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ORIGINAL ARTICLE **OPEN ACCESS**

Bio fabrication and Characterization of Silver Nanoparticles Using Leaf and Stem Extract of *Withania sominifera* (L)Dunal.

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ABSTRACT
Nano biotechnology has become a fundamental area of contemporary science and is gaining worldwide popularity due to its diverse applications. Nanomaterial's can be synthesized using various physical, chemical, and biological methods, including the use of bacteria, fungi, actinomycetes, yeasts, and plants. Plant-based biosynthesis of nanoparticles has emerged as an effective method to develop a non-toxic, quick, eco-friendly, and clean technology. This study aimed to synthesize silver nanoparticles from Withania sominifera and analyse them using UV-Visible spectrophotometry, SEM, EDX, and TEM. The results indicated that the size of the synthesized nanoparticles ranged from 6-22 nm in the leaf extract and 9-32 nm in the stem extract. The average size of nanoparticles in both extracts was estimated to be 13 nm in the leaf extract and 22 nm in the stem extract according to the size distribution.
Keywords: *Bio fabrication, Silver nanoparticles, Withania sominifera, SEM, TEM.*

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INTRODUCTION

Nanotechnology is concerned with various technological systems, the development of novel materials and devices, which have immense potential for various applications. Nanoparticles possess exceptional physicochemical characteristics, making them ideal for use in different fields, including chemistry, biology, industry, pharma, electronics, agriculture, chemical catalysisand many other industries [1]. Among the nanoparticles, silver nanoparticles (AgNPs) have gained widespread popularity due to their novelty and ability to control their size, which has led to their use in numerous consumer products [2]. Green nanotechnology aims to create eco-friendly and sustainable nanotechnology-based products that are safe for all living beings and commercially viable. One of the methods used in green nanotechnology is the green synthesis of metal nanoparticles, which is gaining increasing attention due to the unique optical, chemical, photochemical, and electronic properties of these nanoparticles. Among the various methods used for green synthesis, the biosynthetic method using plant extracts is particularly popular because it does not require an aseptic environment (3). In this study, *Withania somnifera*, commonly known as ashwagandha, was used to synthesize silver nanoparticles. Ashwagandha is a small, evergreen, tomentose woody shrub that grows up to two feet in height. Its primary chemical constituents include alkaloids, steroidal lactones, iron, withanolides, and glycosides (4) The *Withania somnifera* plant possesses significant medicinal properties and is used as an aphrodisiac, liver tonic, anti-inflammatory agent, and astringent. Recent studies have shown its efficacy in treating a variety of conditions, such as bronchitis, asthma, ulcers, emaciation, insomnia, and senile dementia. Traditionally, the plant has been used to treat chronic fatigue, bone weakness and tension, tooth loss, thirst, impotence, premature aging, debility, constipation, senility, rheumatism, nervous exhaustion, memory loss, neurodegenerative disorders, and spermatorrhoea (5)

MATERIALS AND METHODS

Extract preparation

The fresh leaves and stems of *Withania somnifera* were harvested, dried in an oven, and subsequently ground into a powder. A quantity of 10 grams of the powdered form was added to 100 millilitres of sterilized distilled water and boiled for 15 minutes. The mixture was then decanted, filtered through Whatman filter paper No. 01, and centrifuged. The resulting extract was stored at 4°C and used within a week.

Preparation of Silver Nanoparticles

A solution of 1mM Silver nitrate (Merck) was prepared by dissolving it in 50 millilitres of distilled water. Subsequently, 10 millilitres of the leaves and stem extract was added to the solution at room temperature, and the reaction was allowed to occur. The mixture was then centrifuged to obtain a pellet, which was washed twice with distilled water to remove any unbound ligands. After the final wash, the pellet was collected in a petri plate, dried, and scraped for further characterization. The silver nanoparticles synthesized using the leaves and stem extract of *Withania somnifera* were characterized using various techniques. The UV-Visible spectrophotometer UV-2450 (Shimadzu) was used to analyse the nanoparticles. For SEM analysis, thin films of the sample were prepared on a carbon copper grid by dropping a small amount of the sample on the grid, and imaging was performed on a ZIESS S-4500 SEM instrument. The atomic and weight percent of the biosynthesized silver nanoparticles were determined using EDX equipped within the SEM instrument. The size of the nanoparticles was measured using TEM, for which the sample was sonicated for 20 minutes. A drop of the solution was loaded onto a carbon-coated copper grid, and the solvent was allowed to evaporate under infrared light for 30 minutes. TEM measurements were performed on a Philips Model CM 200 instrument operated at an accelerating voltage of 200kv. XRD analysis was conducted on the samples submitted to the USIC, University of Rajasthan.

