

ECO – INSPIRED SYNTHESIS OF SILVER NANOPARTICLES UTILIZING *Acacia nilotica* EXTRACT: A FOCUS ON ANTIOXIDANT PROPERTIES

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ABSTRACT:

The present work on green synthesis of silver nanoparticles (AgNPs) are notable for their simplicity and cost-effectiveness, which yield stable nanomaterials and serve as a viable alternative for the large-scale production of AgNPs. The *Acacia nilotica* extracts helped to reduce and stabilize AgNPs during their formation process. The synthesized AgNPs utilizing leaf extracts for reduction and stabilization were thoroughly reported and evaluated. The findings indicated a significant increase in the rate of AgNP synthesis when detergents and elevated temperatures were used. Following the synthetic procedure, the properties of the synthesized AgNPs were thoroughly analyzed using UV-visible, FTIR, and SEM. Furthermore, an in vitro DPPH assay was conducted to evaluate the antioxidant properties of synthesized AgNP.

Keywords: *A. nilotica* leaf Extract, silver nanoparticles, UV-vis, FTIR, Antioxidant DPPH invitro, SEM.

INTRODUCTION:

Acacia nilotica (L.) is classified under the Fabaceae family, Mimosoideae subfamily. The genus *Acacia* is the second largest (Fabaceae) family, with a vast diversity of species that contribute significantly to ecosystems worldwide. *Acacia* comprises more than 1,350 species (Seigler, 2003). *A. nilotica* is a remarkable species within the pantropical and subtropical genus *Acacia*, flourishing abundantly across Asia, Australia, Africa, and the Americas. In India, nine species, 6 - African tropics, and three others, are native to the Indian subcontinent (Shittu & Solomon, 2010). In its natural habitat, *A. nilotica* is the key to traditional rural and agro-pastoral practices. This species is crucial for treating various illnesses and offers multipurpose applications. In Ayurvedic practice, natural medicinal plants are vital for self-healing, well-being, and building resilience (Rather & Mohammad, 2015). It has been recognized that *A. nilotica* offers nutritional and therapeutic properties that can prevent, alleviate, or treat numerous diseases or conditions (Singh et al., 2009). The historical use of herbs for the treatment of animal and human ailments is well established. It has been reported to be highly effective in treating conditions such as diarrhoea and cough in humans. *A. nilotica* grows quickly and functions as a nitrogen-fixing species, which contributes positively to soil fertility. The leaves of this plant are frequently used as fodder, providing approximately 7-15 % crude protein, 20.1 –

33.3 % crude fiber, 1.2-2.6 % calcium, and 0.1 – 0.2 % phosphorus. The leaves are consisting of 3-6 pairs of pinna measuring 4 cm in length, with each pinna containing 10-30 leaflets (Ali et al., 2012).

Nanoparticles are integral components in a wide variety of applications, including medicine, semiconductors, catalysis, and energy. The preparation of these materials is accomplished through chemical, photoinduced, and microwave-assisted reduction techniques, with chemical reduction methods being the most prevalent. The reduction in nanoparticle procedures depends on the toxicity of the chemicals. To address this issue, numerous researchers have proposed the implementation of environment-friendly processes. In the green process, plant leaf extracts are used as a reducing agent for metal nanoparticle preparation, and the green method is cost-effective and utilizes ambient conditions for the reduction reaction. The creation of metal nanoparticles utilizing natural extracts is regarded as the most suitable approach because of clear environmental considerations (Adeyemi et al., 2022).

Recent researches indicate that AgNPs exhibit superior wound healing properties, enhanced aesthetic qualities, and the ability to promote scarless healing in animal models. The creation of metal nanoparticles utilizing natural extracts is regarded as the most suitable approach, owing to clear environmental considerations. It is straightforward, cost-effective, and yields stable products (Adeyemi et al., 2022). Plant extracts and microorganisms can serve as alternatives to traditional methods of converting metal ions into metal nanoparticles. The present study indicates that the mechanism by which silver nanoparticles (AgNPs) induce bacterial cell death involves their effect on membrane morphology, resulting in altered permeability and disrupted transport of materials. The current study aimed to explore the significant role of plant extracts in the biosynthesis of AgNPs, along with their antibacterial and cytotoxic effects, because plants are rich in phytochemicals (Mustapha et al., 2022).

