Effect of Phyto-mediated Silver Nanoparticles of *Walsura trifoliata* (A. Juss) Harms on Seed germination and Seedling growth

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Abstract

The present study highlights the eco-friendly production of silver nanoparticles (AgNPs) using leaf extract as a promising alternative to conventional chemical and physical synthesis methods. Specifically, we synthesized AgNPs using an aqueous extract from the medicinal tree *Walsura trifoliata* and evaluated their effect and seedling growth. The green-synthesized AgNPs were characterized by UV–Vis spectroscopy and transmission electron microscopy (TEM). A characteristic absorption band confirmed the formation of AgNPs at 434 nm in the UV–Vis spectra. TEM analysis revealed that the nanoparticles were predominantly spherical, with sizes ranging from 25.81 to 29.43 nm and an average diameter of 27.65 nm. Application of these AgNPs resulted in a positive impact, notably enhancing all experimental growth parameters on seeds of Ground nut. The results indicate that the green synthesized AgNPs may be used to remove the seed dormancy and enhance the vigour of agricultural crops.

Key words: AgNPs, UV–vis spectroscopy, TEM, *Walsura trifoliate*, ground nut.

Introduction

Nanoparticles (NPs) have been integrated into nanotechnology and used as versatile tools in a large diversity of scientific and technic practices due to their remarkably advanced physicochemical properties (Ravindran A *et al.,* 2013). Silver nanoparticles (AgNPs) have prominent roles in areas of medicine, Nano sensors, food chemistry, agriculture, food packing, cosmetics and textiles (Abou El-Nour KMM *et al.,* 2010; Paret ML *et al.,* 2016). Every year it is estimated that nearly 320 tons of silver nanoparticle are used in different applications (Ahamed M *et al.,* 2010). To date, several methods have been used to synthesize NPs. Although, chemical, photochemical and physical methods have been commonly used in NPs production, they are time consuming, labour intensive and environmentally harmful procedures. An eco-friendly and cost-effective nanoparticle synthesis method with no toxic chemicals is required. Green synthesis methods using plants have attracted great attention because of their biocompatible procedures (Le Ouay B and Stellacci F, 2015). Plants are regarded as a highly desirable system for nanoparticle synthesis due to their great ability to produce a wide range of bioactive secondary metabolites with high reducing potential. As matched to bacteria and algae and, plants are lesser vulnerable to metal toxicity, for this reason offering a green substitute for the biosynthesis of silver nanoparticles (Philip D *et al.,* Plants has become the ideal and best alternative as a safe, non-toxic technique of NP production since natural capping agents are more in such samples that can also be easily extracted from plants (Ingale AG *et al.,* 2013). The phytochemicals present, including flavonoids, proteins, and polyphenols, are instrumental in reducing silver ions. Consequently, the plant-mediated synthesis of silver nanoparticles (AgNPs) not only facilitates the creation of these nanomaterials but also ensures enhanced biocompatibility, making them highly suitable for various biomedical applications (Mishra VK *et al.,* 2019).

Walsura trifoliata (A. Juss.) Harms, a member of the Meliaceae family, is an evergreen tree that is widely distributed across the tropical regions of Asia, including Southern China, India, Malaysia, and Indonesia (Chetty *et al.,* 2008). This species typically flourishes in dry deciduous forests at elevations ranging from 200 to 300 meters. This tree holds significant importance in traditional medicine, particularly among tribal communities, where it is employed to address various health concerns, such as skin allergies, acting as an astringent, and treating diarrhoea (Pullaiah and Rani, 1999). The bark of *Walsura trifoliata* is recognized for its stimulant, expectorant, emmenagogue, and emetic properties, while the fruit pulp has historically been used as a fish poison (Anonymous, 1976). Furthermore, research has indicated that the bark extract exhibits antimicrobial activity against pathogenic microorganisms (Sri Rama Murthy and Nagamani, 2008). Silver nanoparticle formation has already been reported from various plant extracts such as *Azadirachta indica* (Neem), *Emblica Officinalis* (Amla), *Cinnamomum camphora* (Canizal *et al.,* 2001; Chandran *et al.,* 2006; Ankamwar *et al.,* 2005; Krishnaraj *et al.,* 2010), *Syzyzium alternifolium* (Yugandhar P and Savithramma N, 2015), *Boswellia ovalifoliata* (Ankanna S *et al.,* 2010), and *Adansonia digitata* (Chennareddy Maruthi Kesava Kumar *et al.,* 2016).

