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Antimitotic Activity of Green Synthesized Silver Nanoparticles by Seed Extract of *Sesbania Sesban*

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Abstract: A green method was used to prepare silver nanoparticles by aqueous *Sesbania sesban* seed extract. The study explained the phytochemical analysis to determine phenols, alkaloids, flavonoids, and carbohydrates. The prepared nanoparticles were characterized for particle size distribution, zeta potential, scanning electron microscopy, transmission electron microscopy. The present study used the Bengal gram seed method to evaluate Sesbania sesban Linn (*Fabaceae*) for antimitotic activity. This Bengal gram seed germination assay did the preliminary antimitotic screening. The results specified that the seed extract of *Sesbania sesban* is apt for preparing silver nanoparticles.

Keywords: *Sesbania sesban*; green synthesis; silver nanoparticles; antimitotic activity.

1. Introduction

In an emerging field of nanoscience, nanobiotechnology has increased the prospect of using therapeutic nanoparticles in the detection and treatment of human cancers. Many of them are used for therapeutic drives. Due to their peculiar biological effects imposed by the structure and size, nanoparticles are a possible option for treating diseases [1]. Owing to the specific attributes and therapeutic potential for treating a disease, silver nanoparticles (AgNPs) have drawn attention in nanotechnology [2]. The literature suggests that AgNPs and ions have intrinsic cytotoxic activity [3]. Various methods like physical, chemical, and biological are used to prepare AgNPs. The reduction from Ag (+) to Ag (0) has gained significant importance due to the effective and eco-friendly nature of plant extracts [4,5]. This approach is more effective than other approaches because the plant extracts behave as a stabilizing agent [6-9]. Extracts from various plants like [10], *Cycas* [11], *Brucea javanica* [12], *Carob* [13], *Jatropha curcas* [14], *Rhinacanthus nasutus*, *Cassia angustifolia* [15], *Amla* [16], *Azadirachta indica* [17], *Acacia nilotica* [18], *Coriandrum sativum* [19], *Solanum lycopersicums* [20] were used for synthesis of AgNPs. *Sesbania Sesban* is an evergreen tree which fits the Fabaceae family. This herb has antioxidant, antinociceptive, antidiabetic, anti-inflammatory, antimicrobial, antifertility, sleeping pills, and anthelmintic activity [21,22]. Phytochemical research on the seeds of *Sesbania Sesban* has led to the isolation of alpha-ketoglutaric, oxaloacetic, and pyruvic acids and the presence of myoinositol kaempferol disaccharide, cyanidin, campesterol, β-sitosterol, etc. [23]. The extracts found were high in phenols, flavonoids, B vitamins, and anthocyanins. Myoinositol was observed responsible for the anticancer activity; polyphenols like tannins were reported to have apoptotic activity [24-26]. Hence, efforts are made to determine the antimitotic activity of an aqueous extract and its silver nanoparticles from seeds of a *Sesbania Sesban* plant.

2. Materials and Methods

The seeds of *Sesbania Sesban* were gathered from Sangli, Maharashtra, India. Silver nitrate was received from Sigma-Aldrich. The chemicals used were of analytical grade. Each glassware was washed and rinsed meticulously with distilled water and mopped up in an oven [25,27].

2.1. Plant extract preparation.

The *Sesbania sesban* seeds used were thoroughly washed with distilled water to avoid dirt and any other foreign materials. The seed extract was ready by boiling 5 g of finely powdered seeds in 250 ml of distilled water for 10 minutes. The prepared crude extract was cooled and filtered through the muslin cloth, followed by Whatman filter paper until obtained a clear extract. The extract was stored at 4°C. The freshly prepared seed extract was used to perform the experiments [27].

2.2. Phytochemical studies.

2.2.1. Detection of phenol.

Less extract was attained in water, and a test was performed to check the presence of phenolic compounds using a 1% solution of gelatin containing 10% Sodium Chloride. The development of white precipitate specified the existence of phenols [23,28,29].

2.2.2. Detection of flavonoids.

Fewer extract amounts are dispersed in an aqueous solution of sodium hydroxide. Yellow color formation represented a flavonoid presence [28].

2.2.3. Detection of alkaloids.

Mayer’s test: 2 mg of extract was applied to a few droplets of reagent. A white or yellow precipitate is formed, suggesting the presence of alkaloids [23].

