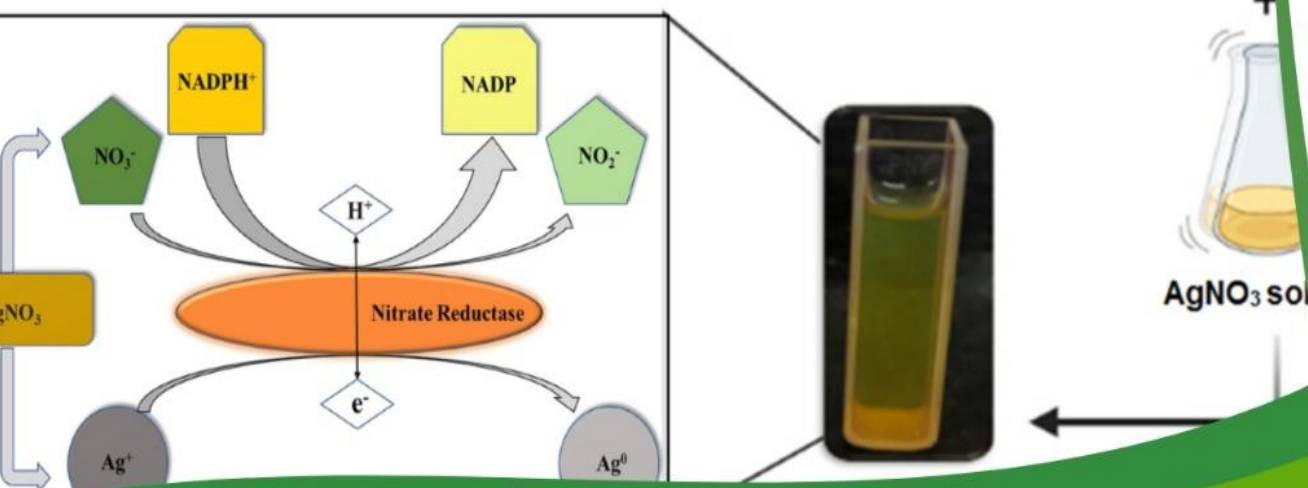




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Green synthesis of silver nanoparticles from *Eclipta alba* (bhringraj) and its characterization

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Green synthesis of silver nanoparticles from *Eclipta alba* (bhringraj) and its characterization

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ABSTRACT

Green synthesis uses a clean, safe, cost-effective and environment friendly nanoparticles production process. Microorganisms such as bacteria, yeast, fungi, algae species, and certain plants act as substrates in the biological synthesis of nanoparticles. The green synthesis method is used to prepare nanoparticles and these methods help to reduce toxicity, increase stability, eco-friendly and cost-effective methods. Green synthesis methods have a more favorable response in environment and biomedical applications. *Bhringraj* (*Eclipta alba*) (medicinal herbs) belongs to the family Asteraceae and has previously been used for the production of metal/metal oxide nanoparticles such as silver nanoparticles (AgNPs). It is a tropical or subtropical weed that can be found worldwide. This plant has some pharmacological properties such as antimicrobial, antifungal, antiviral, anti-inflammatory, analgesic, hepatoprotective, and immuno-modulatory properties. Characterization of these nanoparticles is done with these processes which we used: The UV-VIS spectroscopy was used to investigate the silver nanoparticles synthesized through the leaf extract. Silver colloids were identified with absorption bands at 298 and 598 nm, with the increase of the concentration of the aqueous solution causing a corresponding increment of the absorption band. Scanning electron microscope (SEM) technology allows for high-resolution images of silver nanoparticles that are less than 10nm in size. Using scanning electron microscopy, we gained a deeper understanding of silver nanoparticles' morphology and size detail. During research we found nanoparticles of size ranging between 15-25nm.

