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Research Article

Eco-Friendly Green Synthesis of Silver Nanoparticles Using *Senna occidentalis* Leaf Extract and Evaluation of their Antimicrobial, Antiviral, and Larvicidal Properties

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Abstract

Nanotechnology is a branch of science that deals with nano-scale particles. Currently, nanotechnology in medicine has developed into something that's making it possible to incorporate nanoparticles into medical appliances, boosting the treatment's effectiveness. The present study deals with the cost-effective green synthesis of silver nanoparticles using the leaf extract of *Senna occidentalis,* an important medicinal plant. The synthesized AgNPs were characterized by UV-Vis spectrophotometer, the functional groups were confirmed by FTIR, and size and shape were determined by scanning electron microscopy. The silver nanoparticles exhibited diverse biological applications: antibacterial, antifungal, antiviral, and larvicidal properties. The present work is evident that the silver nanoparticles possess good antibacterial activity against gram-positive and gram-negative bacteria, antifungal activity on *A. niger*, antiviral activity on *Sesbania mosaic* virus. The AgNPs could be used as therapeutic agents to treat microbial diseases for modern agricultural and medical sectors.

Keywords: *Senna occidentalis; Silver Nanoparticles; Antimicrobial; Antiviral; Larvicidal Properties*

Introduction

Silver nanoparticles are widely used in the pharmaceutical industry to fabricate ointments and creams to heal burns and infections [1]. Biological or green synthesis is one of the eco-friendly methods to synthesize nanoparticles [2]. Recently, silver nanoparticles have gained considerable use as a potential drug carrier in the treatment of cancer. Nanoparticles can be synthesized by using a variety of chemicals and physical methods, which involve chemical reduction [3], electrochemical reduction, photochemical reduction, and heat vaporization [4]. However, the synthesis of nanoparticles using these methods involves the use of certain toxic chemicals for the reduction of metals [5]. Synthesis of nanoparticles by biological method, using plant extracts, produces less or no toxicity, so in recent years, the synthesis of nanoparticles has focused on the biological method [6].

In this work, the plant *Senna occidentalis* leaves were collected and ground into a fine paste, and silver nanoparticles were synthesized with it. The synthesized nanoparticles were characterized to determine their absorbance range (UV-Vis Spectrometer), presence of functional group (FTIR analysis), Size, and shape (Scanning electron microscope). Following the characterization, it was used to assess its functions in various activities such as antibacterial, antifungal, antiviral, and larvicidal activities. After carrying out each process three times, the findings were evaluated, and conclusions were drawn.

Plant used for the synthesis of silver nanoparticles

Senna Occidentalis L. is a shrubby, erect, can grow up to 2 meters tall. leaves are 15 - 17 cm long, including ribbed petioles 20 - 40 mm long, and leaves are in 3 - 7 pairs with sharp tips. *Senna occidentalis* is a flowering plant with 2 to 4 flowers, petals are yellow, seeds are dull brown, and its seed pods are dark brown. The descriptions and taxonomy studied by Sarath., *et al.* [6].

Experiments

Collection of plants

The plant *Senna occidentalis* was collected from Puttur, Chittoor District, A.P., India.

Preparation of plant leaf extract

Healthy plant leaves were plucked and ground (10 grams) in a motor and pestle, filtered through Whatman filter paper, and made up with 100 mL distilled water.

Synthesis of silver nanoparticles

The silver nanoparticles were synthesized using a green, eco-friendly method. For this, silver nitrate (1 mM) was added to 100 mL of plant leaf extract (filtrate) [7-12].

Characterization of AgNPs

UV-visible spectrophotometry

The formation of AgNPs was determined by measuring the optical density in the UV-Visible spectrophotometer at 350 nm to 490 nm [13].

Scanning electron microscopy

The size and shape of AgNPs were observed under a scanning electron microscope [14].

FTIR analysis

Fourier Transform Infrared spectrum was used for the determination of amino and carboxyl groups, and aqueous solution of AgNPs was recorded in the range of 500 - 4000 cm^{-1} [15,16].

Antibacterial activity

The antibacterial activity of AgNPs was studied on both gram-positive and gram-negative bacterial strains, *E. coli, S. aureus, Klebsiella pneumoniae,* and *Bacillus subtilis* by using a well diffusion method [17]. The active bacterial culture suspension was spread evenly on the nutrient agar plate using with sterilized L-rod; later, different concentrations of AgNPs suspension were loaded into the wells of the nutrient agar medium, and the plates were incubated at 37°C for 24 hours in an incubator. Streptomycin was used as a control. After incubation, the zone of inhibition was measured [18,19].