2.2.4. Detection detection for carbohydrates.

Less extract was dispersed in 4 ml of distilled water and percolated. The percolate was subjected to detect the subsistence of carbohydrates [23,29].

Fehling’s test: The mixture was processed and heated in boiling water with 1 ml of Fehling solution A and B. A reddish precipitate revealed the existence of carbohydrates [23,28,29].

2.3. Fourier transforms infrared spectroscopy of aqueous extract.

The spectra of prepared aqueous seed extract of *Sesbania Sesban* were verified on a Shimadzu FT-IR-8400. Approximately 2-3 mg of the sample was combined with 100 mg of dry potassium bromide, and the samples were scanned at 2 cm^{-1} resolution from 4000-400 cm^{-1} wave amounts [27].

2.4. Formulation of silver nanoparticles.

The prepared 1 mM solution of silver nitrate (50 ml) was subjected to magnetic stirring in a 100 ml beaker. Fresh seed extract (5 ml) was added dropwise with constant stirring to reduce Ag^+ ion until the color change was observed. The color variation of the mixture from low light yellow to dark reddish-brown color was monitored after 6 hrs. The experiment was performed at room temperature in the dark to avoid the activation of $AgNO_3$ owing to light. The reduction of $AgNO_3$ to Ag^+ ions was confirmed in 6 hours by the color change [25,27,30].

2.5. Characterizations of AgNPs.

2.5.1. UV-Visible spectroscopy.

UV-visible spectroscopy was performed characterizing the AgNPs. Before recording the absorbance spectrum, a reference of distilled water was recorded to set the reference value. The spectrum of prepared AgNPs was supervised by Lambda 35 Perkin Elmer spectrophotometer in the wavelength region 200–800 nm [31].

2.5.2. Particle size distribution and zeta potential.

Analysis of particle size was supported by laser diffraction technique (Malvern 2000 SM, Instruments, UK), which allows measurement of samples in the range of 0.1-10000 nm. The size was assessed at a 90º scattering angle. Prepared AgNPs were released in distilled water and calculated the mean particle size. Zeta potential values of AgNps were ascertained by electrophoretic light scattering using Malvern Zetasizer 3000 (UK) [15,32].

2.5.3. Transmission electron microscopy.

The behavior of prepared of AgNps was determined by transmission electron microscopy (Technai G2 ultra twin FEI, Netherlands). The sample for TEM was made by placing a drop of the sample on a copper grid and drying it under vacuum pressure [33,34].

2.5.4. Scanning electron microscopy.

The surface morphology was confirmed by scanning electron microscopy (Oxford Instruments, INCA X Sight, UK). Samples were placed on double-faced adhesive tape and were examined by a sputter-coated unit with a thin gold-palladium film and surface topography [33].

2.5.5. Determination of antimitotic activity.

High-quality Bengal gram seeds were collected and soaked in water at room temperature to accelerate [35-37]. The seeds were distributed in a group of 10 seeds on filter paper moistened in each Petri dish. 1% DMSO was considered as a control. Paclitaxel was considered standard. Paclitaxel, Aqueous extract, and AgNPs of concentrations ranging from 100 µg/ml, 500 µg/ml, to 1000 µg / ml were prepared in 1% DMSO considered as standard, test solutions 1 and 2 respectively. 3-4 drops of previously prepared solutions of Paclitaxel (standard), Aqueous extract, and AgNPs were added to different concentrations in Petri plates. Seed germination was allowed for 5 days taking care to wet the filter paper with DMSO and solutions of Paclitaxel (standard), aqueous extract, and AgNPs every 24 hours. The average root length was measured in cm at the end of the 5th day, and the average root length of DMSO and % growth inhibition were calculated. The formula used for calculating % Growth Inhibition is (Mean growth of test / standard÷ Mean growth of control) ×100 [36,38-40].

3. Results and Discussion

This paper describes the preparation of *Sesbania Sesban* AgNPs from the seed extract and the performance on phytochemical, physicochemical, and antimitotic activity. As 5 ml of the aqueous seed extract was introduced to the 1 mM, 50 ml $AgNO_3$ solution under stirring, color shifted from low light-yellow color to dark reddish-brown color. This suggested that aqueous seed extract reduces Ag^+ ions, making silver nanoparticles extremely stable in water. The reduction may be attributed to the phytochemicals in the extracts from the plants (Figure 1). From Table 1, the phytochemicals, phenolic, carbohydrates, flavonoids material served as a powerful reduction agent through the donation of electrons to Ag^+ ions and reduced the formation of Ag° nanoparticles [29,41].