Keywords: *Eclipta alba*, Medicinal herb, Green synthesis

INTRODUCTION

Nanotechnology, originating from Richard Feynman's 1959 speech, is prevalent in various scientific fields, including engineering, health, and agriculture. It uses biological and inorganic materials, and metals like copper and silver are being explored for use in consumer goods (Dawadi *et al.*, 2021). Nanotechnology has revolutionized biological research and medicine applications. Green chemistry, involving plants, is a sustainable and eco-friendly method for synthesizing nanoparticles. This research explores the history, characteristics, synthesis techniques, benefits and drawbacks of different approaches, and their implementation in an ecologically benign and low-cost environment. Plants and plant extracts are considered as the best biological substitutes (Parveen *et al.*, 2016).

GREEN APPROACH FOR SYNTHESIS OF NANOPARTICLES

Green synthesis techniques, such as plant extract, have gained popularity due to their low costs, ease of characterization, and less failure potential in synthesizing nanoparticles. These methods are cost-effective and easy to scale up, making them a preferred choice over conventional methods. Greenly orchestrated nanoparticles are preferred over conventional ones due to their remarkable qualities and potential to reduce NP toxicity. The use of vitamins, amino acids, and plant extracts has become increasingly common in modern times (Balaji *et al.*, 2009). *Eclipta prostrata*, a medicinal plant with therapeutic properties, is being explored for nanoparticle synthesis due to its antimicrobial, antimutotoxicity, and immunomodulatory properties. There are studies that uses a green synthesis

approach, minimizing hazardous chemicals and promoting sustainability. Careful handling and disposal of nanomaterials are ensured to prevent adverse effects on human health and the environment (Gnanasekaran *et. al.*, 2024).

PROPERTIES OF SILVER NANOPARTICLES

Physical and synthetic properties of AgNPs, including surface science, size distribution, shape, molecule morphology, structure, and agglomeration, are crucial for cytotoxicity assurance (Zhang *et. al.*, 2016). AgNPs can be blended in various shapes, and their size and shape affect their toxicity. The presence of synthetic and natural coatings on the nanoparticle's surface also influences their toxicity. Positive surface charges make AgNPs more soluble, allowing them to remain in the circulation longer, which is important for anticancer drug development (Liu *et. al.*, 2016).

CHARACTERIZATION OF NANOPARTICLES

Scanning electron microscopy (SEM) is a 3D imaging technique that has an electron beam and creates a sample image. However, many nanoparticles are undetectable by the electron microscope and hence, a thin layer of metal coating is wire applied to the sample for the purpose of forming a conducting layer. SEM images can reveal the presence of nanoparticles and their relative to each other, aggregate, and even the degree of the pure samples (Gamboa *et. al.*, 2019). While atomic force microscopy (AFM) is useful for investigating surface morphology, as well as sensory forces, a downside is that the preparation process is destructive and not very cost effective. Another technique that is appropriate for characterisation of shape and size is transmission electron microscopy (TEM) while dynamic light scattering (DLS) is also useful for determining size of nanoparticles. DLS makes the assumption of spherical particles and is capable of resolving size distributions composed of several modes with high resolution. Since each of AFM, DLS and SEM incorporates structural information from different levels, their combination is mutually beneficial. Analysis of surface properties includes zeta potential, energy dispersive spectroscopy (EDS), photoelectronic X-ray spectroscopy (XPS), infrared Fourier transform spectroscopy (FTIR), and Raman spectroscopy (Sadeghi *et. al.*, 2010). These techniques assist in defining the chemical composition of the nanoparticle, while microscopic techniques enable defining the shape and size in detail. Determining surface charge and stability are provided by analysis of zeta potential, while EDS maps samples using X-ray spectra. FT-IR and Raman spectroscopy are employed in the examination of structural features with the former being mainly applied for the characterization of different nanoparticles.

MATERIAL AND METHODOLOGY

MATERIALS

Conical flask, beaker, whattman filter paper, dropper, aluminium foil paper, spatula, measuring cylinder, mortar n pestle, Bhringraj dried leaves, silver nitrate, double distilled water, weighing machine, magnetic stirrer, centrifuge, hot air oven, UV-spectrophotometer, SEM.

METHODS

Leaf Extract Preparation

Eclipta alba leaves were collected from a pot and then washed thoroughly with Double distilled water. After washing the leaves were shade dried for 3-5 days and then grinded with the help of mortar n pestle forming a fine powder. 10 g fine powder of Bhringraj leaves are added into 100 mL double distilled water. Then, at that point, it was warmed at 30°C for 20 to 30 minutes on magnetic stirrer. To get extraction we used whatman No. 1 filter paper (Ahmad *et. al.*, 2019).

