RESEARCH | Egyptian Journal of Agricultural Research | Plant Pathology

Leaf-mediated green synthesis of silver nanoparticles from *Azadirachta indica* and *Ficus religiosa*: characterization and bioactive properties

Anitha Buggana[*1], Kedharnath Bandari*[1], Sandhya Rani E.*[1], Narasimha Golla*[1] * and Kamel A. Abd-Elsalam*[2]

Address:

[1] *Department of Virology, Sri Venkateswara University, Tirupati, 517502, India*

[2] *Plant Pathology Research Institute, Agricultural Research Centre, 12619, Giza, Egypt*

*Corresponding author: ***Narasimha Golla*** email: dr.g.narasimha@gmail.com

Received: 27-04-2024 ; **Accepted:** 21-07-2024 ; **Published:** 03-08-2024 **DOI:** 10.21608/EJAR.2024.285666.1541

ABSTRACT

Silver nanoparticles are nano-sized metallic particles widely used in various fields, including medicine, pharmaceutical, food, and agriculture. This study carried out the green synthesis of AgNPs using leaf extracts from *Azadirachta indica* (Neem) and *Ficus religiosa* (Peepal) plants. The silver nanoparticles were characterized using sophisticated instruments. UV-visible spectroscopy confirmed the formation of AgNPs, while FTIR identified biomolecules (amino and carboxyl groups) acting as stabilizing agents. SEM determined the size and morphology of AgNPs. Particle size analysis was conducted using DLS, and zeta potential measurements provided information on the size and charge of the silver nanoparticles. The AgNPs were tested for antibacterial efficacy against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria. Similarly, the AgNPs exhibited high fungicidal activity against the fungal strain *A. niger*. The free radical scavenging activities of nanoparticles were studied using DPPH and Hydrogen Peroxide in vitro assays. The antiviral activity of AgNPs was investigated against the *Sesbania mosaic virus* (SeMV), which infects *Sesbania* plants. The AgNPs exhibited excellent antiviral properties, decreasing the viral infection in Sesbania plants, indicating the antiviral efficacy of silver nanoparticles in the agricultural sector.

Keywords: Silver nanoparticles, Characterization, Antimicrobial activity, Antioxidant activity, Antiviral activity, Sesbania *mosaic virus* (SeMV)

INTRODUCTION

Nanostructured metallic nanoparticles are becoming increasingly popular due to their high surface area-to-volume ratio, especially in material science and nanomedicine (Fernando *et al.,* 2024). This technique involves atomic and molecular research and development that produces particles with diameters ranging from 1 to 100 nm (Asefian and Ghavam, 2024). Applications of nanomaterials are significant in our daily lives, and maximizing or minimizing their benefits and downsides is possible. Nanomaterials' exceptional physical and chemical properties are due to their size, shape, and composition (Elwakil *et al.,* 2024). The creation of silver nanoparticles (NPs) presents several advantages over physicochemical techniques, including cost-effectiveness, environmental friendliness, ease of scaling up, and absence of the requirement for high energy, harmful chemicals, or temperatures (Patel & Patel, 2022). Nanoparticles synthesized using biological systems such as bacteria, fungi, algae, and plants offer an alternative method (Avilala and Golla, 2019)

Various metals such as silver, gold, zinc, copper, etc., are synthesized as metallic nanoparticles in biological systems (Golla, 2018). Around 5000 years ago, silver was used in various forms to store food items by Egyptians, Persians, Greeks, and Romans (Tarannum *et al.,* 2019). Silver nanoparticles are widely used among all metal nanoparticles due to their unique properties such as high electrical conductivity and optical, antimicrobial, and biological characteristics (Ahmed *et al.,* 2021). Due to their unique properties, AgNPs have a wide range of applications in the biomedical field, including the textile industry (Gokarneshan and Velumani, 2017), climate change and contamination control (Pulit-Prociak & Banach, 2016), the cosmetic industry (Naidu et al., 2015), wound dressing (Della Sala *et al.,* 2022), antiseptic fabrics (Swilam and Nematallah, 2020), pharmaceuticals (Kumar *et al.,* 2011), water purification (Lin *et al.,* 2013), sensing of food adulterants (Asthana *et al.,* 2016), and adsorption of metals and pesticides (Das *et al.,* 2012; Ping *et al.,* 2012). Researchers have extensively synthesized AgNPs using various plant leaf extracts, including *Ocimum sanctum* (Mallikarjuna et al., 2011), *Pepper* (Mallikarjuna *et al.,* 2012), *Plectranthus amboinicus* (Purusottam Reddy *et al.,* 2017), *Ziziphus nummularia* (Golla, 2018), *Acalypha hispida* (Selvakumar *et al.,* 2018), *Rose canina* (Gulbagca *et al.,* 2019)**,** *aloe vera* (Burange *et al.,* 2021), and *Cinnamomum camphora* (Li *et al.,* 2021).

Plants are the main resource used in traditional medicine to make medications (Sarath Kumar *et al.,* 2024). Since ancient times, medicinal plants have been of great importance for their ability to treat various diseases and are aptly mentioned in Ayurveda (Azaizeh *et al.,* 2003). Medicinal plants are becoming more widely acknowledged for their potential as useful sources for novel therapeutic compounds, particularly in light of the failure of most conventional synthetic medications (Goni *et al.,* 2021). A common and significant medicinal plant in these areas is *Azadirachta indica*, also known as neem. It is a member of the *Meliaceae* family and is widely distributed in tropical and subtropical regions, including Bangladesh, India, Nepal, and Pakistan. Neem leaves contain compounds such as nimbin, nimbanene, ascorbic acid, n-hexacosanol, nimbolide, nimbandiol, and the amino acid nimbiol; 7-desacetyl-7-benzoylgedunin;17-hydroxyazadiradione; and7-desacetyl-7benzoylazadiradione (Kokate *et al.,* 2007; Hossain *et al.,* 2011). After being isolated from fresh neem leaves, the polyphenolic flavonoids quercetin and ß-sitosterol were found to possess antibacterial and antifungal qualities (Govindachari et al., 1998). Previous studies have validated the anti-inflammatory, anti-arthritic, antipyretic, hypoglycemic, anti-gastric ulcer, antifungal, antibacterial, and antitumor properties of neem (Bandyopadhyay *et al.,* 2004; Sultana *et al.,* 2007; Ebong *et al.,* 2008; Paul *et al.,* 2011), Biswas *et al.,* 2002) reported the multiple medicinal uses of Neem plant. The two crucial phytochemicals, terpenoids, and flavanones present in neem, act as reducing agents and stabilize the nanoparticles (Banerjee *et al.,* 2014).

Ficus religiosa, commonly known as peepal and belonging to the *Moraceae* family, is an important medicinal plant. It is known by various local names in India, including Asvatthah in Sanskrit, Sacred fig in Bengal, Peepal in Hindi, Arayal in Malayalam, Ravi in Telugu, and Arasu in Tamil. Often found near Indian temples alongside neem trees, a ceremonial marriage symbolizing a union between the male-representing peepal tree, and the female-representing neem tree is observed. This form of worship is linked to fertility and serves as a prayer and ritual to the almighty. Peepal trees are highly valued in Indian societies as they are believed to continuously generate oxygen. On their lower leaf epidermis, they have a unique type of stomata known as sunken, which are larger than normal stomata. These stomata retain water and gas molecules for extended periods. The peepal tree is a valuable source of traditional medicine for treating asthma, diabetes, gastric problems, and inflammatory and infectious diseases (Singh *et al.,* 2011). It has been studied for its potential to treat neurological diseases like Parkinson's and Huntington's in animal models, such as rats (Bhangale & Acharya, 2016), and to prevent ulcers in albino mice (Gregory *et al.,* 2013). The medicinal properties of the peepal tree are attributed to the presence of phenols, tannins, steroids, alkaloids, flavonoids, and lanosterol. These phytochemicals are water-soluble and act as reducing and capping agents (Choudhari *et al.,* 2011).

Earlier, there were reports on the synthesis of AgNPs (Chinnasamy *et al.,* 2021; Murugesu *et al.,* 2021; Alharbi and Alsubhi, 2022; Hasan *et al.,* 2022; Riyas *et al.,* 2022; Pawar *et al.,* 2022). However, no reports existed on the synthesis of AgNPs from the leaf extracts of *Azadirachta indica* and *Ficus religiosa*. The present work focuses on investigating the eco-friendly green synthesis of AgNPs, their characterization by various instruments such as UV, FTIR, Zeta Potential, and SEM, and their antimicrobial (both gram-positive and gram-negative bacteria) and antiviral efficacy against the *Sesbania mosaic virus (*SeMV), a causative agent of SeMV disease in sesbania plants.

MATERIALS AND METHODS

Collection of plant leaves:

Fresh leaves of *Azadiractha indica* (Neem) and *Ficus religiosa* (Peepal) were collected from the Sri Venkateswara University Campus, Tirupati, Andhra Pradesh, India. The collected leaves were used in the preparation of extracts for the green synthesis of AgNPs, and the evaluation of their biological activities.