Recent global challenges related to environmental issues have prompted an emphasis on green chemistry as a sustainable approach within the field of chemistry to address various health concerns. The rise in antibiotic-resistant bacteria is closely linked to the increased use of antibiotics and represents a significant contemporary health crisis. The synthesis of nanoparticles utilizing biogenic materials, including plants, microorganisms, and natural biomolecules, exemplifies green chemistry and may serve as a viable alternative to conventional methods because of their eco-friendly attributes (Sastry et al., 2004; Mohanpuriya et al., 2008; Gurunathan and Raman, 2013).

MATERIALS AND METHODS:

Acacia nilotica

(TAMIL: Karuvelam & HINDI: Babul, Kikar).

Taxonomical classification :

Kingdom	Plantae
Sub kingdom	Tracheobionta

Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Sub class	Rosidae
Order	Fabaces
Family	Fabaceae
Genus	<i>Acacia</i>
Species	<i>nilotica</i>

Collection of plant materials:

The *A. nilotica* leaves were attained from the local environment in the Karmankudi forest, (Latitude: 11.46426° or 11° 27' 51" N and Longitude: 79.34898° or 79° 20' 56" E) located in Virudhachalam, Cuddalore district, Tamil Nadu, India. The freshly collected leaves were thoroughly washed with tap water to eliminate any adhering dust, after which they were shade-dried for a week until adequately dried for grinding. Following the drying process, the plant materials were finely ground using a mechanical blender and labeled for future use.

Preparation of Silver Nanoparticles using plant extract:

Silver nanoparticles were synthesized using silver nitrate in conjunction with plant powder (Fig. 1-3). Briefly, the leaves were finely crushed into powder. 10-gram of this powder was then dissolved in 100 milliliters of double-distilled water. The resulting mixture was then heated to 60°C for a period of 20 minutes. Furthermore, a 0.01 M solution of silver nitrate was created by dissolving it in dd H₂O. Different ratios of Silver nitrate and the powdered sample were prepared, including 5:5, 6:4, 7:3, 8:2 and 9:1. From these different ratios, a concentration of 8:2 ratio was selected for the bulk preparation, because it shows the higher production than other ratios.

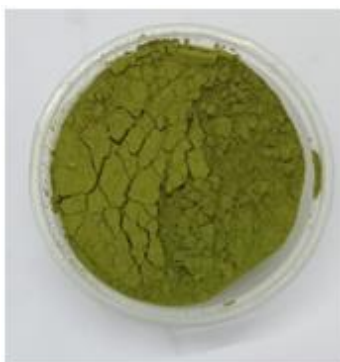


Fig 1: Sample (PB)

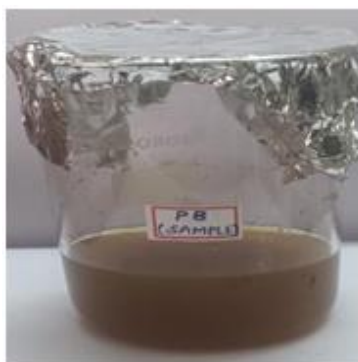


Fig 2: Sample solution



Fig 3: Precursor solution

The following approaches were adopted for the assessment of AgNPs:

I. Uv-visible:

The confirmation of AgNPs synthesis was first achieved through UV-Visible spectrophotometry (Perkin -Elmer, lambda 35, Germany). To monitor the reduction of silver ions (Ag⁺) to AgNPs was tracked over time by collecting sample at regular intervals. These samples were analyzed by their absorbance spectra across the wavelength range of 200-800 nm, allowing for the observation of characteristics surface plasmon resonance peaks indicative of AgNP formation.

II. FTIR Analysis:

Fourier Transform Infrared Spectroscopy (FTIR, perkin- Elmer) was employed to investigate the roles of various functional groups in the green synthesis and stabilization of silver nanoparticles (AgNPs). The FTIR Spectra were recorded over a range of 400-4000 cm⁻¹, facilitating the identification and assignment of specific vibrational modes to corresponding functional groups present in both the plant extract and the synthesized AgNPs. A comparative analysis of the spectra was performed to elucidate changes and confirm the involvement of functional group in the synthesis and stabilization process (Ravikumar & Angelo, 2015).

III. Evaluation of Antioxidant Activity:

The DPPH assay is widely utilized to study the antioxidant properties of natural products (MacDonald-Wicks et al., 2006). The underlying principle of this assay is that antioxidants function as hydrogen donors (Moon & Shibamoto, 2009; Kumar & Nair, 2023). It quantifies the presence of radical scavenging compounds. The mechanism by which DPPH accepts hydrogen from an antioxidant is illustrated in the fig 4 given below (Lewis et al., 2012). DPPH, a stable and commercially accessible organic nitrogen radical, serves as a stranded tool for assessing antioxidant activity. The reduction of DPPH, directly correlated to the antioxidant capacity of a sample, is effectively measured using a UV spectrometer due to its accuracy and simplicity. With a prominent absorption pieck at 517 nm (appearing purple), DPPH transitions to yellow as it reacts with hydrogen atoms from an antioxidant. This reaction occurs stoichiometrically, ensuring precise determination of antioxidant activity (Sadiq et al., 2017).