Figure 1. Synthesis of *Sesbania Sesban* silver nanoparticles – color change in the reaction mixture.

Table 1. Phytochemical Estimation of aqueous extract.

Sr. No.	Chemical Constituent	Observation	Present
1.	Phenols	Gives white precipitate	Yes
2.	Flavonoids	Yellow color	Yes
3.	Alkaloids	No yellow precipitate	No
4.	Carbohydrates	Reddish precipitate	Yes

The synthesized green AgNPs FTIR spectrum is given in Figure 2. In the FTIR spectra of the *Sesbania Sesban* seed extract, a large peak was found in the range of 3200 to 3600 cm^{-1} indicating the stretching vibration OH (intermolecular hydrogen, binding). The following band was observed at 2850-3000 cm^{-1} representing an aromatic stretch of C-H. Furthermore, a band at 1620-1680 cm^{-1} shows the occurrence of a C = C alkene stretch. The peak at 1670-1820 cm^{-1} denotes the occurrence of a C = O carbonyl group stretch, while the observed peak at 1000-1300 cm^{-1} indicates the occurrence of the C-O carboxyl group. Also, a strong absorption peak in the range of 1000-1400 cm^{-1} is allotted to the CF-alkyl halide group, the C-Cl group in the 600-800 cm^{-1} range, the C-Br group in the 500- range. 600 cm^{-1} and the CI group shows an absorption peak at 500cm^{-1} [27].

Figure 3 displays UV – visible spectra of prepared AgNps. In the visible range, the particles absorbed light. They gave a peak called surface plasmon resonance (SPR) attributed to the charging density at the conductor interface with the UV-visible spectroscope insulator. The conduction band and the valence band are very parallel for silver nanoparticles. The free electrons give rise to SPR because of the oscillation of electrons of silver nano in equilibrium with the light wave. The absorption peak at 447 nm suggests that the reduced nanoparticles are silver [31].

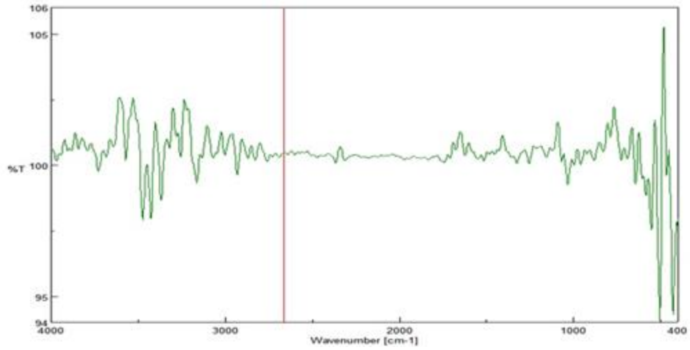

Figure 2. FTIR spectrum of *Sesbania Sesban* aqueous seed extract.

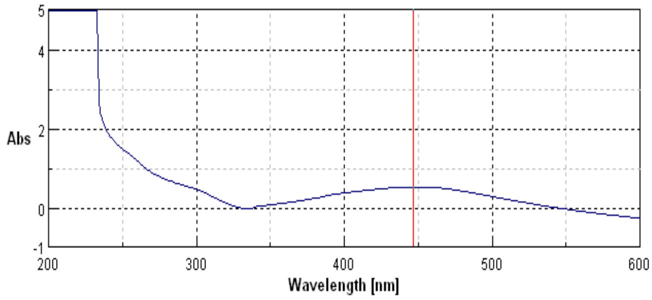

Figure 3. UV-Visible spectra of Silver nanoparticles.

The particle size distribution ranged from 251.7 nm to 623.3 nm, and most of them were observed as monodispersed. Such behavioral difference in the size obtained in the TEM and SEM may be due to the presence of bioactive molecules of *Sesbania Sesban* available for reducing $AgNO_3$ Figure 4 [34].