Preparation of leaf extract:

The collected leaves were thoroughly washed with normal tap water followed by double distilled water to remove dirt from the surface of the leaves. The leaves were air-dried to avoid excessive weight during weighing. 5grams of Neem leaves and 5 grams of Peepal leaves were weighed separately on balance. Both types of leaves were chopped into small pieces and macerated with distilled water using a motor and pestle. The leaf extracts were filtered through muslin cloth, and the filtrate was made up to 100 mL by adding distilled water. The prepared leaf extracts were stored at room temperature for further experiments.

Synthesis of Silver nanoparticles:
For the synthesis of AgNPs, 0.1 mM of $AgNO_3$ is added to the prepared leaf extract and incubated at room temperature. After some time, the color of the leaf extract changes from green to dark brown, indicating the formation of silver nanoparticles. The formed silver nanoparticles are then characterized.

Characterization of silver nanoparticles:
UV-Visible spectrophotometer:
The Ag+ ions were reduced by plant leaf extracts and observed with a UV-visible spectrophotometer (UV-1800). The absorption spectrum was recorded at wavelengths ranging from 250 to 500 nm.

Fourier transmission infrared (FTIR) spectroscopy:
The functional groups, such as amino and carboxyl groups in the nanosuspension, were determined by FTIR spectral analysis of the synthesized AgNPs using a Perkin Elmer spectrophotometer. The analysis employed the NaCl method, and the spectrum for the analysis was recorded in the range of 3800-650 cm^-1.

Scanning electron microscopy (SEM):
The size and shape of AgNPs were confirmed using Scanning Electron Microscopy.

DLS particle size analysis and zeta potential:
DLS intensity and laser diffraction methods were employed to determine the particle size of the synthesized AgNPs. This analysis was used to determine the hydrodynamic diameter and polydispersity index (PDI). Zeta potential analysis was also performed to study the negative potential values of the AgNPs that contribute to their reduction and stability. These analyses were carried out using laser diffractometry with an SZ-100 instrument at Sri Venkateswara University, Tirupati.

Antibacterial activity:
The antibacterial effectiveness of AgNPs was studied against the bacterial strains *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) using the agar well diffusion method (Singh *et al.,* 2010). To conduct the study, 0.5mL of bacterial culture suspension was spread onto solidified nutrient agar plates using a sterile spreader, followed by the punching of 4 wells with a borer. AgNPs of different concentrations (10, 15, 20, 25 µL) were placed in the wells. Streptomycin antibiotic was used as a control. The Petri plates were then placed in an incubator at 37^0C, and the antibacterial activity of AgNPs was evaluated by measuring the diameter of the zone of inhibition (in cm) surrounding the wells, indicating the antimicrobial effectiveness of the silver nanoparticles.

Antifungal activity:
The antifungal activity of AgNPs has been tested against the fungus *Aspergillus niger* using the agar well diffusion method (Khadri *et al.,* 2013). For this assay, 20 mL of sterilized potato dextrose agar (PDA) medium was transferred to sterilized plates and allowed to solidify. Then, 0.1 mL of *A.niger* spore suspension was seeded onto the plates using a sterile spreader. Four wells were bored into the plate, and different concentrations of the colloidal nanosuspension (15, 25, and 50 µL) were added to three wells while the fourth well received 50 µL of leaf extract without AgNPs. The plates were incubated at 25^oC for 4 to 5 days. The zone of inhibition was measured in centimeters to determine the activity of AgNPs.

Antioxidant activity of synthesized AgNPs:
Hydrogen Peroxide Scavenging Assay:
The radical scavenging activity of the AgNPs was determined by following the method described by Ruch et al., 1989. Different concentrations of AgNPs (100, 150, 200, and 250 µL) were mixed (0.6 mL) of hydrogen peroxide (43 mM) and 3.4 mL of phosphate buffer (pH 7.4; 0.1 M). The mixture was incubated for 10 minutes, and the absorbance was measured at 230 nm using a UV-visible spectrophotometer. Ascorbic acid was used as a standard. The percentage of radical scavenging activity was calculated using the following formula:
Percentage of RSA = [(Ac-As/Ac)] x 100
where Ac is the absorbance of the control (phosphate buffer), and as is the absorbance of the sample (AgNPs). The tests were carried out in triplicate.

DPPH radical scavenging activity:
The antioxidant activity of the AgNPs was determined by the 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) assay (Gupta, 2014). One milliliter of DPPH solution was added to 1 mL of methanol solution, and different concentrations of

AgNPs (1, 2, 3, and 4 mL) were added. The reaction mixture was kept in a dark place for 30 min. Later, the absorbance was measured at 517 nm in a UV-visible spectrophotometer. Ascorbic acid was used as a standard. The percentage of radical scavenging activity was calculated by using the following formula:

Percentage of RSA = [(Ac-As/Ac)] x 100

Where Ac represents the absorbance of the control reaction, and as represents the absorbance of a sample (AgNPs). The tests were carried out in triplicate.

Antiviral activity:

Sample collection:

The *Sesbania grandiflora* plants, infected with the *Sesbania mosaic virus* and exhibiting mosaic symptoms on the leaves, were collected from the Department of Virology Garden at Sri Venkateswara University in Tirupati, Andhra Pradesh, India.

Preparation of virus inoculum:

The virus inoculum was prepared by crushing infected *Sesbania grandiflora* leaves with a known amount of phosphate buffer (pH 7.0; 0.1 M) in a mortar and pestle. Thus, the sap extract was strained through a muslin cloth, and the resulting filtrate was used for future studies.

Viral activity:

The *Sesbania grandiflora* plant was used to assess antiviral activity. Seeds of *Sesbania* were sown in ten plastic cups filled with soil. Among these, one was healthy (negative control), one was infected (positive control) and eight were test plants. Two concentrations of AgNPs (25 and 200 µL) were tested for their antiviral activity. The AgNPs at different concentrations were incubated with virus sap at room temperature for various time intervals: 30, 60, 90, and 120 minutes. Subsequently, the virus sap suspension was mechanically inoculated onto the test plants. Following inoculation, the plants were grown for approximately four weeks and monitored daily for the development of symptoms.

Chlorophyll estimation:

The chlorophyll estimation of healthy and infected leaves was carried out according to (Kamble *et al.,* 2015) with some modifications. Four or five leaf samples from healthy, infected, and nanoparticle-treated plants were gently ground with a mortar and pestle. To the homogenized material, 1 mL of 80% acetone mixed with 0.5 g of $CaCO_3$ was added. The materials were further macerated gently. These samples were then filtered through muslin cloth, and the filtrate was collected. The absorbance of the solution was measured by a spectrophotometer at wavelengths of 645 nm and 663 nm against a blank using 80% acetone. To calculate the amount of chlorophyll content, the following formula was utilized:

$Chl\ a = 11.75 \times A_{662.6} - 2.35 \times A_{645.6}$

$Chl\ b = 18.61 \times A_{645.6} - 3.96 \times A_{662.6}$

Where, Chl a and Chl b are chlorophyll a and chlorophyll b, A is absorbance.

RESULTS

Collection of plant leaf material and preparation of silver nanoparticles:

The leaf materials of *Azadirachta indica* (Neem) and *Ficus religiosa* (Peepal) were collected from the Sri Venkateswara University campus in Tirupati, Andhra Pradesh, India [**Fig. 1 (A)**]. The collected leaves were rinsed with double-distilled water to remove any surface dirt and then chopped into small pieces. They were then allowed to dry in the shade. A crude leaf extract was prepared at a concentration of 10 mg/ml using distilled water. To this extract, 10 ml of 1 mM Silver Nitrate ($AgNO_3$) solution was added. The color change of the leaf extract solution from green to dark brown indicates the formation of silver nanoparticles [**Fig. 1 (B)**].

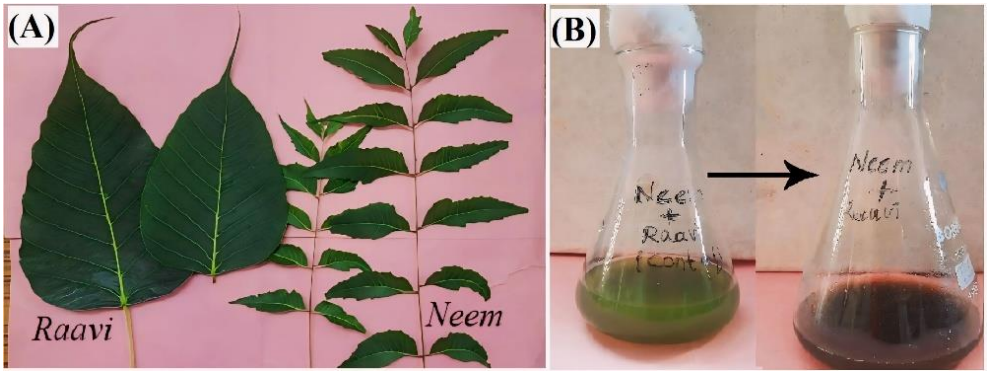

Fig. 1. (**A**) *Ficus religiosa* and *Azadirachta indica* leaves (**B**) Bio reduction of silver nitrate

Characterization of synthesized nanoparticles:

UV-Visible Spectroscopy and FTIR analysis:

The AgNPs were primarily characterized by UV-visible spectroscopy exhibiting a strong absorption band in the visible range from 250 to 500 nm. The UV-visible spectrum [**Fig. 2 (A)**] displays the Surface Plasmon Resonance (SPR) peak of the AgNPs at 422 nm. The FTIR of the formed silver nanoparticles is shown in [**Fig. 2 (B)**]. The peak at 3435 cm^-1 corresponds to the O-H stretch of alcohols. The peaks at 2953 cm^-1 and 2853 cm^-1 correspond to C-H stretching vibrations of alkane. The peak at 2725 cm^-1 corresponds to the C-H stretch of aldehyde. The peak at 1644 cm^-1 corresponds to the C=C stretching vibration of alkenes. The peak at 1459 cm^-1 corresponds to the C-H bending of methyl groups. The peak at 1376 cm^-1 corresponds to the C-N stretching of aromatic amines (Mallikarjuna et al., 2011). The peak at 1219 cm^-1 corresponds to the C-O stretching vibration of phenols. The peaks at 721 cm^-1 and 772 cm^-1 correspond to the C=C bending of aromatic groups, indicating the presence of secondary metabolites.