RESULTS AND DISCUSSION

UV–Visible Spectroscopy

The reduction of pure Ag+ ions was observed through a visible change in the colour of the solution from transparent to colour. The physical appearance of dark brown coloured solution from light yellow colour (Fig.1) is due to the surface plasmon resonance phenomenon (SPR), which is the preliminary indication of formation of nanoparticles (6). The observation from visual examination was further confirmed by UV–visible spectrum analysis. The UV-Visible spectra analysis indicated the presence of a single, strong absorption peak centered at about 430 nm in the leaf extract and 380 nm in the stem extract, which suggests that the nanoparticles are uniform in size and isotropic in shape (7).

SEM

The size and presence of silver nanoparticles were confirmed through SEM analysis using a software-controlled scanning electron microscope. A thin film of the sample was prepared on a carbon-coated copper grid, which was allowed to dry under a mercury lamp for five minutes. The average size of the nanoparticles was estimated to be 13 nm in the leaf extract and 22 nm in the stem extract (Fig 2). This finding is similar to the results obtained from *Morinda lucida* leaf extract conducted by (***8)***.

To determine the shape and size of the silver nanoparticles, TEM analysis was conducted. A carbon-coated copper grid was used to prepare a sample of the nanoparticle suspension by allowing the water to evaporate inside a vacuum dryer. The sample was then examined using a Transmission Electron Microscope (Philip). It was observed that most of the Ag nanoparticles were somewhat globular and spherical in shape, but there was some variation in the size of the particles. The size of particles in leaf extract ranged from 6 nm to 22 nm, while in the stem extract it ranged from 9 nm to 32 nm. Based on the size distribution, the average size of the nanoparticles was estimated to be 13 nm in leaf extract and 22 nm in stem (Fig 3). These findings coincided with the results of study conducted by (9).

EDX ANALYSIS

To confirm the presence of silver nanoparticles, Energy Dispersive X-ray Spectroscopy (EDX) analysis was conducted. The results showed a characteristic optical absorption peak at 2.5 keV in leaf extract and 2 keV in stem extract, confirming the presence of metallic nanoparticles. This finding was similar to the results obtained in a previous study on the plant extract of *Peganum harmala* (10). The other elemental signals were recorded, possibly may be due to elements from enzymes or proteins present within the plant.

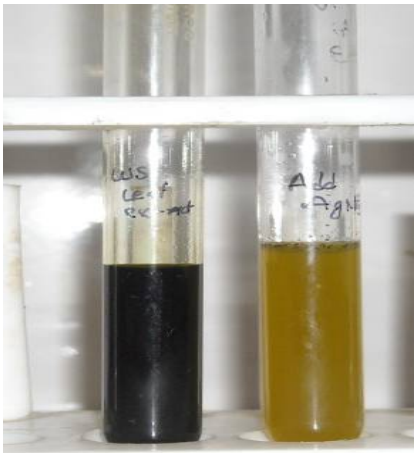

Figure 1: Colour change in extract of *Withania somnifera* and silver nitrate solution (from light yellow to dark brown*)*

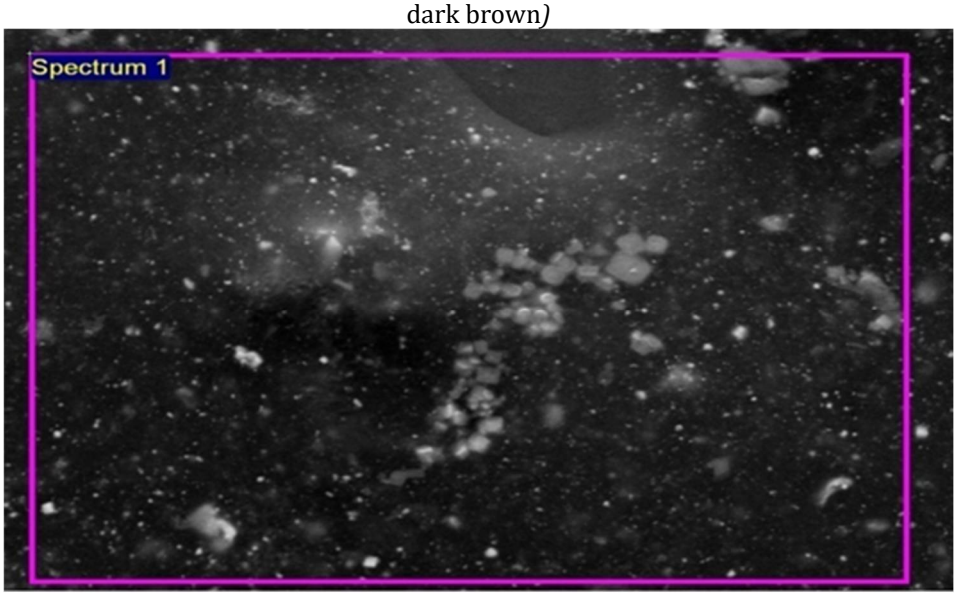

Figure 2: SEM images of AgNPs synthesized using *Withania somenifera* extract.