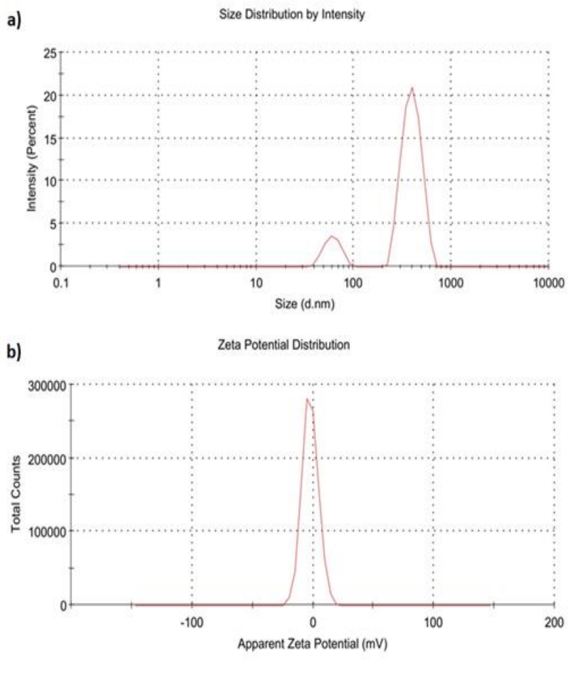

Figure 4. Particle size distribution and Zeta potential of Silver nanoparticles.

Figure 5 provides the SEM picture of synthesized AgNPs. Particle morphology was smooth, irregularly shaped, tiny particles on the surface [27].

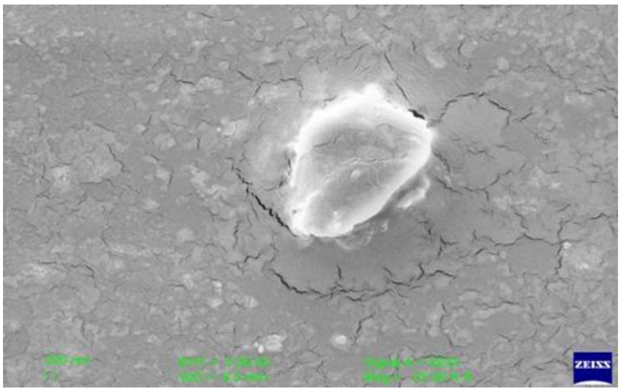

Figure 5. SEM image of Silver nanoparticles.

The morphology of silver nanoparticles of *Sesbania Sesban* seed extract was observed from a higher magnification TEM study at 20nm and 100 nm scales. These nanoparticles were uniform and are uniformly distributed in Figure 6 [42].

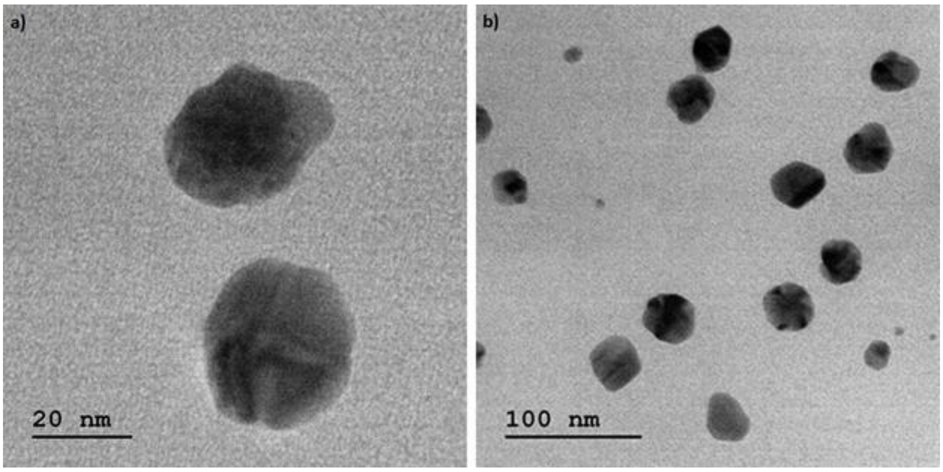

Figure 6. TEM image of Silver nanoparticles.