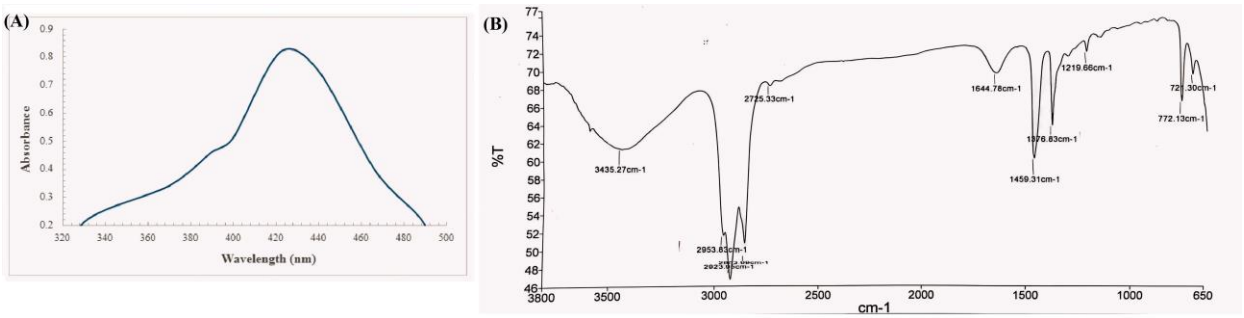

Fig. 2. (**A**) UV-Visible spectra of AgNPs (**B**) FTIR spectrum of AgNPs

Scanning Electron Microscope:

The morphology and shape of the synthesized nanoparticles capped with biomolecules were confirmed by the SEM micrograph (**Fig. 3**). The SEM micrographs revealed that the AgNPs are spherical, with sizes ranging from 50 to 100 nm. They are also polydispersed and do not conglomerate in the solution.

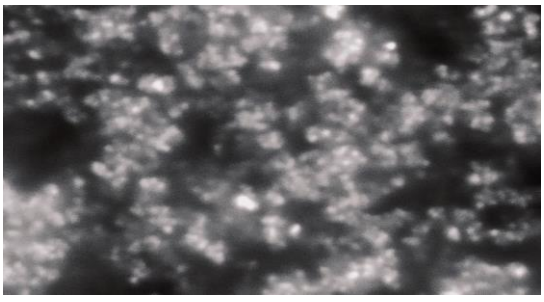

Fig. 3. SEM image of AgNPs

DLS particle size analysis and zeta potential:

The size distribution of the AgNPs is measured due to their Brownian motion in a liquid solution. The Z-average and polydispersed index values of the AgNPs were 83.8 nm and 3.633, respectively [**Fig. 4 (A)**]. Another important parameter in characterization is Zeta potential analysis, which indicates the presence of charge on the silver nanoparticles. The Zeta potential value of the AgNPs was -19.9 mV [**Fig. 4 (B)**].

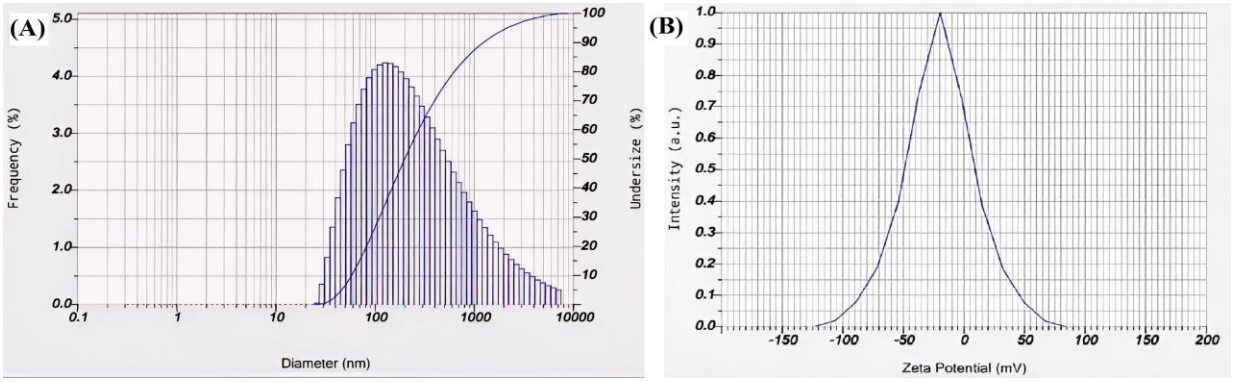

Fig. 4. (**A**)The particle size of AgNPs (**B**) Zeta potential of AgNPs

Antibacterial activity:

The antibacterial efficacy of the silver nanoparticles was confirmed by the formation of a zone of inhibition around the well (**Fig. 5**). The nanosuspension demonstrated significant antibacterial activity against *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) bacteria, with the zones of inhibition measuring 1.9 cm and 2 cm, respectively (**Fig. 6**).

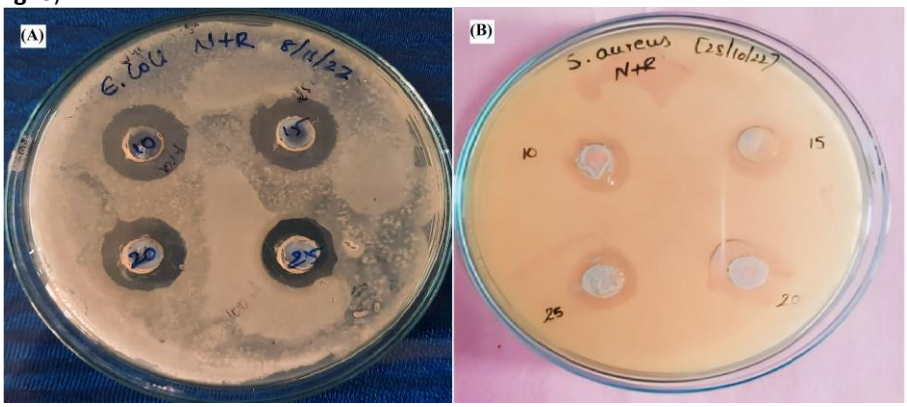

Fig. 5. AgNPs antibacterial efficacy on *E. coli* (**A**)and *S. aureus* (**B**) at different concentrations

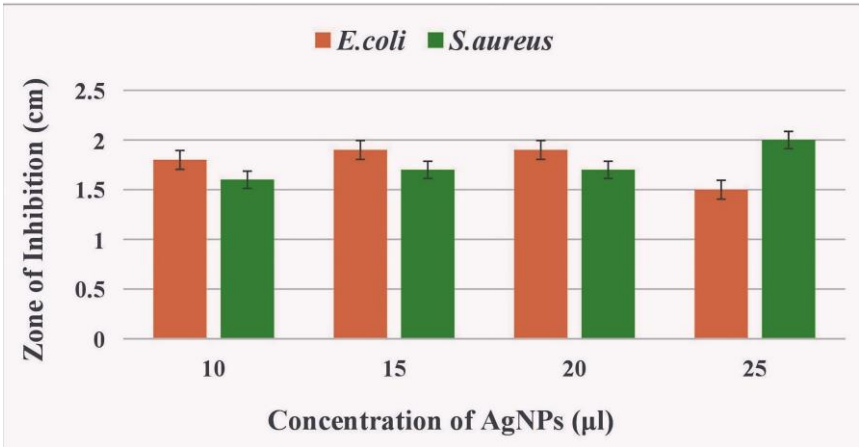

Fig. 6. Graph illustrating the zone of inhibition of AgNPs on bacterial strains

Antifungal activity:

The AgNPs exhibit strong antifungal activity on *Aspergillus niger* growth (**Fig. 7**). The result shows that *A.niger* was sensitive to the AgNPs nanoparticles with a zone of inhibition up to 2.6 cm, compared to 1.5 cm for the leaf extract (**Fig. 8**). This suggests that an increase in the concentration of AgNPs leads to a larger zone of inhibition and it is an indication of fungicidal efficacy of silver nanoparticles.