Silver Nitrate Solution Preparation

50 ml of aqueous solution (0.1M) of silver nitrate (AgNO_3) was prepared in 100 ml conical flask and then it was heated for 15 to 20 minutes till it became light in texture.

Synthesis of silver nanoparticles

Plant extract solution was kept on magnetic stirrer after settle down of solution form. Silver nitrate solution was added drop wise with help of dropper into plant extract. We kept it on temprature range from 30-40°C. After continuous stirring of solution for 2-3hours we obtain a color change from green to dark brown. Hence, one identification of formation of nanoparticles is color further solution was kept down for cooling and attaining its normal room temperature. After cooling down the solutuion was then transferred into centrifuge tube for centrifugation at 10000 rpm at 30°C for 15 minutes and washed every

time after every 15min and the centrifuge again 3 times at same condition. After centrifugation the pellet particles were transferred to the petri plate and then dried at 60°C for 4-5 hours (Forough *et. al.*, 2010).



Fig 1: Green synthesis of silver nanoparticles

Characterization of Nanoparticles

UV Vis spectral analysis was done by using Shimadzu UV-visible spectrophotometer. UV-Visible absorption spectrophotometer with a resolution of 1 nm between 200 and 800 nm was used. One millilitre of the sample was pipetted into a test tube and subsequently analysed at room temperature.

Scanning Electron Microscope was used to determine the average size of synthesized silver nanoparticles. SEM focuses a beam of electrons on to the surface of particles to determine their size. These electrons interact with atoms present in the sample, resulting in various signals that tell the scanner about the topography of the surface and the composition of the sample (Premasudha *et. al.*, 2015).

RESULT

Plant extract

Collecting *Eclipta Alba* leaves from the field, washing thoroughly with double distilled water. After washing, *Eclipta* leaves are shade dried for a period of 3 to 5 days. Gently grind the leaves with motor n pestle, till a fine powder is formed. Add 100 ml Double distilled water containing 10 g fine *Bhringraj* leaves. At this point, heat at 30 °C for 20-30 minutes on a magnetic stirrer for extraction. Whatman No.1 filter paper is used.



Fig 2: Plant Extract Preparation

Synthesis of nanoparticle

Aqueous solution (0.1M) of silver nitrate (AgNO_3) was prepared in 100 ml conical flask and *bhringraj* extract was added for reduction into Ag^+ ions. The mixture was then heated on a hot plate for complete

bio-reduction at 30-40°C with constant stirring for 3-4 hours till we obtained a color change from green to dark brown. Hence, one identification of formation of nanoparticles is color further solution was kept down for cooling and attaining it's normal room temperature. After cooling down the solution was then transferred into centrifuge tube for centrifugation at 10000 rpm at 30°C for 15 minutes and washed every time after every 15min and the centrifuge again 3 times at same condition. After centrifugation the pellet particles were transferred to the petri plate and then dried at 60°C for 4-5 hours.

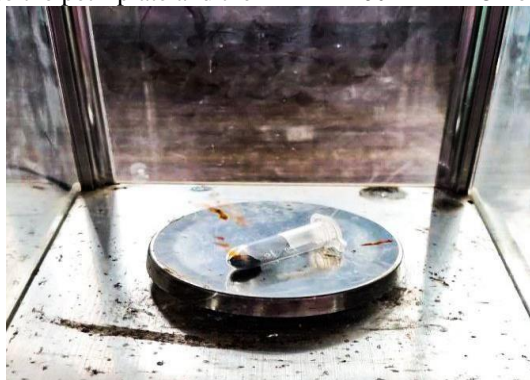


Fig 3: Synthesis of Nanoparticles

CHARACTERIZATION

UV- Visible spectrophotometer:

Spectral analysis of aqueous silver nanoparticles: Fig.4 shows UVVis spectra that capture the evolution of leaf soup over time when exposed to 1 mM aqueous Ag NO₃ solution. The presence of metal nanoparticles in the solution results in observing their characteristic surface plasmon resonance (SPR) absorption band. The absorption band is formed by the collective oscillations of free electrons in resonance with an incident light wave. Different absorption bands at 298 and 598 nm, indicating the presence of silver colloids, were identified by UVVis spectra. In particular, an increase in the concentration of the aqueous solution has been shown to increase the intensity of the absorption band. Consequently, over time the concentration increased resulting in a more pronounced absorption pattern. The analysis demonstrates the dynamic nature of metal nanoparticles in aqueous solutions, illuminating their optical properties and behaviour under a variety of environmental conditions.