Antifungal activity

The antifungal activity of silver nanoparticles was tested on fungal strain, *Aspergillus niger* by well diffusion method [17]. The spore suspension of *A. niger* was spread evenly on a potato dextrose medium by using sterilized L-rod and the wells were made in the PDA medium using a sterile borer then different concentrations of silver nanoparticle suspension were added. The plates were incubated at room temperature for 5 - 7 days. After incubation, the zone of inhibition was measured [20,21].

Antiviral activity

Isolation and propagation of virus

The virus inoculum of the *SeMV* was collected from the greenhouse in the Department of Virology, S.V. University, Tirupati. The inoculum was suspended in 0.01M phosphate buffer (P^H 7.1) in a 1:9 ratio and mechanically inoculated onto the leaves of the *Sesbania* plant by using carborundum powder. After inoculation, for the appearance of symptoms, the plants were observed every day [22].

Effect of AgNPs on virus

The infected leaves of the *Sesbania* plant were collected and macerated in a mortar and pestle in cold conditions with 0.01M phosphate buffer (P^H 7.1), and the leaf sap was suspended in 0.01M phosphate buffer (P^H 7.1) in a 1:9 ratio. Later equal volume of the prepared virus sap was transferred to test tubes, treated with different concentrations of silver nanoparticles, and incubated for 1 hour at room temperature. A positive control was maintained without the addition of silver nanoparticles (negative control as a healthy plant). After incubation, the nano-treated virus sap and positive control were mechanically inoculated onto the leaves of the *Sesbania* Plant, the plants were observed every day [23].

Chlorophyll content in infected and AgNPs-treated *Sesbania* plant leaves

Chlorophyll estimation of *Sesbania* plant

The estimation of chlorophyll content in the positive control, negative control, and nano-treated virus in the *Sesbania* plant, the leaves were macerated and treated with 80% acetone and then centrifuged at 3000 RPM. The supernatant was collected, and the OD was measured at 645 and 663 nm in a UV spectrophotometer (blank 80% acetone). The OD values were calculated in the following formula [24]:

Total chlorophyll = 20.2 × OD at 645 nm + 8.02 × OD at 663 nm

Chlorophyll a = 12.7 × OD at 663 nm - 2.69 × OD at 645 nm

Chlorophyll b = 22.9 × OD at 645 nm - 4.68 × OD at 663 nm.

Protein content in healthy, infected, and AgNPs-treated *Sesbania* plant leaves

The Standard folin phenol method was used for the estimation of protein content in the positive control, negative control, and nano-treated virus in the *Sesbania* plant leaves. The obtained optical density values were used to plot a graph to determine the protein content in the positive, negative control, and test (AgNPs and virus sample of infected Sesbania plant leaves [25].

Larvicidal activity

The larvicidal activity of AgNPs was determined on *Culicidae larvae*. The larvae were collected from stagnant water around the local area of S.V. University. The collected larvae were carefully transferred to Petri plates containing fresh water with different concentrations of AgNP suspension and incubated at room temperature and incubated. After incubation, the mortality rate was calculated by using the formula [26,27]:

Results and Discussion

Collection of plant

The plant *Senna occidentalis* was collected from Puttur, Chittoor District, Andhra Pradesh, India (Figure 1). The collected plant leaves were used for further analysis.

Figure 1: *Senna occidentalis plant.*

Preparation of plant leaf extract

The healthy leaves of *Senna occidentalis* were macerated in a motor and pestle, filtered, and made up to 100 mL with distilled water, and used for further studies.

Synthesis of silver nanoparticles

Upon the addition of one millimole of silver nitrate to 100 mL of leaf extract, the color of the solution was changed from green to dark brown (Figure 2 and 3) [7] is an indication of the formation of silver nanoparticles in the plant extract. Similar reports have been observed in the work of other researchers and stated that due to plasmon resonance, it changed its color from colorless to yellowish brown [7-12].