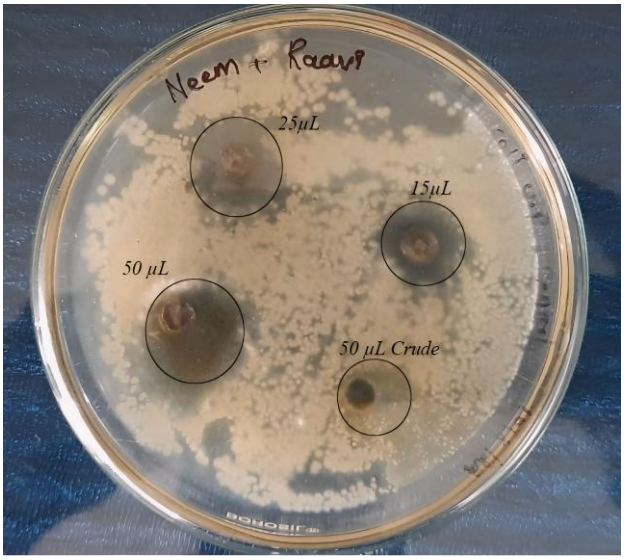

Fig. 7. Antifungal activity of AgNPs on *A. niger*

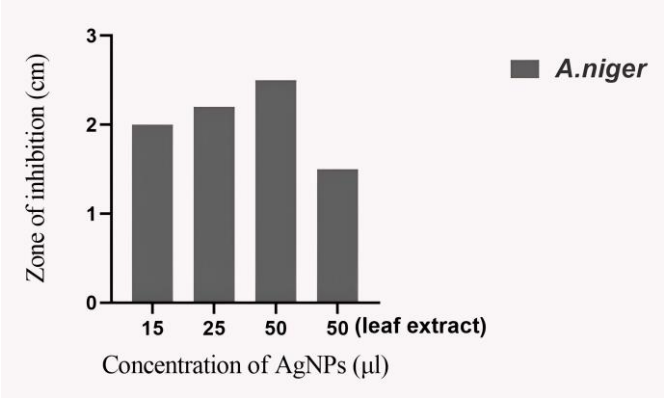

Fig. 8. Zone of inhibition of AgNPs on *A. niger*

Antioxidant activity:

Hydrogen peroxide scavenging activity:

The scavenging ability of AgNPs on hydrogen peroxide is demonstrated (**Fig. 9**). The result shows that the hydrogen peroxide quenching activity of AgNPs with 83.7%, whereas ascorbic acid has 92.7%.

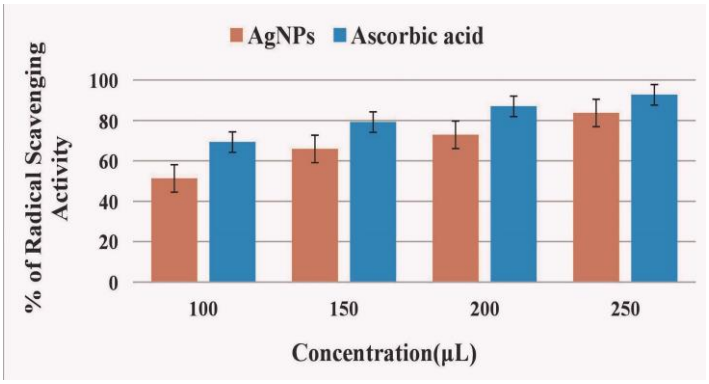

Fig. 9. Graph depicting the Hydrogen peroxide scavenging activity of AgNPs

DPPH activity:

Consequently, the antioxidant activity of AgNPs was demonstrated using DPPH as a free radical. The in vitro antioxidant studies (**Fig. 10**) showed that the free radical scavenging activity increased with a higher concentration of AgNPs. This demonstrates that at higher concentrations (2 mL), DPPH activity was 90.7 % whereas ascorbic acid (standard) was found to be 100 %.

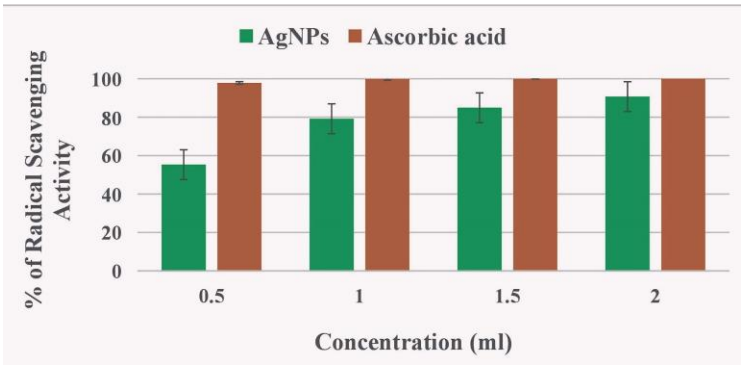

Fig. 10. Graph illustrating the DPPH activity of AgNPs

Antiviral activity in *vivo:*

Silver nanoparticles have been found to exhibit antiviral activity against the plant pathogenic virus *Sesbania mosaic virus* (SeMV). The plant shows symptoms such as stunted growth, drying of leaves and mosaic pattern on small leaves observed five days after mechanical inoculation. However, in plants treated with AgNPs, these symptoms are observed only after two weeks of inoculation. Test plants treated with AgNPs along with virus sap demonstrate that as incubation times increase, the rate of infection or symptom severity decreases. At low concentrations (25 µL), AgNPs with virus tither incubated for 30 minutes resulted in the plants displaying yellowing of basal leaves, leaf desiccation, and mosaic symptoms on young leaves. However, at 120-minute incubation, the plant leaves appear greenish and erect, with only slight yellowing of basal leaves [**Fig. 11(A)**]. At a higher concentration of AgNPs (200 µL), along with virus sap at 30-minute incubation time, the plants exhibit a slight yellowish base leaf with mosaic symptoms in young leaves. In contrast, at a 120-minute incubation time, the plants are healthy and erect [**Fig. 11(B)**].

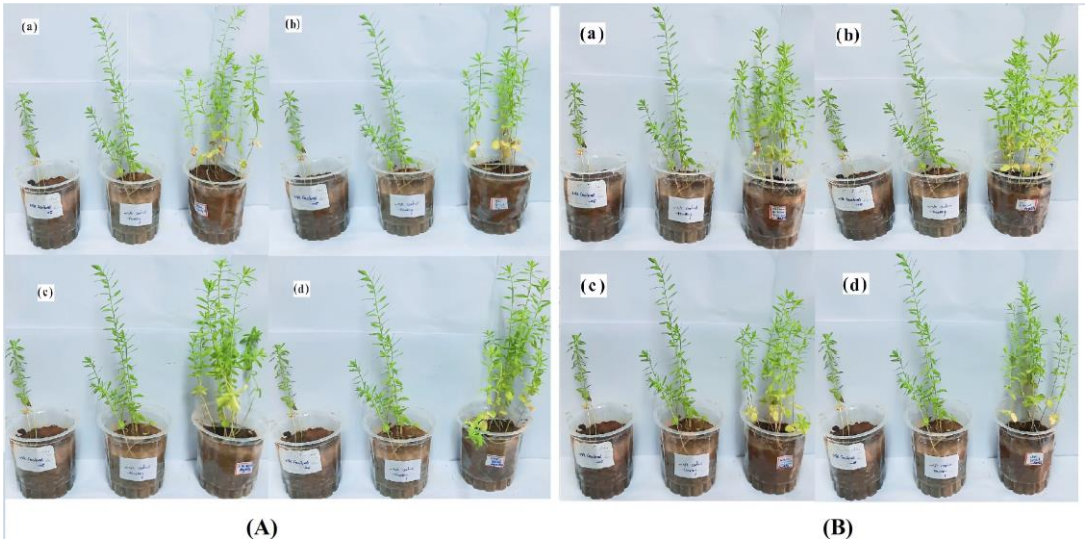

Fig. 11. (**A**) Test plants treated with AgNPs were incubated for various durations: (a) 30 minutes, (b) 60 minutes, (c) 90 minutes, (d) 120 minutes, each in 25 µL (**B**) Test plants treated with AgNPs were incubated for various durations: (a) 30 minutes, (b) 60 minutes, (c) 90 minutes, (d) 120 minutes, each in 200 µL volumes compared with healthy and infected plants.

Chlorophyll estimation:

The total chlorophyll content in healthy and infected sesbania plants was estimated and depicted in [**Fig. 12**]. In healthy leaves, the total chlorophyll is 33.7 mg/ml, whereas in virus-infected leaves is 11.8 mg/ml. The results show that as the incubation time increases, the chlorophyll content also increases in the test plants. At 120 minutes of incubation time, both concentrations (25 and 200 µL) of test plants have chlorophyll contents of 30.8 mg/ml and 24.1 mg/ml, respectively. This result is nearly equal to the chlorophyll content of healthy leaves.