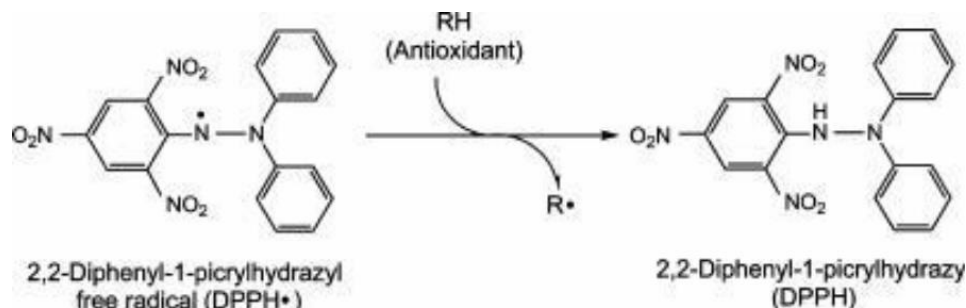


Fig. 4

IV. SEM:

Scanning electron microscope (SEM) was employed to analyse the morphology and structure of AgNP. To assess the shape and bonding configuration of the biosynthesized AgNP, dried plant powder sample was placed on a carbon grid coated with copper and examined using a scanning electron microscope (Hitachi – s – 4500).

RESULTS:

II. Characterization of AgNPs:

Physical characterization

In the present study, the color of the AgNP solution is shifted from light green to yellowish brown fig 5. This change in color was noticed within 30 minutes to 1 hour. The reaction mixture was heated to a temperature that did not exceed its boiling point and was continuously stirred at a speed of 800 rpm with a magnetic stirrer. After 1 hour, the mixture was transformed into a brown color. The entire reaction was taken place in a dark environment. The result in suspension was centrifuged at 3,500 rpm for a period of 10 min. The silver nanoparticles in the pellet were washed 3-4 times with deionized water to eliminate impurities. The result in nanoparticles were stored in a cool, dry, and dark environment for future use (Yugal *et al.*, 2017).

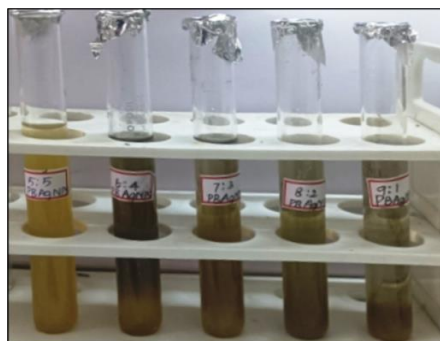


Fig. 5

i) UV-Visible Analysis:

The AgNPs' UV-visible spectrum displays a distinctive absorption band at 517 nm that is suggestive of silver nanoparticles fig. 6. The successful synthesis of AgNPs is confirmed by the lack of other peaks within this range. The range reported for AgNPs is consistent with the recorded value.

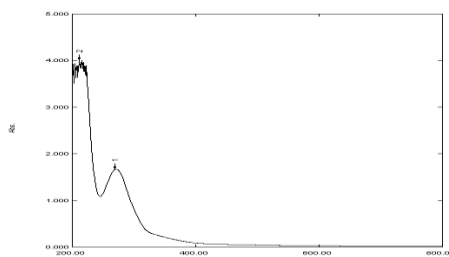


Fig. 6

ii) **FTIR Analysis:**

The derived silver nanoparticles were analyzed using Fourier Transform Infrared (FTIR) spectroscopy, specifically employing a Perkin Elmer device from the USA, to investigate the chemical groups present in the nanoparticle. The spectra of both the extract and AgNPs exhibited distinct stretching vibration patterns of the functional groups. The wide band observed at 3315 cm⁻¹ corresponds to the stretching vibration of the OH group, while the bands at 619, 1050, 1372, 1653, 2930, and 3413, respectively, were noted fig. 7.

Based on the literature review, the functional groups that play a role in the reduction and stabilization of synthesized silver nanoparticles (AgNPs) include phenolic, alcoholic, and carboxylic acid groups.