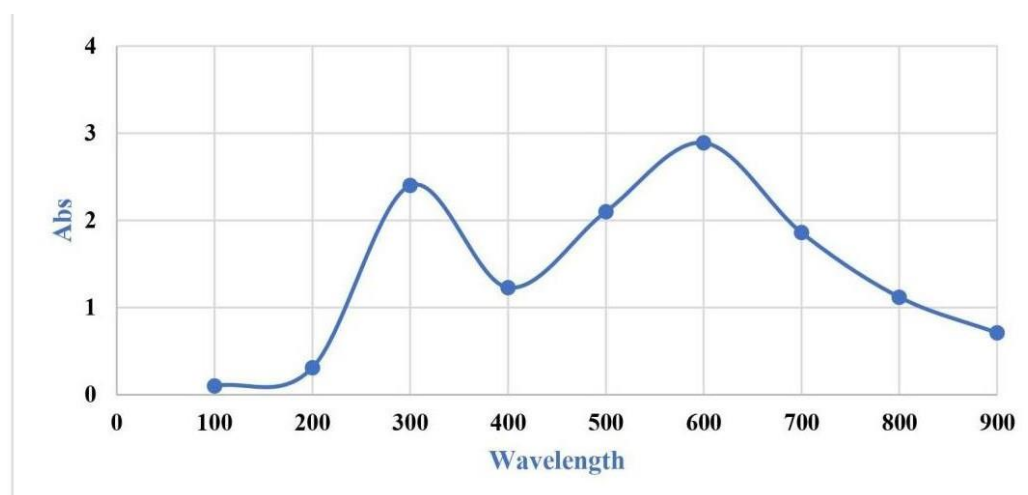


Fig 4: UV-visible spectra of silver nanoparticles Scanning

electron microscope:

SEM profiles of Ag NPs drop coated films with different magnifications are shown in Fig.5. These images show the aggregate Ag NPs with a spherical form by both methods. The aggregate form in SEM analysis is linked to the sample preparation method to the sample. The nanoparticles must be filtered and dried prior to measurement. The different surface morphology of particles is found from different methods. Microwave assisted Ag NPs show the flake type while the aging method shows spherical-like aggregates.