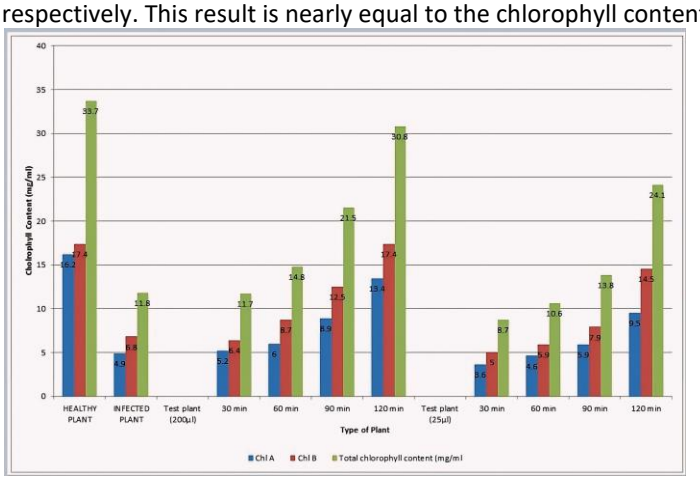

Fig. 12. Chlorophyll content in healthy, infected, and nano-treated plants

DISCUSSION

The bioreduction of silver ions into AgNPs was confirmed by a color change. This change is due to the excitation of surface plasmon resonances (SPR), a characteristic property of silver nanoparticles. Similar reports were described by (Vaishnavi *et al.,* 2020; Palithya *et al.,* 2022), where plant extracts of *Pterocarpus santalinus* and *Areva lantana* reduced the filtrate from green to dark brown. The exact mechanism of the synthesis of silver nanoparticles is yet to be fully understood. However, it is hypothesized that the NADH-dependent nitrate reductase enzyme present in plant cells reduces Ag^+ ions to Ag atoms (Labrenz *et al.,* 2000; Roh *et al.,* 2001). The UV-visible spectrum displays the Surface Plasmon Resonance (SPR) peak of the AgNPs at 422 nm a finding consistent with that of Hamouda et al. (Hamouda *et al.,* 2019). The size and shape of AgNPs influence the absorption peak, with the size of nanoparticles having a linear correlation with peak intensity (Kerker 1985; Sosa *et al.,* 2003). FTIR analysis was conducted to detect various functional groups, such as amino and carboxyl groups, which aid in the reduction of Ag^+ ions to form silver nanoparticles. These biomolecules act as capping and stabilizing agents for the synthesized nanoparticles. The peak at 3435 cm^-1 corresponds to the O-H stretch of

alcohols. The peaks at 2953 cm^-1 and 2853 cm^-1 correspond to C-H stretching vibrations of alkane. The peak at 2923 cm^-1 corresponds to C-H vibrations of biomolecules (Mallikarjuna *et al.,* 2018). The shape of nanoparticles greatly affects their conjugation with specific drug molecules and targeting of cells (Dauthal and Mukhopadhyay, 2016). Particle size is crucial for cellular transport; smaller particles can easily pass through the plasma membrane of cells. Nanoparticles smaller than 100 nm are particularly useful for various applications in drug delivery and biosensor development (Mukherjee *et al.,* 2014). These nanoparticles generally exhibit very good antibacterial activity, as they interact with bacterial cell walls (Prasad and Swamy, 2013; Shaikh *et al.,* 2019; Anand *et al.,* 2021). The charge on the surface of the nanoparticles creates a repulsive force that prevents agglomeration and contributes to the long-term stability of the nanoparticles. The obtained result is consistent with the zeta potential value of -19.7 mV for silver nanoparticles produced by the fungus *Trichoderma longibrachiatum* (Elamawi *et al.,* 2018), indicating that the AgNPs are highly stable and resist agglomeration in the medium. Many reports have documented the antibacterial activity of silver nanoparticles against bacteria (Narasimha, 2013), fungi (Narasimha *et al.,* 2011), and plants (Seku et al., 2018). Several studies have described the mechanisms of action of AgNPs (Lok *et al.,* 2006; Durán *et al.,* 2010, 2016). One probable mechanism is that AgNPs attach to the cell membrane, destabilizing respiratory function and permeability in bacterial cells (Kvítek *et al.,* 2008). Regular interaction of AgNPs with sulfur and phosphorus groups disrupts DNA replication and subsequently disintegrates microbial systems (Ramesh *et al.,* 2015; Singh *et al.,* 2014).

Numerous reports have documented the antifungal activity of AgNPs in seeds (Simangunsong et al., 2022), fungi (Jaidev and Narasimha, 2010), and plant extracts (Mallikarjuna *et al.,* 2013). Dorau *et al.* (2004) reported that the antifungal activity of AgNPs is attributed to the formation of insoluble compounds through the inactivation of sulfhydryl groups in the fungal cell wall and the rupture of membrane-bound enzymes and lipids, leading to cell lysis. Hydrogen peroxide, while not highly reactive, can damage cells by inducing hydroxyl radicals. Thus, it is crucial to eliminate these reactive species from cells or food systems. Previous reports have confirmed that biosynthesized AgNPs are effective scavenging agents (Keser *et al.,* 2012; Mohanta *et al.,* 2017). Free radicals are primary therapeutic targets due to their role in causing inflammatory diseases. Antioxidants, as potential agents, inhibit free radicals and possess anti-cancer, anti-aging, and antifungal properties. They can also prevent rheumatoid arthritis and inflammation (Khan *et al.,* 2019; Shanmugapriya *et al.,* 2016; Sharmila *et al.,* 2019). Many reports have been published on the DPPH activity of AgNPs synthesized from various plant extracts (Khorrami *et al.,* 2018; Ahn *et al.,* 2019; Küp *et al.,* 2020; Khuda *et al.,* 2022). Khandelwal *et al.* (2014) reported that the antiviral activity of AgNPs and other metal nanoparticles is due to interference with the glycoproteins of the viral envelope, which prevents virus invasion and replication in host cells. The results of the present study agree with previous reports that silver nanoparticles aggregate extensively and interfere with viral envelope proteins, thereby reducing disease severity in infected plants (Elbeshehy *et al.,* 2015; Noha and El-Dougdoug *et al.,* 2018; Abdelkhalek *et al.,* 2022; El Gamal *et al.,* 2022).

AgNPs are potent antiviral agents. The present study suggests that AgNPs potentially inhibit virus growth. There are differing opinions on whether the virus destroys chlorophyll or inhibits its synthesis. According to Sheffield (1993), the Tomato *acuba mosaic* did not affect the chlorophyll in mature leaves at the time of infection; rather, it prevented the formation of plastids in young leaves. Viruses such as cucumber mosaic and tomato spotted wilt produce chlorosis, indicating the destruction of chlorophyll. Earlier reports documented the reduction of chlorophyll content in various host plants infected with different viruses (Arora *et al.,* 2009; Sinha and Srivastava, 2010; Narasimha and Papaiah, 2014). Nanotechnology is used in agriculture to improve food production by increasing nutritional value, quality, and safety (Devra, 2022). Silver nanoparticles exhibit the slow release of agrochemicals and are very stable and biodegradable. Hence, they can be utilized in the preparation of nanocapsules for the gradual and effective administration of fertilizers, pesticides, and agrochemicals in farming activities (Chowdappa and Gowda, 2013). Nano fertilizers (NFs) are useful in controlling nutrition because of their exceptional ability to increase nutrient utilization efficiency (Reshma Anjum et al., 2024). Extensive chemical fertilizer application destroys soil structure, mineral cycles, plants, microbiological flora in the soil, and other food chains in ecosystems, ultimately leading to heritable mutations in subsequent consumer generations. Focusing on the macro elements (N, P, and K) with nano fertilizers is a better course of action because it might replace most of these nutrients with ones that have significant positive environmental effects (Abd-Elsalam and Alghuthaymi, 2024). Nano pesticides, nano fertilizers, food packaging, pest control, crops, and food protection are various uses for AgNPs (Khan *et al.,* 2023).

CONCLUSION

The present work focuses on the green synthesis of silver nanoparticles (AgNPs) using leaf extracts of *Azadirachta indica* and *Ficus religiosa*. The bio-reduced AgNPs were characterized by UV-visible spectrophotometry and FTIR, indicating that functional groups such as amines, aldehydes, and carboxylic acids are responsible for the reduction and stabilization of the nanoparticles. The size of the AgNPs was measured to be 83.8 nm, with a

spherical shape. The silver nanoparticles exhibited good antibacterial activity against both Gram-positive and Gram-negative bacteria. The bio-reduced AgNPs exhibit fungicidal activity against *A. niger*. Their antioxidant properties, including hydrogen peroxide and DPPH scavenging, demonstrate that the silver nanoparticles are effective free radical scavengers. Furthermore, the nanoparticles display significant antiviral activity against Sesbania mosaic virus (SeMV). With increased concentration and incubation time of AgNPs, the viral infection rate in Sesbania plants decreases, indicating the antiviral efficacy of silver nanoparticles.

REFERENCES

Abdelkhalek, A., El-Gendi, H., Alotibi, F. O., Al-Askar, A. A., Elbeaino, T., Behiry, S. I., Abd-Elsalam, K. A., & Moawad, H. (2022). *Ocimum basilicum*-mediated Synthesis of Silver Nanoparticles Induces Innate Immune Responses against Cucumber Mosaic Virus in Squash. *Plants*, *11*(20), 2707.

