hot-air oven after 10 minutes reaction kinetics.

3.2 AFM Analysis of the Silver Nanoparticles

The silver nanoparticles extracted from three different leaf extracts were also analyzed using AFM. Fig. 5 contains the micrographs obtained from the AFM measurements of the silver nanoparticles obtained under different conditions using *Clerodendrum Inerme* leaf extracts. Fig. 5(a) illustrates an AFM micrograph of silver nanoparticles synthesized from fresh leaf extracts scanned in an area of 1.7 μm × 1.7 μm, while both Fig. 5(b) and Fig. 5(c) were scanned in an area of 5 μm × 5 μm. Figs. 6, 7, and 8 contain the average lengths (measured along the longer axis of nanoparticles), widths (measured along the shorter axis of nanoparticles), and areas calculated for the silver nanoparticles obtained under different conditions of the leaves. Three different AFM micrographs of equal areas were obtained for the three different leaf conditions and average lengths, widths, and areas [fresh leaves (n=9), hot-air oven dried leaves (n=5), and sun dried leaves (n=3)] of the nanoparticles were calculated. The silver nanoparticles prepared from the extracts of fresh leaves possessed the smallest sizes, while the largest sizes were possessed by the

nanoparticles from sun-dried leaf samples and the sizes of the silver nanoparticles obtained from the hot-air oven dried leaf samples were in between the sizes of the nanoparticles obtained under the other two conditions. It is clear from the above results that the *Clerodendrum Inerme* leaf extracts processed under different conditions are highly promising in the development of silver nanoparticles of different sizes of nanoparticles that could be tailored for specific applications. However, the processing conditions need to be further optimized to extract spherical and mono-dispersed nanoparticles.



(a) (b) (c)

Fig. 5 AFM micrographs of silver nanoparticles synthesized from the leaf extracts of Clerodendrum Inerme – (a) fresh leaves, (b) sun dried leaves, and (c) hot-air oven dried leaves

Fig. 6 Average lengths of silver nanoparticles synthesized from the leaf extracts of Clerodendrum Inerme under different conditions

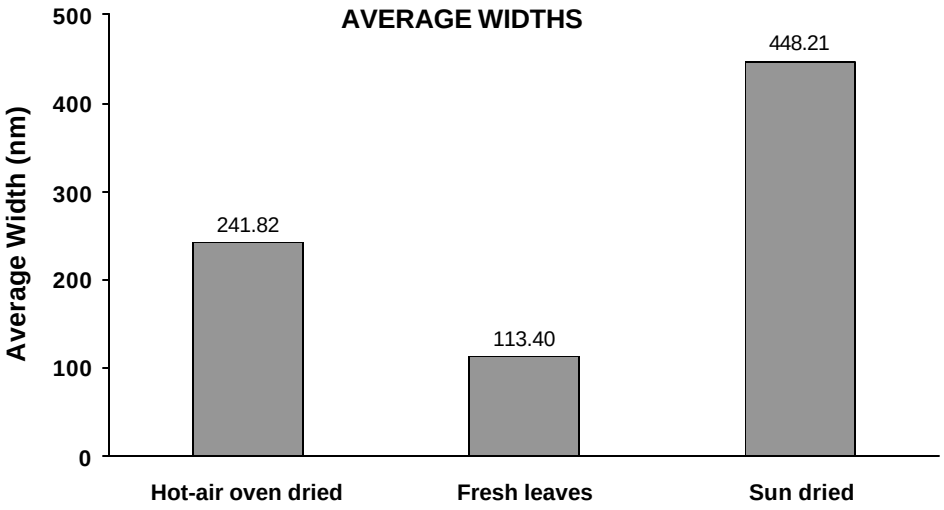


Fig. 7 Average widths of silver nanoparticles synthesized from the leaf extracts of Clerodendrum Inerme under different conditions

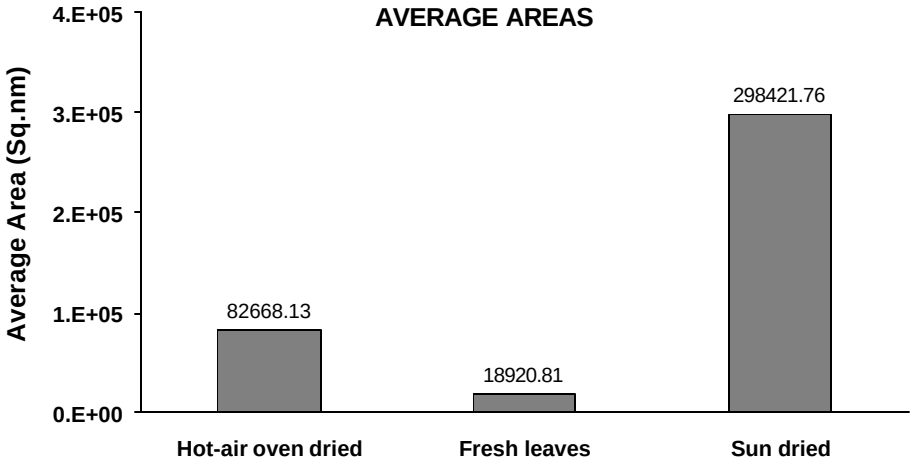


Fig. 8 Average areas of silver nanoparticles synthesized from the leaf extracts of Clerodendrum Inerme under different conditions

4. Conclusions

In conclusion, this study has successfully demonstrated for the first time the use of *Clerodendrum inerme* leaf extracts in extracting silver nanoparticles. Although silver nanoparticles of fairly well-defined dimensions were generated using this method, the exact reason for the differences in the sizes of nanoparticles synthesized using leaves processed under the three different conditions studied here (fresh leaves, sun-dried leaves, and hot-air oven dried leaves) has to be further investigated. The results indicate that the *Clerodendrum inerme* plant which is grown as an ornamental plant in many gardens in India and other parts of the world can be beneficially used in the nanobiotechnology-based industries for bioinspired synthesis of silver

nanoparticles. Further studies are underway to synthesize extract mono-dispersed and spherical nanoparticles from *Clerodendrum inerme* leaves.

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