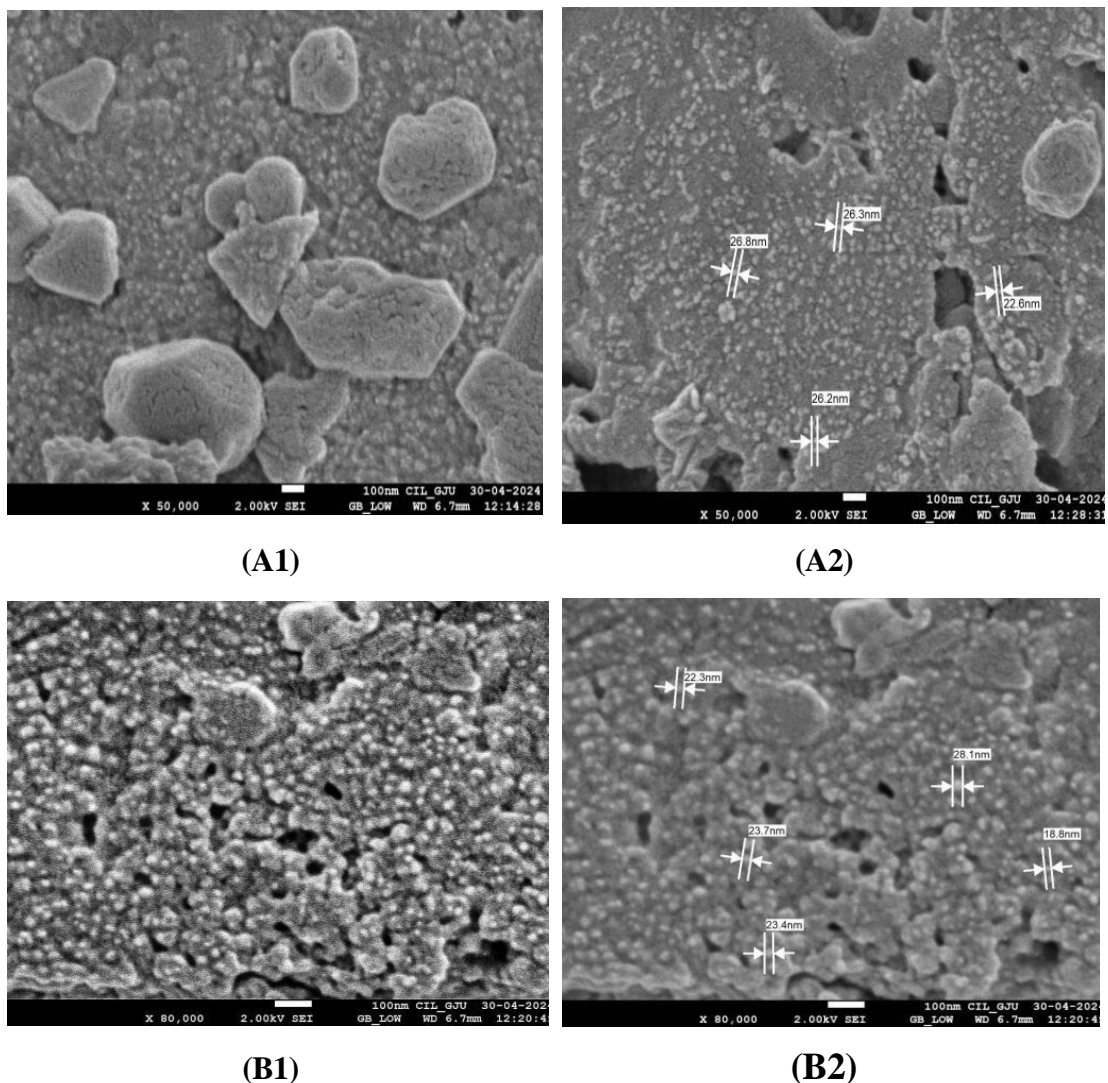


Fig 5: SEM results (A1) Shape of nanoparticles at 50,000x (A2) Size of silver nanoparticles at 50,000x (B1) Shape of silver nanoparticles at 80,000x (B2) Size of silvernanoparticles at 80,000x

DISCUSSION

Green synthesis refers to synthesizing various metal nanoparticles by using bioactive agents (e.g., plant material, microorganisms) and different biowaste sources such as vegetable residues, fruit peel residues, eggshell residues, agricultural residues, etc. Green synthesis aims to encourage innovative chemical technologies to minimize or eliminate the production and use of hazardous substances while designing, manufacturing, and using chemical products. This includes reducing or eliminating the generation of pollution in the synthesis process, eliminating the consumption and waste of non-renewable raw materials, reducing the use of hazardous or polluting raw materials in product manufacturing processes, and decreasing the synthesis time. Paul J. Anastas, widely regarded as "the father of green chemistry," defined green synthesis as "the work philosophy that involves alternative tools and pathways for pollution prevention," including designing the synthetic strategy and treating the potential secondary products arising from that route.

In the present study, Bhringraj was employed to synthesize silver nanoparticles because it is associated with substantial antimicrobial and antifungal properties. Bhringraj was chosen for the present research work as it was readily available with a short growing period compared to other plants and has a compact size of up to 30-40cm height, which was previously employed for green synthesis. Bhringraj belongs to the Eclipta genus, and there are usually several bioactive constituents in the Eclipta genus.