MATERIAL AND METHODS

COLLECTION OF THE PLANT MATERIAL

The medicinal plant *Walsura trifoliata* plant leaf material was collected from the medicinal plant garden-1, department of Botany, Sri Venkateswara University, Tirupati.

Preparation of Plant Extract

The fresh collected leaf material of *Walsura trifoliata* was washed with running tap water thrice for the removing of admixture on the material, then washed by double distilled water twice and moisture was removed with the help of tissue paper then the leaf was kept in shade dried 14 days. Then the material made ground in to fine powder with the help of electric mixture. 10 mg of the leaf powder was taken in to 250 ml conical flask, 100 ml of double distilled water added to this and rinse well for 5 min, it was kept on water bath for 60 min. After cooled it was filtered through the Whatman no.1 filter paper and it was kept in to 4 ^{0} C until the synthesis.

Preparation of the 1 mM Ag $(NO_3)_2$ solution

10 gr of Ag $(NO_3)_2$ was purchased by Sigma Aldrich, 1 mM Ag $(NO_3)_2$ solution was prepared by the adding of 100 ml of Milli-Q water.

Synthesis of Silver nanoparticles

10 ml of aqueous fruit extract was taken into 250 ml conical flask and heated on water bath with 100 ml of silver nitrate with heating between 60-80^{o}C for 60 min. Colour change from light brown to deep brown indicated formations of silver nanoparticles. Then the content was centrifuged at 15,000 rpm for 20 min to remove the presence biological admixture, and it was used for characterization and the effect of AgNPs on growth para meters of ground nut.

Characterization of AgNPs

Number of tree taxa were exploited for green synthesis of nanoparticles, limited work was recorded on *Walsura trifoliata* leaf. Hence the present work study was undertaken to test the efficiency of *Walsura trifoliata* leaf for the synthesis of AgNPs and validated the efficacy on agricultural aspects. UV-Vis absorption spectrum of AgNPs was using with Nano drop 800 nm spectrophotometer. Transmission electron microscopy (TEM) analysis was performed with the using HF-3300 advanced 300 kV TEM from Hitachi.

Growth parameters of seed germination

Dharani variety Ground nut seeds were obtained from Regional Agriculture Research station, S.V. Agricultural College, Tirupati, Andhra Pradesh, India. The seeds were surface sterilized through 0.2% $HgCL_2$ solution for 5 minutes with shaking and washed thoroughly with distilled water. The seeds were pre-soaked in 50 ml of respected treatments up to 12 hours for monitoring the percentage of germination and seeds were shifted and germinated on fluted filter paper towels in bread boxes. The seedlings were exposed to diverse treatments to observe the seedlings growth.

Percentage of seed germination: Seed germination (%) = (Number of germinated seeds/ Number of total seeds) X100.

Root and Shoot length: Root length was taken from the point below the hypocotyls to the end of the tip of the root. Shoot length was measured from the base of the root- hypocotyl transmission zone up to the base of the cotyledons. The root and shoot length was measured with the help of a thread and scale.

Seedling vigour index: The seedling vigour index was determined by using the formula given by Abdul baki and Anderson (1973).

Seedling vigour index = Average root length in cms + Average shoot length X Germination percentage.

Fresh and Dry weight: The fresh weight of root and shoot of seedlings was determined by weigh the root and shoot separately on electric balance. After the fresh weight taken then the seedlings were placed in a hot air oven at 80^0 C for 48 hr then the weight of dry matter was note downed.

Results and Discussion

UV- visible spectroscopy

The UV-visible absorption spectrum of Silver nanoparticles (AgNPs) was analyzed using a Nano Drop 800 nm Spectrophotometer. Notably, the solution exhibited a colour transition from light brown to deep brown, which serves as a definitive indicator of successful AgNPs synthesis. This alteration in colour is a primary confirmation of the Surface Plasmon Resonance (SPR) characteristic of these nanoparticles. The absorbance peak was distinctly observed at 434 nm (**Fig. 1)**, specifically in the context of *Walsura trifoliata*, thereby providing further validation of the formation of Silver nanoparticles. Similar type of results observed in Plant-mediated synthesis of silver nanoparticles using *Alcea rosea* leaf aqueous extract (Indra Ojha *et al.,* 2026).