Figure 2: *Senna occidentalis leaf extract.*

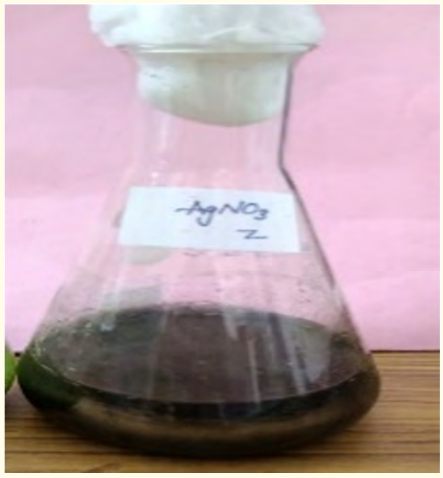

Figure 3: *Bio reduction of $AgNO_3$.*

Characterization of AgNPs

UV-visible spectrophotometry

The UV spectrum of silver nanoparticles was taken from 350 nm to 490 nm, and the formation of a peak was observed between 380 - 430 nm. A strong, narrow peak was observed at 420 nm. From the results of the UV - Vis spectrum (Figure 4), the presence of silver nanoparticles was confirmed. Similar reports were made [13] for silver nanoparticles, which stated that the reduction of silver nanoparticles was observed at 420 nm.

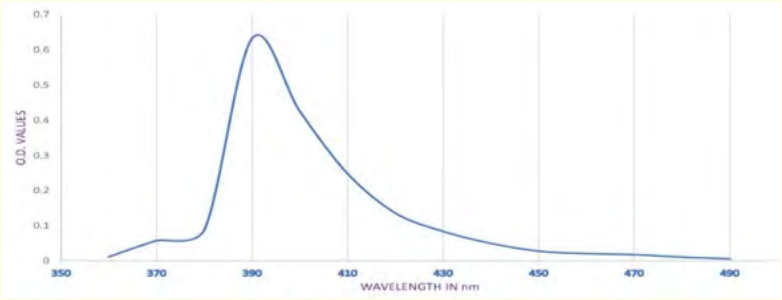

Figure 4: *UV-Vis spectrum of AgNPs.*

Scanning electron microscopy

From the scanning electron microscopy, the size and shape of the silver nanoparticle were confirmed. The shape of the AgNPs is spherical and triangular, well dispersed. The size of the AgNPs ranges from 10 nm to 199 nm (Figure 5). There is a white halo layer around the nanoparticle; it is thought to possess phage contrast properties [14]. Other researchers have performed scanning electron microscopy of AgNPs and reported the size and shapes of AgNPs from various sources.

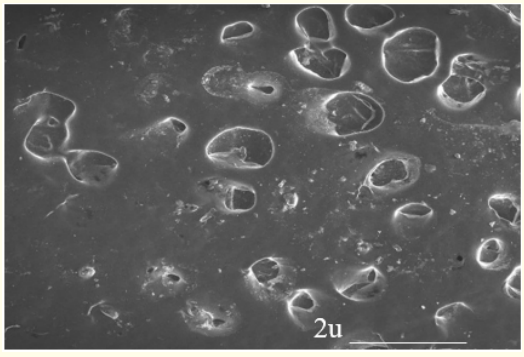

Figure 5: *SEM image of AgNPs.*

FTIR analysis

The FTIR spectrum of silver nanoparticles was measured from 4000 to 500 cm^{-1} (Figure 6), which reveals the presence of O-H stretching (3340 cm^{-1}, broad peak) and C=C stretching (1636 cm^{-1}, sharp, small peak) in the silver nanoparticle [15]. Similar results have been referred to, stating that the nanoparticle's surface was capped and stabilized by flavonoids and other phenolic compounds [16].