Bhringraj can tackle various health issues in humans, but due to a lack of experiments on Bhringraj, we,

as humans, need more information. Bhringraj is helpful in the most concerning issue nowadays, i.e., cholesterol, as the use of bhringraj can lower the (TC) total cholesterol in the body, (LDL) low-density lipoprotein cholesterol. Bhringraj is a plant of immense value as it cures many diseases without any side effects. In contrast to curing disease, bhringraj improves the overall general health of the human body. In present study nanoparticles were prepared by washing *Eclipta alba* leaves, drying them, and grounding them into a fine powder. Bhringraj leaves were added to the water, warmed, and then added to the plant extract. Silver nitrate was added, stirring for 2-3 hours. The solution turned green to brown, forming nanoparticles. The solution was then centrifuged at 10000 rpm for 15 minutes, washed, and centrifuged again three times. The pellets were dried at 60°C for 4-5 hours. After drying, the formation of nanoparticles is done and then sent for characterization using a UV-visible spectrophotometer and Scanning electron microscope.

UV-visible spectroscopy: UV-VIS spectroscopy determines the absorption in the ultraviolet and visible part of the electromagnetic spectrum. UV-VIS can also be used to validate the quality of the material. Spectral analysis confirmed the formation of silver nanoparticles mediated by leaf extract. Continuous scanning was performed using a spectrometer from 200 nm to 800 nm using a Spectrometer, and baseline correction was performed using distilled water as a reference.

SEM: Scanning electron microscope (SEM) technology allows for high-resolution images of silver nanoparticles that are less than 10nm in size. T-SEM analysis of silver nanoparticles in the thin-film supports offers many advantages over analyzing nanoparticles in bulk substrates. Using scanning electron microscopy, we gained a deeper understanding of silver nanoparticles' morphology and size detail.

A comparison of experimental results revealed an accurate evaluation of the diameter of the prepared nanoparticles.

CONCLUSION

The use of biological materials extracts to synthesize silver nanoparticles (AgNPs) from green methods specifically AgNPs from *Eclipta alba* leaves is a great achievement especially in the field of nanotechnology (Rafique et.al., 2017). The current study seeks to evaluate the effectiveness of *Eclipta alba* for AgNP synthesis as an alternative green and eco-friendly resource, maximizing efficacy, cost-efficiency and environmental safety, even in terms of biowaste management. The sustainable use of plant extracts in synthesis provides avenues for production of nanomaterials, reducing the risk in usage of harmful chemicals and energy demanding options. *Eclipta alba* has bioactive compounds that assist in the creation of AgNPs by serving as reducing and stabilizing agents during synthesis (Okafor et. al., 2013). Various analytical approaches were employed in the study in order to analyze the AgNPs that were synthesized and determine their physicochemical properties. UV-visible spectroscopy was used tracking the biosynthesis of silver nanoparticles. Scanning Electron Microscopy (SEM) aided in the description of the physical characteristics of the synthesized AgNPs regarding the form, dimension, and textural aspects. The above characterization techniques are crucial for the evaluation of the structure of AgNPs and inferring how the properties depend on the structure for various applications (Abdelghani et. al., 2018).

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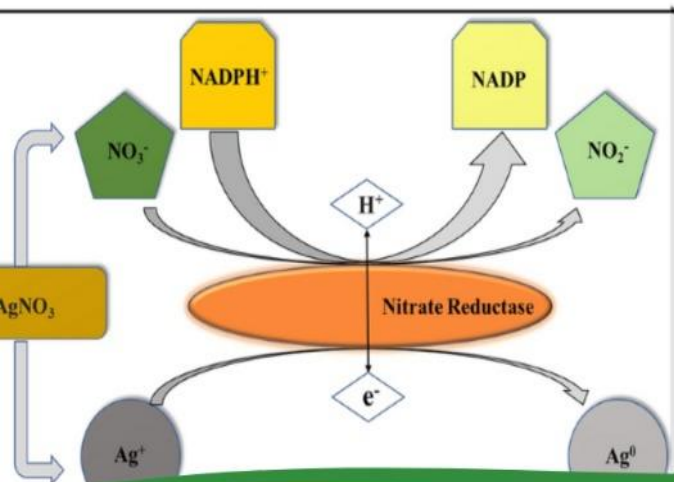
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