Transmission Electron Microscopy (TEM)

The morphological structure and distribution of the synthesized silver nanoparticles (AgNPs) were assessed using high magnification transmission electron microscopy (TEM) at 50 nm. The TEM micrographs indicate that the synthesized nanoparticles are polydispersity and predominantly spherical in shape, with sizes ranging from 25.81 nm to 29.43 nm (**Fig 2A,B)**. Notably, there was no observable agglomeration among the nanoparticles, suggesting that they remain well-dispersed. For the TEM analysis, the silver nanoparticles were coated onto copper grids and examined using a Hitachi HF3300 advanced microscope operating at 300 kV. These results are accordance with earlier study of Synthesis of Silver nanoparticles Using extracts from different Parts of the *Paullinia cupana Kunth* Plant (Lima AKO *et al.,* 2024).

Application of the Silver Nanoparticles (AgNPs) on Ground nut

Seed germination and Seedling growth

Groundnut seeds of the Dharani variety, treated with 1 mM $AgNO_3$, plant extract, and various concentrations (1, 10, 20, 40, 50, and 100 ppm) of biosynthesized silver nanoparticles (AgNPs) using leaf of *W. trifoliata*, showed remarkable improvements in seedling growth compared to the control (water). Growth parameters such as root length shoot length, wet weight, dry weight, and vigour index consistently increased with higher concentrations of AgNPs treatment.

The highest root length was observed at 50 ppm AgNPs (16.6 ± 0.12cm), while the lowest was at 1 ppm (11.5 ± 0.03cm). The control group had a root length of 8.7 ± 0.14 cm, while the silver nitrate and plant extract treatments yielded 12.5 ± 0.06 cm and 9.7 ± 0.05 cm, respectively. The highest shoot length was recorded at 40 ppm AgNPs (9.8±0.03 cm), whereas the control, plant extract, and AgNO3 treatments showed lower values (6.1±0.08 cm, 5.2±0.15 cm, and 5.3 ± 0.03 cm, respectively). Leaf length in the control was 0.8 ± 0.06 cm, while AgNPs at 40 ppm resulted in 1.7 ± 0.05 cm, significantly less than the plant extract (1.2 ± 0.03 cm) and marginally higher than the 1 mM silver nitrate treatment (**Fig.4**). Leaf width was 0.9 ± 0.05 cm in the control, 0.8 ± 0.06 cm with plant extract, 0.4 ± 0.03 cm with silver nitrate, and 1.0 ± 0. 12cm, at 40 ppm AgNPs. Seedling wet weight was highest at 40 ppm AgNPs (1.92 ± 0.01 g), compared to water (1.11 ± 0.01 g), plant extract (1.06 ± 0.01 g), and silver nitrate (1.01 ± 0.02 g). The highest seedling dry weight was also observed at 40 ppm AgNPs (0.54 ± 0.025 g), exceeding the control (0.32 ± 0.014 g), plant extract (0.37 ± 0.012 g), and 1 mM silver nitrate (0.46 ± 0.006 g) Details depicted in Table 1; Fig.3&4. These kinds of results were observed in effects of silver nanoparticles on seed germination and growth performance of pea (Saidur Rahman .Md *et al.,* 2023).

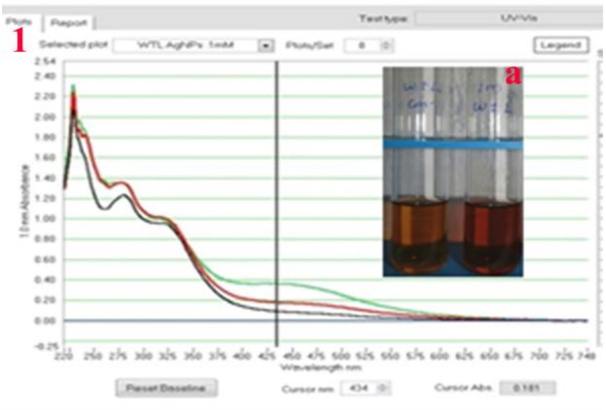

Figure 1. UV-Vis Spectrosocpy analysis of synthesized AgNPs absorption peak obtained at 434nm, a). Vissible confirmation between water extract and Synthesized AgNPs.