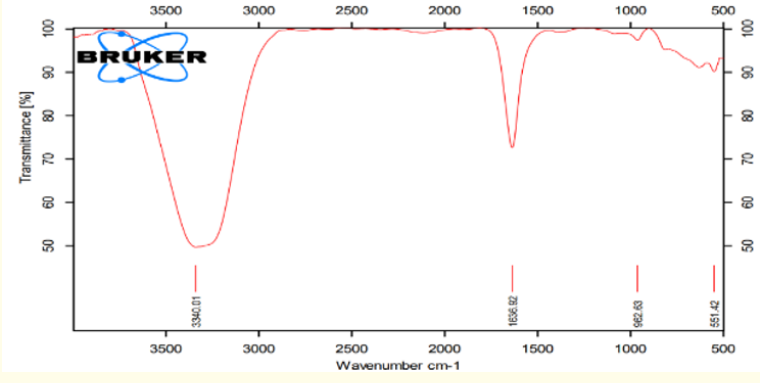

Figure 6: *FTIR spectrum of AgNPs.*

Antibacterial activity

The antibacterial activity of AgNPs on bacterial strains was studied, and the results are shown in table 1. The silver nanoparticles exhibited antibacterial activity against both gram-positive and gram-negative bacteria. With increasing the concentration of AgNPs, the zone of inhibition also increased. The zone of inhibition of both gram-positive bacteria and gram-negative bacteria, on average, was 2.2 cm to 3.2 cm. Similar reports have stated that a combination of medicine and nanotechnology can bring the ability to prevent infections and to eliminate multidrug-resistant pathogens [18,19].

Conc of AgNPs (in µl)	Zone of inhibition (in cm)			
	B. subtilis	***Klebsiella***	***S. aureus***	***E. coli***
25	2.2	2.6	2.2	2.4
50	2.8	2.6	2.5	2.8
75	3.0	3.0	2.8	3.2
Streptomycin (Control)	1.2	1.4	1.4	1.2

Table 1: *Antibacterial activity of AgNPs.*

Antifungal activity

The antifungal activity of silver nanoparticles against *A. niger*, the silver nanoparticles exhibited fungicidal activity. With increasing the concentration of AgNP suspension, the fungicidal activity was also increased. The results are shown in table 2. The zone of inhibition of *A. niger* on average was 0.8 cm to 1.4 cm as the concentration increased. Similar reports were observed on the antifungal activity of AgNPs and the zone of inhibition exhibiting by the AgNPs exhibited zone of inhibition from 1.6 to 1.9 cm [20] and 1.0 to 2.2 cm [21].

Conc of AgNPs (in µl)	Zone of inhibition (in cm)
	A. niger
50	0.8
75	1.2
100	1.4
Fluconazole (Control)	1.4

Table 2: *Antifungal activity of AgNPs.*

Antiviral activity of AgNPs

Isolation and propagation of virus

On the seventh day, the systemic yellow and white mosaic symptoms were observed. These infected leaves were used to study the antiviral properties of silver nanoparticles. Similar reports have been studied from other work in which they have propagated the *Bean Yellow Mosaic* virus in Faba Bean plants [22].

Effect of AgNPs on virus

The *SeMV-infected* Sesbania mosaic plant leaf sap was suspended in 0.01M phosphate buffer (pH 7.1), treated with different concentrations of AgNPs, and incubated for 1 hour at room temperature. A positive control was maintained without treating the infected plant leaf sap with silver nanoparticles. After incubation, the nano-treated virus suspension was mechanically transmitted onto the *Sesbania* plant, and control and infected plants (6 plants) were observed for the development of symptoms. As the concentration of AgNPs increased, the number of visible symptoms decreased compared to the positive control (Table 3). Similar results were studied from the other reports, they treated the virus suspension in silver nanoparticles and incubated for 24 hours and 48 hours and sprayed onto the 10 days Faba Bean plants and they stated that the infection rate was lesser in 48 hours incubated virus suspension in AgNPs than the 24 hours incubated virus suspension in AgNPs [23].

No. of plants	Conc. of AgNPs (in µl)	Systemic symptoms (Visible)	No. of symptoms
6	Positive Control	Yellow and white patches	28
6	100	Yellow and white patches	12
6	300	Yellow and white patches	5-6
6	500	Yellow and white patches	2-3

Table 3: *Antiviral efficacy of AgNPs on SeMV.*

Chlorophyll estimation of *Sesbania* plant

The chlorophyll estimation of the positive control, negative control, and nano-treated virus in *Sesbania* plant leaves is shown in figure 7. The chlorophyll content in the control (healthy) was 30 µg/ml, while in the positive control (infected), it was 21 µg/ml. The chlorophyll content in the nano-treated virus was 26 µg/ml at a concentration of 100 µl, 27 µg/ml at 300 µl, and 28 µg/ml at 500 µl of AgNPs. As the concentration of the silver nanoparticle suspension increased, the chlorophyll content also increased [24].