Abd-Elsalam, K.A. & Alghuthaymi M.A.(Ed.s) (2024). Nanofertilizers for Sustainable Agroecosystems: Recent Advances and Future Trends. *Springer Nature*, 573

Ahmed, H. M., Roy, A., Wahab, M., Ahmed, M., Othman-Qadir, G., Elesawy, B. H., Khandaker, M. U., Islam, M. N., & Emran, T. Bin. (2021). Applications of Nanomaterials in Agrifood and Pharmaceutical Industry. *Journal of Nanomaterials*, *2021*, 1-10

Ahn, E. Y., Jin, H., & Park, Y. (2019). Assessing the antioxidant, cytotoxic, apoptotic, and wound healing properties of silver nanoparticles green-synthesized by plant extracts. *Materials Science and Engineering C*, *101*, 204-216.

Alharbi, N. S., & Alsubhi, N. S. (2022). Green synthesis and anticancer activity of silver nanoparticles prepared using fruit extract of *Azadirachta indica*. *Journal of Radiation Research and Applied Sciences*, *15*(3), 335-345.

Anand, A. V., Bharathi, V., Bupesh, G., Lakshmi, N. J., Sundaram, K. M., & Saradhadevi, K. M. (2021). Identification of novel potent pancreatic lipase inhibitors from *Ficus racemosa. Biomedicine (India)*, *41*(1), 23-30.

Arora, R., Joshi, U. N., Gupta, P. P., & Singh, J. V. (2009). Effect of the yellow mosaic virus on pathogenesis related enzymes and chlorophyll content in moth bean (*Vigna aconitifolia*). *Acta Phytopathologica et Entomologica Hungarica*, *44*(1), 49-60.

Asefian, S., & Ghavam, M. (2024). Green and environmentally friendly synthesis of silver nanoparticles with antibacterial properties from some medicinal plants. *BMC Biotechnology*, *24*(1), 5.

Asthana, A., Verma, R., Singh, A. K., Susan, M. A. B. H., & Adhikari, R. (2016). Silver Nanoparticle Entrapped Calcium-Alginate Beads for Fe (II) Removal via Adsorption. *Macromolecular Symposia*, *366*(1), 42-51

Avilala, J., & Golla, N. (2019). Antibacterial and Antiviral Properties of Silver Nanoparticles Synthesized by Marine Actinomycetes. *International Journal of Pharmaceutical Sciences and Research*, *10*(3), 1223-1228.

Azaizeh, H., Fulder, S., Khalil, K., & Said, O. (2003). Ethnobotanical knowledge of local Arab practitioners in the Middle Eastern region. *Fitoterapia*, *74*(1–2), 98-108.

Bandyopadhyay, U., Biswas, K., Sengupta, A., Moitra, P., Dutta, P., Sarkar, D., Debnath, P., Ganguly, C. K., & Banerjee, R. K. (2004). Clinical studies on the effect of Neem (*Azadirachta indica*) bark extract on gastric secretion and gastroduodenal ulcer. *Life Sciences*, *75*(24), 2867-2878.

Banerjee, P., Satapathy, M. K., Mukhopadhyay, A., & Das, P. (2014). Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: Synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresources and Bioprocessing*, *1*(1), 1-10.

Bhangale, J. O., & Acharya, S. R. (2016). Anti-Parkinson Activity of Petroleum Ether Extract of *Ficus religiosa* (L.) Leaves. *Advances in Pharmacological Sciences*, *2016*, 1-9.

Biswas, K., Chattopadhyay, I., Banerjee, R. K., & Bandyopadhyay, U. (2002). Biological activities and medicinal properties of Neem (*Azadirachta indica*). *Current Science*, *82*(11), 1336-1345.

Burange, P. J., Tawar, M. G., Bairagi, R. A., Malviya, V. R., Sahu, V. K., Shewatkar, S. N., Sawarkar, R. A., & Mamurkar, R. R. (2021). Synthesis of silver nanoparticles by using Aloe vera and *Thuja orientalis* leaves extract and their biological activity: a comprehensive review. *Bulletin of the National Research Centre*, *45*(1), 1-13.

Chinnasamy, G., Chandrasekharan, S., Koh, T. W., & Bhatnagar, S. (2021). Synthesis, Characterization, Antibacterial and Wound Healing Efficacy of Silver Nanoparticles from *Azadirachta indica. Frontiers in Microbiology*, *12*, 1-14.

Choudhari, A. S., Suryavanshi, S., Ingle, H., & Kaul-, R. (2011). Evaluating the antioxidant potential of aqueous and alcoholic extracts of *Ficus religiosa* using ORAC assay and assessing their cytotoxic activity in cervical cancer cell lines. *Biotechnology Bioinformatics Bioengineering*, *1*(4), 443-450.

Chowdappa, P., & Gowda, S. (2013). Nanotechnology in crop protection: Status and scope. *Pest Management in Horticultural Ecosystems*, *19*(2), 131-151.

Das, S. K., Khan, M. M. R., Guha, A. K., Das, A. R., & Mandal, A. B. (2012). Silver-nano biohybrid material: Synthesis, characterization, and application in water purification. *Bioresource Technology*, *124*, 495-499.

Dauthal, P., & Mukhopadhyay, M. (2016). Noble Metal Nanoparticles: Plant-Mediated Synthesis, Mechanistic Aspects of Synthesis, and Applications. *Industrial and Engineering Chemistry Research*, *55*(36), 9557-9577.

Della Sala, F., Longobardo, G., Fabozzi, A., Di Gennaro, M., & Borzacchiello, A. (2022). Hyaluronic Acid-Based Wound Dressing with Antimicrobial Properties for Wound Healing Application. *Applied Sciences, 12*(6), 3091.

Devra, V. (2022). Applications of Metal Nanoparticles in the Agri-Food sector. *Egyptian Journal of Agricultural Research, 100*(2), 163–183.

Dorau, B. F., Arango, R. A., & Green, F. (2004). An investigation into the potential of ionic silver as a wood preservative.(2), 133-145.

Durán, N., Durán, M., de Jesus, M. B., Seabra, A. B., Fávaro, W. J., & Nakazato, G. (2016). Silver nanoparticles: A new view on mechanistic aspects of antimicrobial activity. *Nanomedicine: Nanotechnology, Biology, and Medicine*, *12*(3), 789-799.

Durán, N., Marcato, P. D., Conti, R. De, Alves, O. L., Costa, F. T. M., & Brocchi, M. (2010). Potential use of silver nanoparticles on pathogenic bacteria, their toxicity, and possible mechanisms of action. *Journal of the Brazilian Chemical Society*, *21*(6), 949–959.

Ebong, P. E., Atangwho, I. J., Eyong, E. U., & Egbung, G. E. (2008). The antidiabetic efficacy of combined extracts from two continental plants: *Azadirachta indica* (A. Juss) (Neem) and *Vernonia amygdalina* (Del.) (African Bitter Leaf). *American Journal of Biochemistry and Biotechnology*, *4*(3), 239-244.

El Gamal, A. Y., Tohamy, M. R., Abou-Zaid, M. I., Atia, M. M., El Sayed, T., & Farroh, K. Y. (2022). Silver nanoparticles as a viricidal agent to inhibit plant-infecting viruses and disrupt their acquisition and transmission by their aphid vector. *Archives of Virology*, *167*(1), 85-97.

Elamawi, R. M., Al-Harbi, R. E., & Hendi, A. A. (2018). Biosynthesis and characterization of silver nanoparticles using *Trichoderma longibrachiatum* and their effect on phytopathogenic fungi. *Egyptian Journal of Biological Pest Control*, *28*, 28.

Elbeshehy, E. K. F., Elazzazy, A. M., & Aggelis, G. (2015). Silver nanoparticle synthesis mediated by new isolates of Bacillus spp., nanoparticle characterization and their activity against Bean Yellow Mosaic Virus and human pathogens. *Frontiers in Microbiology*, *6*, 453.

Noha, K., Bondok, A. M., & El-Dougdoug, K. A. (2018). Evaluation of silver nanoparticles as antiviral agent against ToMV and PVY in tomato plants. *Sciences*, *8*(1), 100-111.

Elwakil, B. H., Eldrieny, A. M., Almotairy, A. R. Z., & El-Khatib, M. (2024). Potent biological activity of newly fabricated silver nanoparticles coated by a carbon shell synthesized by an electrical arc. *Scientific Reports*, *14*(1), 5324.

Fernando, K. M., Gunathilake, C. A., Yalegama, C., Samarakoon, U. K., Fernando, C. A. N., Weerasinghe, G., Pamunuwa, G. K., Soliman, I., Ghulamullah, N., Rajapaksha, S. M., & Fatani, O. (2024). Synthesis of Silver Nanoparticles Using Green Reducing Agent: Ceylon Olive (Elaeocarpus serratus): Characterization and Investigating Their Antimicrobial Properties. *Journal of Composites Science, 8*(2), 43.

Gokarneshan N., and Velumani K. (2017). Application of Nano Silver Particles on Textile Materials for Improvement of Antibacterial Finishes. *Global Journal of Nanomedicine*, *2*(3), 42-45.

Golla, N. (2018). Phytosynthesis and antimicrobial studies of silver nanoparticles using *Ziziphus nummularia* leave extracts. *MOJ Drug Design Development and Therapy*, *2*(5), 261-264.