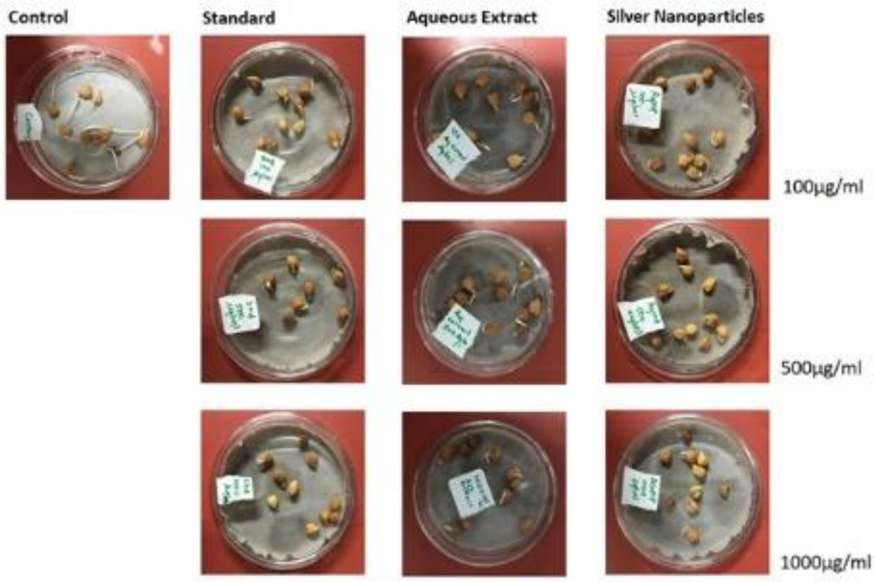

Figure 7. Morphological changes in Bengal gram seeds treated with: control, standard, aqueous seed extract, and silver nanoparticles at different concentrations.

In the present research, using germinating Bengal gram seeds, we assessed the antimitotic activity of aqueous extract of *Sesbania Sesban* seeds and synthesized AgNPs nanoparticles (Figure 7). The results show that the extract completely inhibits germination at a dose of more than 500 µg/ml. The results also indicate that the extract is sensitive to the Bengal gram seeds. These research compounds displayed varying concentrations of antimitotic activity [35].

Germination of Bengal gram seeds is the common method to test antimitotic activity. The AgNPs can inhibit the germination of Bengal gram seeds as depicted in Table 2 with the doses of 100, 500, and 1000 µg/ml, respectively, indicating the potential antimitotic activity. AgNPs showed a substantial antimitotic effect in all concentrations resulting in the growth inhibition after the 5th day. The anticancer drug functions by interjecting the division of cells into dividing cells. Mitosis inhibition in gram seed root tips is a sensitive and simple tool for assessing drug cytotoxicity. Research on the inhibition of mitosis in gram seeds has shown a disturbance in the formation of mitotic spindles and disruption of the formation of cell plates that may be due to the arrest of cell division. Therefore, we propose that AgNPs' cytotoxic activity can disrupt mitotic processes in the rapidly dividing cancer cells that will be beneficial for cancer control [43-45].

Table 2. Antimitotic activity of *Sesbania Sesban* on Bengal gram seeds assay.

Sr. No.	Groups of Sample	Concentration (µg/ml)	No. of Roots	Average Root of Length (In cm)	% Growth of Inhibition (In %)
1.	Control (DMSO)	-	10	2.19±0.49	-
2.	Standard (Paclitaxel)	100	6	0.51±0.3	23.28
		500	7	0.44±0.28	20.18
		1000	3	0.36±0.11	16.71
3.	*Sesbania Sesban* Aqueous Extract	100	7	0.55±0.25	25.43
		500	7	0.68±0.23	31.27
		1000	7	0.71±0.19	32.60
4.	*Sesbania Sesban* Silver Nanoparticles	100	9	0.72±0.27	32.96
		500	7	0.57±0.29	26.07
		1000	6	0.45±0.25	20.54

4. Conclusions

This study verified an ecological, fast, cost-effective, and environmentally friendly method to prepare AgNPs with *Sesbania Sesban* seed extract. The secondary metabolites were mainly responsible for the biosynthesis of AgNPs, probably flavonoids or phenols or carbohydrates. Therefore, the seeds of *Sesbania Sesban* can be considered to have significant antimitotic activity.

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Conflicts of Interest

The authors declare no conflict of interest.

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