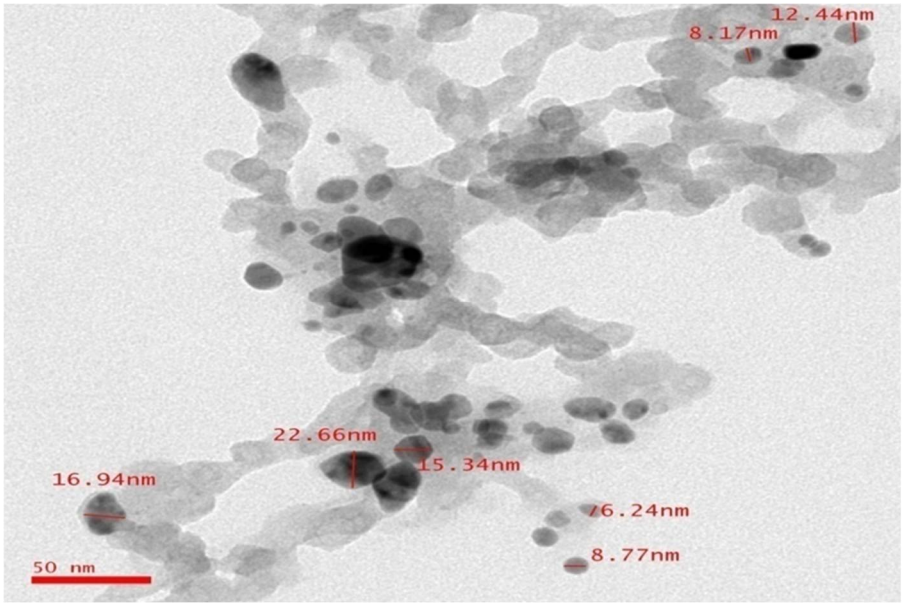

Figure 3: TEM images of AgNPs synthesized using *Withania somenifera* extract.

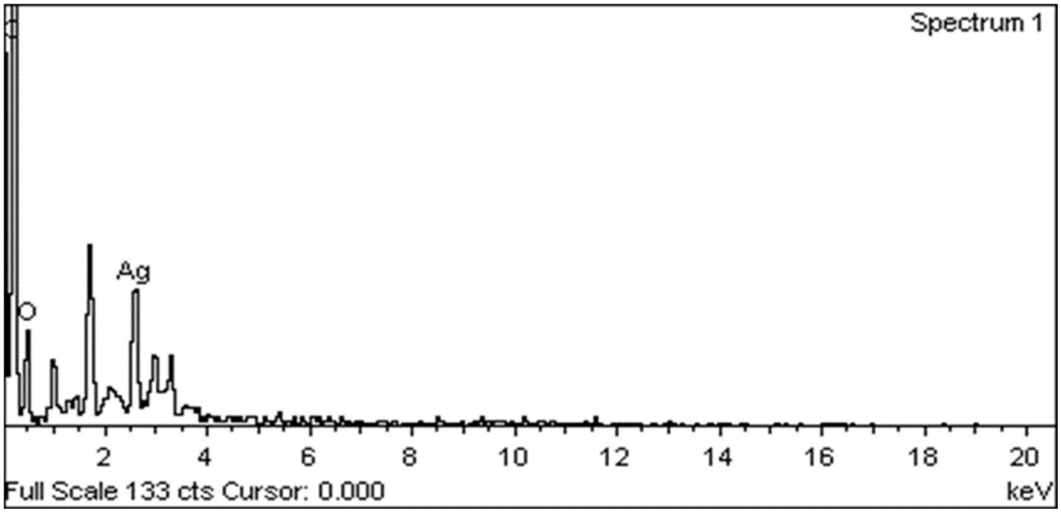

Figure 4: EDX graph of AgNPs synthesized using Withania somenifera extract.

CONCLUSION

The silver nanoparticles synthesized in this study are environmentally friendly and considered to be safe. This biosynthesis approach is aligned with the principles of nature since it involves natural processes that occur in biological systems. In fact, there are numerous examples of nanoparticle formation in algae, fungi, yeast, and higher plants. These findings suggest potential applications of the synthesized nanoparticles in drug formulation and biomedical fields.

The production of environmentally friendly silver nanoparticles is highly significant in the field of nanotechnology, as demonstrated by previous research [11, 12]. The utilization of silver nanoparticles has great potential in various disease therapies and medical treatments, especially those derived from plant extracts. Therefore, these research findings shed light on the future possibilities of exploring natural systems that hold some of the most intriguing secrets of nature. Additionally, these studies offer the prospect of an environmentally friendly method for the remediation of mining waste.

Conflict of interest: The authors declare no conflict of interest

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