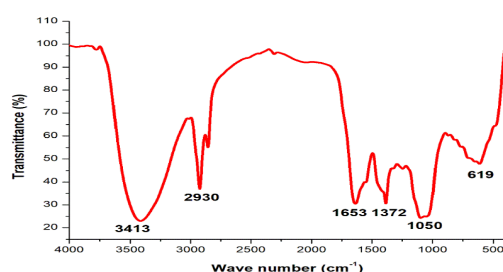


Fig. 7

III. **DPPH Assay:**

The herbal extract of *A. nilotica* leaves was found to contain significantly more reductants (antioxidants) than the corresponding methanal extract. In general, an increase in absorbance value is directly correlated with an increase in extract concentration. Therefore, an increasing trend of decreasing power is implied by the increasing OD value at 517 nm, the control mean OD value of 1.669 (fig. 8), the percentage of inhibition (fig. 9), and the tested sample's IC₅₀ value of 86.39 mg/ml (fig. 10). A methanolic solution of the stable free radical DPPH was used to test the different extracts' capacity to scavenge radicals. In general, a freshly prepared DPPH solution has a deep purple hue.

OD Value at 517 nm

Control Mean OD value: 1.699

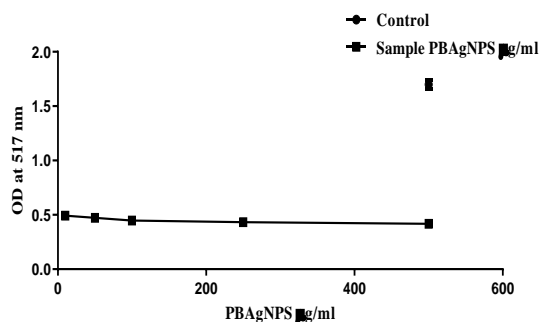


Fig. 8

Percentage of inhibition:

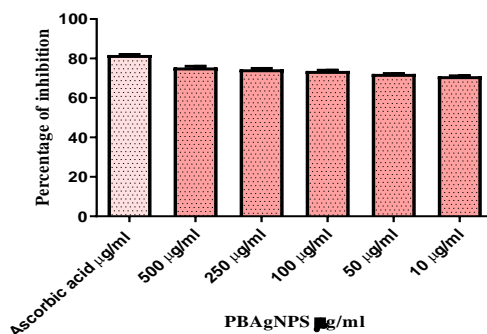


Fig. 9

IC50 Value of tested sample: 86.39μg/ml

DPPH

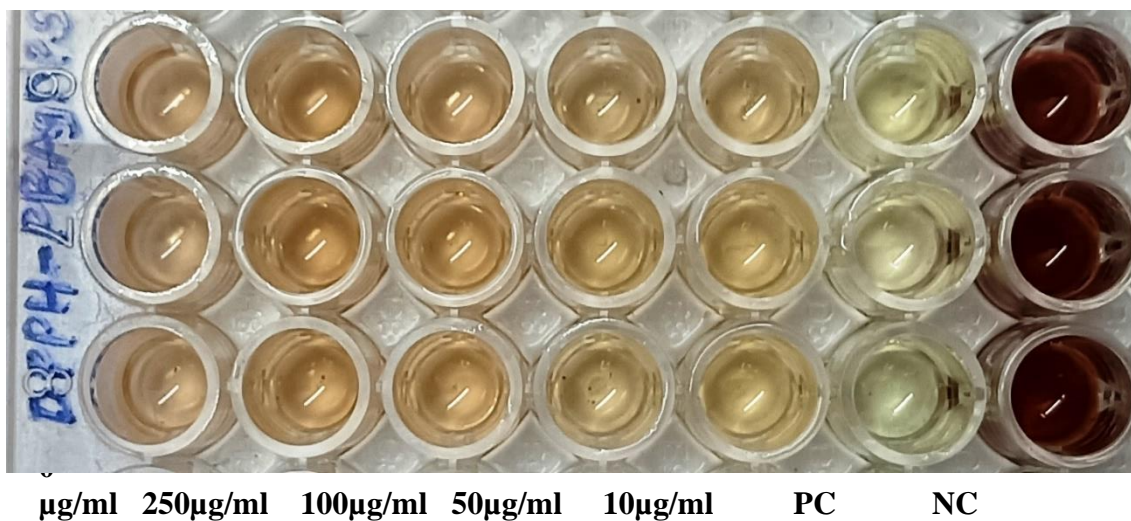


Fig. 10 Shows the results of DPPH assay

IV. SEM Assay:

A scanning electron microscope was used to assess the elemental makeup of powdered materials. The morphology of the green synthesized silver nanoparticles (AgNPs) made with the aqueous extract of *A. nilotica* was visible in the scanning electron microscopy. The SEM image shows that aggregation of nanoparticles, which may be caused by solvent evaporation during sample preparation, results in the formation of larger AgNP particles. The various particle sizes may be explained by this way fig. 11.