Goni, O., Khan, M. F., Rahman, M. M., Hasan, M. Z., Kader, F. Bin, Sazzad, N., Sakib, M. A., Romano, B., Haque, M. A., & Capasso, R. (2021). Pharmacological insights on the antidepressant, anxiolytic, and aphrodisiac potentials of *Aglaonema hookerianum* Schott. *Journal of Ethnopharmacology*, *268,* 113664

Govindachari, T. R., Suresh, G., Gopalakrishnan, G., Banumathy, B., & Masilamani, S. (1998). Identification of antifungal compounds from the seed oil of *Azadirachta indica*. *Phytoparasitica*, *26*(2), 109-116.

Gregory, M., Divya, B., Mary, R. A., Viji, M. M. H., Kalaichelvan, V. K., & Palanivel, V. (2013). Anti-ulcer activity of Ficus religiosa leaf ethanolic extract. *Asian Pacific Journal of Tropical Biomedicine*, *3*(7), 554-556.

Gulbagca, F., Ozdemir, S., Gulcan, M., & Sen, F. (2019). Synthesis and characterization of Rosa canina-mediated biogenic silver nanoparticles for anti-oxidant, antibacterial, antifungal, and DNA cleavage activities. *Heliyon*, *5*(12), e02980.

Gupta, S. (2014). Effect of *Andrographis serpyllifolia* Leaves Extract on Experimentally Induced Typhoid Using Salmonella Typhi. *British Journal of Pharmaceutical Research*, *4*(2), 230-239.

Hamouda, R. A., Hussein, M. H., Abo-elmagd, R. A., & Bawazir, S. S. (2019). Synthesis and biological characterization of silver nanoparticles derived from the cyanobacterium *Oscillatoria limnetica. Scientific Reports*, *9*(1), 13071.

Hasan, K. M. F., Xiaoyi, L., Shaoqin, Z., Horváth, P. G., Bak, M., Bejó, L., Sipos, G., & Alpár, T. (2022). Functional silver nanoparticles synthesis from a sustainable point of view: 2000 to 2023 – A review on game-changing materials. *Heliyon*, *8*(12), e12322.

Hossain, M. A., Shah, M. D., & Sakari, M. (2011). Gas chromatography-mass spectrometry analysis of various organic extracts of *Merremia borneensis* from Sabah. *Asian Pacific Journal of Tropical Medicine*, *4*(8), 637-641.

Jaidev, L. R., & Narasimha, G. (2010). Fungal mediated biosynthesis of silver nanoparticles, characterization, and antimicrobial activity. *Colloids and Surfaces B: Biointerfaces*, *81*(2), 430-433.

Kamble, P. N., Giri, S. P., Mane, R. S., & Tiwana, A. (2015). Estimation of Chlorophyll Content in Young and Adult Leaves of Some Selected Plants. *Universal Journal of Environmental Research and Technology*, *5*(6), 306-310.

Kerker, M. (1985). The optics of colloidal silver: something old and something new. *Journal of Colloid and Interface Science*, *105*(2), 297–314.

Keser, S., Celik, S., Turkoglu, S., Yilmaz, Ö., & Turkoglu, I. (2012). Hydrogen Peroxide Radical Scavenging and Total Antioxidant Activity of Hawthorn. *Chemistry Journal*, *2*(1), 9-12.

Khadri, H., Alzohairy, M., Janardhan, A., Kumar, A. P., & Narasimha, G. (2013). Green Synthesis of Silver Nanoparticles with High Fungicidal Activity from Olive Seed Extract. *Advances in Nanoparticles*, *2*(3), 241-246.

Khan, S., Zahoor, M., Sher Khan, R., Ikram, M., & Islam, N. U. (2023). The impact of silver nanoparticles on the growth of plants: The agriculture applications. *Heliyon*, *9*(6), e16928 .

Khan, Z. U. H., Sadiq, H. M., Shah, N. S., Khan, A. U., Muhammad, N., Hassan, S. U., Tahir, K., safi, S. Z., Khan, F. U., Imran, M., Ahmad, N., Ullah, F., Ahmad, A., Sayed, M., Khalid, M. S., Qaisrani, S. A., Ali, M., & Zakir, A. (2019). Greener synthesis of zinc oxide nanoparticles using *Trianthema portulacastrum* extract and evaluation of its photocatalytic and biological applications. *Journal of Photochemistry and Photobiology B: Biology*, *192*, 147-157.

Khandelwal, N., Kaur, G., Kumar, N., & Tiwari, A. (2014). Application of silver nanoparticles in viral inhibition: A new hope for antivirals. *Digest Journal of Nanomaterials and Biostructures*, *9*(1), 175-186.

Khorrami, S., Zarrabi, A., Khaleghi, M., Danaei, M., & Mozafari, M. R. (2018). Selective cytotoxicity of green synthesized silver nanoparticles against the MCF-7 tumor cell line and their enhanced antioxidant and antimicrobial properties. *International Journal of Nanomedicine*, *13*, 8013-8024.

Khuda, F., Jamil, M., Ali Khan Khalil, A., Ullah, R., Ullah, N., Naureen, F., Abbas, M., Shafiq Khan, M., Ali, S., Muhammad Umer Farooqi, H., & Ahn, M. J. (2022). Assessment of the antioxidant and cytotoxic potential of silver nanoparticles synthesized from the root extract of *Reynoutria japonica* Houtt. *Arabian Journal of Chemistry*, *15*(12), 104327.

Kokate C. K., Purohit A. P., & Gokhale S. B. (2007). Pharmacognosy (40th ed.). *Nirali Prakashan*, 637.

Kumar, V. G., Gokavarapu, S. D., Rajeswari, A., Dhas, T. S., Karthick, V., Kapadia, Z., Shrestha, T., Barathy, I. A., Roy, A., & Sinha, S. (2011). Facile green synthesis of gold nanoparticles using leaf extract of antidiabetic potent Cassia auriculata. *Colloids and Surfaces. B, Biointerfaces*, *87*(1), 159-163.

Küp, F. Ö., Çoşkunçay, S., & Duman, F. (2020). Biosynthesis of silver nanoparticles using leaf extract of *Aesculus hippocastanum* (horse chestnut): Evaluation of their antibacterial, antioxidant and drug release system activities. *Materials Science and Engineering. C, Materials for biological applications*, *107*, 110207.

Kvítek, L., Panáček, A., Soukupová, J., Kolář, M., Večeřová, R., Prucek, R., Holecová, M., & Zbořil, R. (2008). Effect of surfactants and polymers on stability and antibacterial activity of silver nanoparticles (NPs). *Journal of Physical Chemistry C*, *112*(15), 5825-5834.

Labrenz, M., Druschel, G. K., Thomsen-Ebert, T., Gilbert, B., Welch, S. A., Kemner, K. M., Logan, G. A., Summons, R. E., De Stasio, G., Bond, P. L., Lai, B., Kelly, S. D., & Banfield, J. F. (2000). Formation of sphalerite (ZnS) deposits in natural biofilms of sulfate-reducing bacteria. *Science (New York, N.Y), 290*(5497), 1744-1747.

Li, W., Qu, F., Chen, Y., Sun, Y., Zhang, J., Xie, G., You, Q., & Xu, H. (2021). Antimicrobial activity of silver nanoparticles synthesized by the leaf extract of *Cinnamomum camphora*. *Biochemical Engineering Journal*, *172*, 108050.

Lin, S., Huang, R., Cheng, Y., Liu, J., Lau, B. L., & Wiesner, M. R. (2013). Silver nanoparticle-alginate composite beads for point-of-use drinking water disinfection. *Water Research*, *47*(12), 3959-3965.

Lok, C.N., Ho, C.M., Chen, R., He, Q. Y., Yu, W. Y., Sun, H., Tam, P. K. H., Chiu, J. F., & Che, C. M. (2006). Proteomic analysis of the mode of antibacterial action of silver nanoparticles. *Journal of Proteome Research*, *5*(4), 916-924.

Mallikarjuna, K., Balasubramanyam, K., Narasimha, G., & Kim, H. (2018). Phyto-synthesis and antibacterial studies of bio-based silver nanoparticles using *Sesbania grandiflora* (Avisa) leaf tea extract. *Materials Research Express*, *5*(1), 015054.

Mallikarjuna, K., John Sushma, N., Subba Reddy, B. V., Narasimha, G., & Deva Prasad Raju, B. (2013). Palladium nanoparticles: Single-step plant-mediated green chemical procedure using Piper beetle leaves broth and their anti-fungal studies. *International Journal of Chemical and Analytical Science*, *4*(1), 14-18.

Mallikarjuna, K., Narasimha, G., Dilip, G. R., Praveen, B., Shreedhar, B., Sree Lakshmi, C., Reddy, B. V. S., & Deva Prasad Raju, B. (2011). Green synthesis of silver nanoparticles using *Ocimum* leaf extract and their characterization. *Digest Journal of Nanomaterials and Biostructures*, *6*(1), 181-186.

Mohanta, Y. K., Panda, S. K., Bastia, A. K., & Mohanta, T. K. (2017). Biosynthesis of silver nanoparticles from *Protium serratum* and investigation of their potential impacts on food safety and control. *Frontiers in Microbiology*, *8*, 626.

Mukherjee, S., Chowdhury, D., Kotcherlakota, R., Patra, S., Vinothkumar, B., Bhadra, M. P., Sreedhar, B., & Patra, C. R. (2014). Potential theragnostic application of bio-synthesized silver nanoparticles (4-in-1 system). *Theranostics*, *4*(3), 316-335.