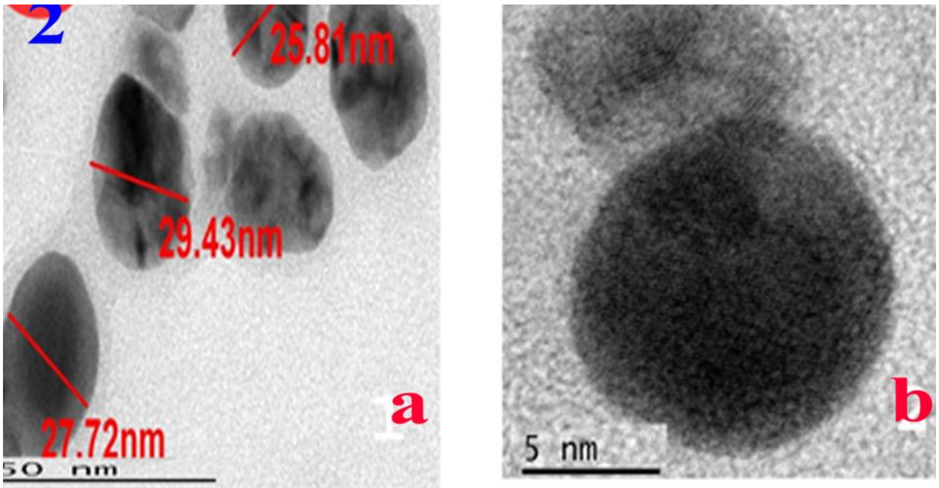

Figure 2. TEM Images: **a) 1.** SNPs Average size of AgNPs at 50 nm 27.65nm and **b).** Sphericle shape of AgNPs without agglomeration at 5 nm.

Figure 3. Seedling growth of ground nut using with AgNPs synthesized from leaf of *W. trifoliata.*

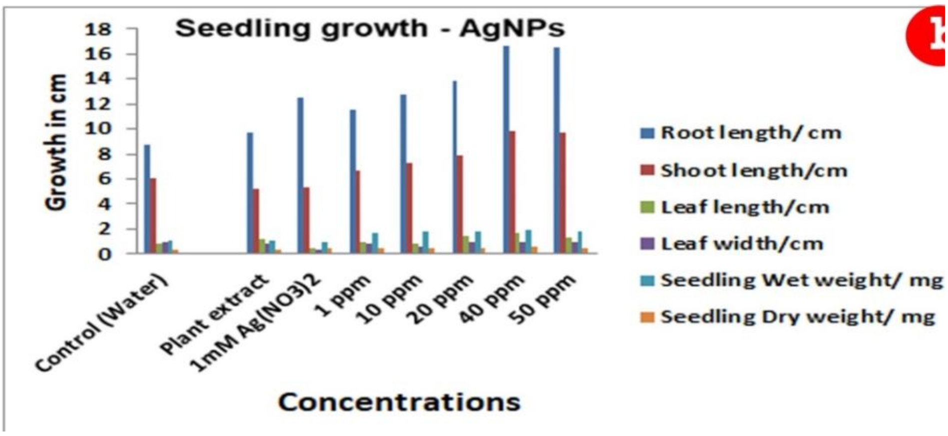

Figure 4. Graphical representation of In-vitro study on Dharani ground nut seedlings by using AgNPs synthesized from leaf of *W. trifoliata.*

Conclusion:

Silver nanoparticles (AgNPs) were successfully synthesized using an aqueous leaf extract of *Walsura trifoliata* A. Juss (Harms) through a green synthesis approach. The selected medicinal plant leaf extract acted as both a reducing and capping agent in the formation of AgNPs, owing to its rich phytochemical content. The formation of AgNPs was evidenced by a noticeable colour change and confirmed by surface Plasmon resonance (SPR) observed in the UV-Vis spectrum at 434 nm. Transmission electron microscopy (TEM) analysis revealed that the nanoparticles were predominantly spherical in shape, with sizes ranging from 25 to 29.43 nm. No agglomeration was observed, which can be attributed to the high resolution and magnification capabilities of TEM, as well as the effective capping by phytochemicals. The silver nanoparticles (AgNPs) synthesized from *Walsura trifoliata* demonstrated a significant positive effect on *in-vitro* seed germination and seedling growth in the selected Dharani ground nut variety. This suggests that the biosynthesized AgNPs may enhance early plant development and could be beneficial for agricultural applications.

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AUTHOR CONTRIBUTION

Practical design and manuscript preparation were supervised by my research supervisor Prof. N. Savithramma, Department of Botany, Sri Venkateswara University, Tirupati. The first author, K. Venkata Subbaiah, conducted field and laboratory work assisted by Prof. N. Savithramma throughout the research.

CONFLICT OF INTEREST STATEMENT

The authors declared that they have no interest of conflicts.

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