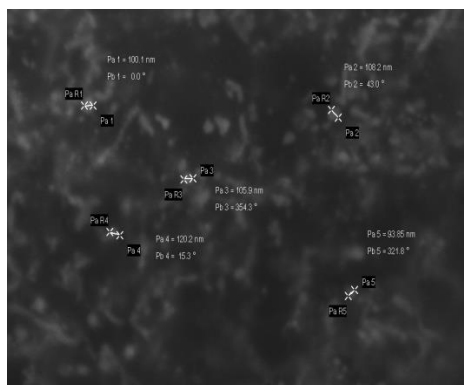


Fig. 11 shows the results of SEM

DISCUSSION:

The synthesis of silver nanoparticles (AgNPs) using green chemistry principles, particularly through the utilization of *A. nilotica* leaf extract, represents a significant advancement in the field of nanotechnology and materials science. This study demonstrates the efficacy of plant extracts as a reducing and stabilizing agent for the production of AgNPs, aligning with global efforts towards sustainable and environmentally friendly manufacturing processes (Arya, 2018).

The simplicity and cost-effectiveness of the green synthesis approach, as highlighted in this work, not only offer a viable alternative for large-scale production but also contribute to the reduction of hazardous waste associated with traditional chemical synthesis methods. The findings from the UV-visible, FTIR, and SEM analyses provided comprehensive insights into the characteristics and quality of the synthesized AgNPs, affirming their potential for various applications (Ravikumar & Angelo, 2015).

The significant increase in the rate of AgNP synthesis observed when detergents and elevated temperatures were employed suggests that these factors can be optimized to enhance production efficiency. This optimization could lead to more effective industrial applications, making the large-scale manufacture of AgNPs more feasible and economically viable, especially in regions where the *A. nilotica* resources is more abundant in India.

Moreover, the evaluation of antioxidant properties of curcumin analogs through the DPPH assay in this study opens new avenues for the application of AgNPs in the biomedical field, particularly in the development of therapeutic agents with antioxidant capabilities. The antioxidant activity of these compounds, combined with the antibacterial properties of AgNPs, could lead to the development of novel treatments for a range of diseases, offering a dual mechanism of action that enhances efficacy (Saratale et al., 2019).

Comparative analysis with existing literature reveals that the green synthesis of AgNPs using plant extracts not only supports the principles of green chemistry but also offers competitive advantages in terms of particle stability and functional properties. Previous studies

have primarily focused on the antibacterial and cytotoxic effects of AgNPs without extensive exploration of their antioxidant potential. Hence, this study contributes valuable knowledge to the field by highlighting the multifaceted applications of AgNPs synthesized through green chemistry.

Furthermore, the successful use of *A. nilotica*, a widely available and traditionally significant plant, underscores the importance of exploring indigenous resources for nanomaterial synthesis. This approach not only promotes biodiversity conservation but also encourages the integration of traditional knowledge with modern scientific research, fostering innovation in sustainable technologies.

CONCLUSION:

In this study not only validates the effectiveness of using *A. nilotica* extracts in the green synthesis of AgNPs but also emphasizes the potential of these nanoparticles in various applications, including wound healing, aesthetic improvements, and as antioxidant agents. Future research could focus on further optimizing the synthesis process, exploring the mechanisms underlying the observed biological activities of AgNPs, and expanding their applications in other fields such as environmental remediation and energy storage. The leaves display a bipinnate configuration, signifying that they are segmented into leaflets on two occasions. Gum Arabic consists of a complex blend of carbohydrates and finds application in numerous food and industrial sectors, including adhesives, textiles, and cosmetics. It is utilized for the treatment of various health issues, such as coughs, colds, and diarrhea. This tree, found in regions like Australia and the United States, grows at a relatively slow pace but is resilient and highly drought-resistant. The synthesis materials were thoroughly characterized utilizing a wide array of spectroscopic and microscopic techniques. UV, FTIR, SEM were assessed for the NPs demonstrated significant against pathogenic microorganisms. Additional research is required to evaluate the in vitro antioxidant activity of this DPPH before it can be used clinically. Therefore, it is envisaged that these Ag – doped NPs nanoparticles may be exploited in drug delivery, pharmaceutical.

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