Murugesu, S., Selamat, J., & Perumal, V. (2021). Phytochemistry, pharmacological properties, and recent applications of *Ficus benghalensis* and *Ficus religiosa*. *Plants*, 10(12), 2749.

Naidu, K. S. B., Govender, P., & Adam, J. K. (2015). Nanosilver particles in biomedical and clinical applications: Review. *Journal of Pure and Applied Microbiology*, *9*(Issue Special Edition 2), 103-112.

Narasimha, G, Janardhan, M Alzohairy, H Khadri, & K Mallikarjuna (2013). Extracellular synthesis, characterization, and antibacterial activity of silver nanoparticles by Actinomycetes isolative. *International Journal of Nano Dimension*, *4*(1), 77-83.

Narasimha G., Mallikarjuna K & Deva Prasad Raju B. (2011). Mushrooms (*Agaricus bisporus)* mediated biosynthesis of silver nanoparticles, characterization, and their antimicrobial activity. *International Journal of Nano Dimension*, *2*(1), 29-36.

Narasimha, G., & Papaiah, S. (2014). Peroxidase and polyphenol oxidase activities in healthy and viral infected sunflower (*Helianthus annuus* L.) leaves. *Biotechnology: An Indian Journal*, *9*(1), 1-5.

Palithya, S., Gaddam, S. A., Kotakadi, V. S., Penchalaneni, J., Golla, N., Krishna, S. B. N., & Naidu, C. V. (2022). Green synthesis of silver nanoparticles using flower extracts of *Aerva lanata* and their biomedical applications. *Particulate Science and Technology*, *40*(1), 84-96.

Patel, S., & Patel, N. K. (2022). Biogenic Synthesis of Silver Nanoparticles and Investigation of Antibacterial Activity from Leaves of *Duranta erecta. Egyptian Journal of Agricultural Research*, 1*0*(1), 4–12.

Paul, R., Prasad, M., & Sah, N. K. (2011). Anticancer biology of *Azadirachta indica* L (neem): A mini-review. *Cancer Biology and Therapy*, *12*(6), 467-476.

Pawar, A. A., Sahoo, J., Verma, A., Alswieleh, A. M., Lodh, A., Raut, R., Lakkakula, J., Jeon, B.-H., & Islam, Md. R. (2022). *Azadirachta indica*-Derived Silver Nanoparticle Synthesis and Its Antimicrobial Applications. *Journal of Nanomaterials*, *2022*(6), 1–15.

Ping, H., Zhang, M., Li, H., Li, S., Chen, Q., Sun, C., & Zhang, T. (2012). Visual detection of melamine in raw milk by label-free silver nanoparticles. *Food Control*, *23*(1), 191-197.

Prasad, R., & Swamy, V. S. (2013). Antibacterial Activity of Silver Nanoparticles Synthesized by Bark Extract of *Syzygium cumini*. *Journal of Nanoparticles*, *2013*, 6.

Pulit-Prociak, J., & Banach, M. (2016). Silver nanoparticles - A material of the future...? *Open Chemistry*, *14*(1), 76-91.

Ramesh, P. S., Kokila, T., & Geetha, D. (2015). Plant-mediated green synthesis and antibacterial activity of silver nanoparticles using *Emblica officinalis* fruit extract. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *142*, 339-343.

Reddy, B. P., Mallikarjuna, K., Narasimha, G., & Park, S. H. (2017). *Plectranthus amboinicus*-mediated silver, gold, and silver-gold nanoparticles: phyto-synthetic, catalytic, and antibacterial studies. *Materials Research Express*, *4*(8), .

Reshma Anjum, M., Maheshwari, J., Anusha, K., Sravya, B., Narasimha, G., & Abd-Elsalam, K. A. (2024). Maximizing Crop Yield with Macro and Micro Nano Enhanced Fertilizers. In: Abd-Elsalam, K.A., Algathuthaymi, M.A. (eds) Nanofertilizers for Sustainable Agroecosystems.Nanotechnology in the Life Sciences. *Springer*, Cham.1–33.

Riyas, Z. M., Gayathri, R., Prabhu, M. R., Velsankar, K., & Sudhahar, S. (2022). Green synthesis and biomedical behavior of Mg-doped ZnO nanoparticle using leaf extract of *Ficus religiosa*. *Ceramics International*, *48*(17), 24619-24628.

Roh, Y., Lauf, R. J., McMillan, A. D., Zhang, C., Rawn, C. J., Bai, J., & Phelps, T. J. (2001). Microbial synthesis and the characterization of metal-substituted magnetites. In *Solid State Communications*,*118*(10), 529-534.

Ruch, R. J., Cheng, S. jun, & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, *10*(6), 1003-1008.

Sarath Kumar S.V, Latheef P., Rani E.S., & Narasimha G. (2024). Phytochemical analysis, antibacterial and antifungal activity of leaf extract of *Senna occidentalis*. *International Journal of Scientific Research*, *13*(1), 1–3.

Seku, K., Gangapuram, B. R., Pejjai, B., Kadimpati, K. K., & Golla, N. (2018). Microwave-assisted synthesis of silver nanoparticles and their application in catalytic, antibacterial, and antioxidant activities. *Journal of Nanostructure in Chemistry*, *8*(2), 179-188.

Selvakumar, P., Sithara, R., Viveka, K., & Sivashanmugam, P. (2018). Green synthesis of silver nanoparticles using leaf extract of *Acalypha hispida* and its application in blood compatibility. *Journal of Photochemistry and Photobiology B: Biology*, *182*, 52-61.

Shaikh, S., Nazam, N., Rizvi, S. M. D., Ahmad, K., Baig, M. H., Lee, E. J., & Choi, I. (2019). Mechanistic insights into the antimicrobial actions of metallic nanoparticles and their implications for multidrug resistance. In *International Journal of Molecular Sciences*, *20*(10), 2468.

Shanmugapriya, A., Kalaiarasi, G., Kalaivani, P., Dallemer, F., & Prabhakaran, R. (2016). CT-DNA/BSA protein binding and antioxidant studies of new binuclear Pd (II) complexes and their structural characterization. *Inorganica Chimica Acta*, *449*, 107-118.

Sharmila, G., Muthukumaran, C., Saraswathi, H., Sangeetha, E., Soundarya, S., & Kumar, N. M. (2019). Green synthesis, characterization, and biological activities of nanoceria. *Ceramics International*, *45*(9), 12382-12386.

Sheffield, F.M.I. (1933). The development of assimilatory tissue in solanaceous hosts infected with Aucuba mosaic of tomato. *Annals of Applied Biology-AAB*, *20*(1), 57-69.

Simangunsong, T. L., Putra, A., Ginting, S. F., Wardhani, F. M., Abdullah, H., & Ikhtiari, R. (2022). Antifungal Analysis of Papaya Seed Extracts and Biosynthesized Silver Nanoparticles. *Science and Technology Indonesia*, *7*(2), 186-194.

Singh, A., Jain, D., Upadhyay, M. K., Khandelwal, N., & Verma, H. N. (2010). Green synthesis of silver nanoparticles using Argemone Mexicana leaf extract and evaluation of their antimicrobial activities. *Digest Journal of Nanomaterials and Biostructures*, *5*(2), 483-489.

Singh, D., Singh, B., & Goel, R. K. (2011). Traditional uses, phytochemistry and pharmacology of *Ficus religiosa*: A review. In *Journal of Ethnopharmacology*, *134*(3), 565-583.

Singh, K., Panghal, M., Kadyan, S., Chaudhary, U., & Yadav, J. P. (2014). Antibacterial activity of synthesized silver nanoparticles from *Tinospora cordifolia* against multi-drug resistant strains of *Pseudomonas aeruginosa* isolated from burn patients. *Journal of Nanomedicine and Nanotechnology*, *5*(2), 1-6.

Sinha, A., & Srivastava, M. (2010). Biochemical Changes in Mungbean plants infected by Mungbean yellow mosaic virus. *International Journal of Virology*, *6*(3), 150-157.

Sosa, I. O., Noguez, C., & Barrera, R. G. (2003). Optical properties of metal nanoparticles with arbitrary shapes. *Journal of Physical Chemistry B*, *107*(26), 6269-6275.

Sultana, B., Anwar, F., & Przybylski, R. (2007). Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, *Terminalia arjuna, Acacia nilotica,* and *Eugenia jambolana* Lam. trees. *Food Chemistry*, *104*(3), 1106-1114.

Swilam, N., & Nematallah, K. A. (2020). Polyphenols profile of pomegranate leaves and their role in green synthesis of silver nanoparticles. *Scientific Reports*, *10*(1), 14851.

Tarannum, N., Divya, & Gautam, Y. K. (2019). Facile green synthesis and applications of silver nanoparticles: A state-of-the-art review. *RSC Advances*, *9*, 34926-34948.

Vaishnavi, V., Sadasivuni, K. K., Ponnamma, D., & Golla, N. (2020). Green Synthesis of Silver Nanoparticles from *Pterocarpus Santalinus* Leaf Broth and Their Antibacterial and Antioxidant Activities. *Macromolecular Symposia*, *392*(1), 2000079(1-4).