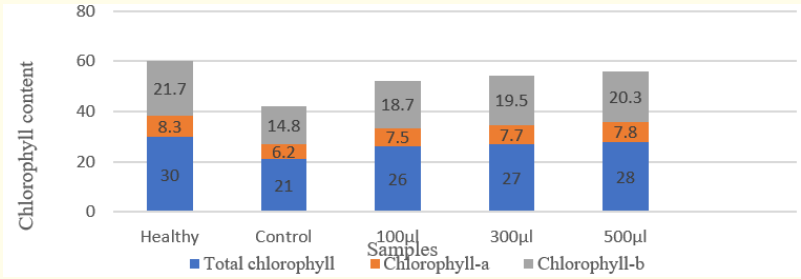

Figure 7: *Chlorophyll estimation.*

Estimation of protein content

The protein content of the positive control, negative control, and nano-treated virus in *Sesbania* plant leaves is shown in figure 8. The protein content in the negative control (healthy) was 560 µg/ml, while in the positive control (infected), it was 290 µg/ml. The protein content in the nano-treated virus was 300 mg/ml at a concentration of 100 µg/ml, 360 mg/ml at 300 µg/ml, and 500 ug/ml at 500 µg/ml of AgNPs. As the concentration of AgNPs increased, the protein content also increased [25].

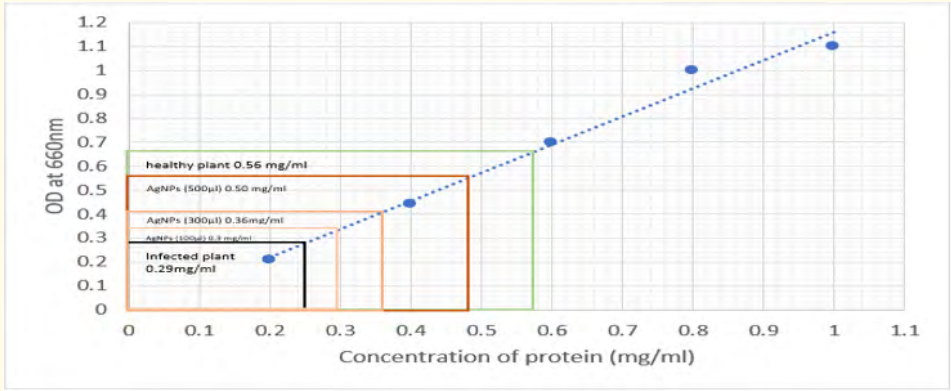

Figure 8: *Estimation of protein.*

Larvicidal activity

The AgNPs are effective against larvae. The results are shown in figure 9. With increasing concentration of AgNPs and incubation time, the mortality rate of the larvae increased. After 12 hours of incubation, most of the larvae were dead; at the end of 24 hours of incubation, all the larvae had died. The percentage of mortality was 40 to 100%. Similar reports were made for silver nanoparticles against larvae and reported that the larvicidal activity of AgNPs increased as the concentration [26], and they exhibited potential larvicidal activity [27].

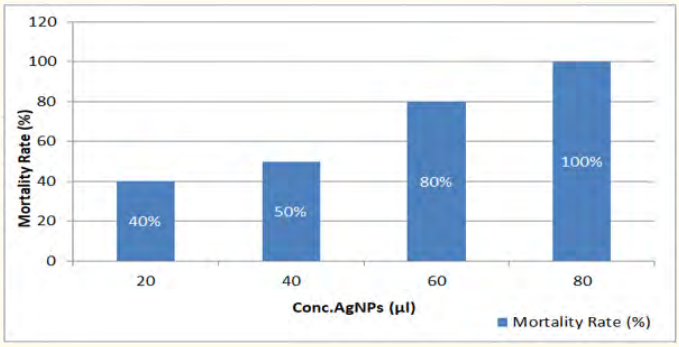

Figure 9: *Larvicidal activity of AgNPs.*

Conclusion

In this study, silver nanoparticles were synthesized through an eco-friendly green synthesis method using *Senna occidentalis* plant leaf extract. The synthesized nanoparticles are characterized by UV spectrophotometer, FTIR, and the size and shape of AgNPs were confirmed by SEM. The AgNPs exhibited significant antibacterial, antifungal, antiviral, and larvicidal properties. Based on their potent bioactive nature, silver nanoparticles could be used as antibacterial, antifungal, antiviral, and larvicidal agents for modern medicine and the agriculture sectors.

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