WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 16, 587-597. Research Article ISSN 2277–7105

GREEN SYNTHESIS, CHARACTERIZATION AND ANTI-INFLAMMATORY ACTIVITY OF SILVER NANOPARTICLE BY USING PHYLLANTHUS EMBLICA LEAF EXTRACT

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Article Received on 21 June 2025,
Revised on 11 July 2025,
Accepted on 01 August 2025
DOI: 10.20959/wjpr202516-37876

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ABSTRACT

The Green synthesis of nanoparticles is a more eco-friendly, cost-effective, and sustainable alternative to physical and chemical approaches of synthesis. Using medicinal herbs like Phyllanthus emblica can improve the therapeutic potential of manufactured nanoparticles. While numerous plant extracts have been utilized to synthesize silver nanoparticles (AgNPs), few investigations have focused on Phyllanthus emblica leaf extracts. The primary goal of this study was to produce silver nanoparticles from Phyllanthus emblica leaf extract and assess their structural characteristics and antibacterial activity. Silver Nitrate is used as metal precursor and phyllanthus emblica is used as reducing and stabilizing agent. After a visual color shift from yellow to brown, the formation was verified by UV-visible spectroscopy. It was then further described using FTIR to determine the functional group's involvement and the Agar well diffusion method to assess its antibacterial activity against both Gram-positive and Gram-negative bacterial strains. A far-off Surface Plasmon Resonance peak at about 440 nm was visible in the UV-Vis Spectra, indicating the production of AgNPs. Hydroxyl (O–H), carbonyl or aromatic (C=O or C=C) and metal ligands (Ag–O or Ag–N) functional groups were detected by FTIR analysis, suggesting their role in the production and stabilization of silver nanoparticles. The produced silver nanoparticle demonstrated significant antibacterial action, particularly against bacteria that are Gram-negative. These results demonstrate the potential of silver nanoparticles mediated by Phyllanthus emblica as strong antibacterial agents. The work encourages further investigation of phytochemical-rich plants for

biomedical research and supports the use of plant-based green synthesis techniques in nanotechnology.

KEYWORDS: Green synthesis, Phyllanthus emblica, Silver Nanoparticles, UV-Vis Spectroscopy, FTIR, Agar well diffusion, Antibacterial activity.

1. INTRODUCTION

The over the last century, nanotechnology has evolved as an important field of research, resulting in numerous significant developments in various scientific domains. It comprises the creation, engineering, and application of materials ranging in size from 1 to 100 nm, also known as nanoparticles.[1,2] Nanoparticles application has grown significantly in the twenty-first century due to their specified chemical, optical, and mechanical capabilities. Among them, metallic nanoparticles are most promising because they show good antibacterial properties due to their large surface area to volume ratio, which is now the current interest of researchers due to the growing microbial resistance to metal ions, antibiotics, and the development of resistant strains.[3-5]

Although a variety of nanoparticles, including copper, zinc, titanium, magnesium, gold alginate, and silver, have been investigated, silver nanoparticles have proven to be the most successful. Their potent antibacterial properties against viruses, bacteria, and other eukaryotic microorganisms have drawn a lot of attention to them.[6] The distinctive physical and chemical characteristics of silver nanoparticles (AgNPs), such as their morphology and distribution, size, shape, and high surface area, make them widely used in a variety of industries, including food, medicine, healthcare, and industry. Along with a broad range of applications connected to organic chemistry, they also demonstrate superior performance in optical, electrical, and thermal devices with high electrical and heat conductivity. They are also used in the food sector, medical device coatings, optical sensors, cosmetics, and many medicinal items. It's also important to note their application as antibacterial, anti-inflammatory, and anticancer agents in drug administration, theranostics, and diagnostics.[7,8] Nanoparticles have been synthesized using a variety of techniques, which can be broadly divided into three categories: physical, chemical, and biological (green) techniques. However, the use of hazardous chemicals, high energy consumption, and costly equipment are common in physical and chemical procedures, which might pose environmental risks. Green synthesis, on the other hand, is seen as a sustainable and environmentally beneficial method. It entails the creation of nanoparticles employing microbes, proteins, or plant extracts as stabilizing or reducing agents.[9,10] Because of its ease of use, affordability, and the inclusion of natural phytochemicals that improve the stability and biological activity of nanoparticles, plant-mediated synthesis has drawn a lot of attention.[11]

2. PLANT INTRODUCTION

Phyllanthus emblica Linn. (Syn. Emblica officinalis), also referred to as Indian gooseberry or amla, is a significant herbal remedy utilized in ayurveda and unani medicine. It belongs to the Euphorbiaceae family. As shown in below Fig. 1

Fig. 1: Phyllanthus emblica Linn.

The herb is used to restore lost strength and vitality as a tonic and as a medication. Phyllanthus emblica is high in nutrients and may be a significant source of minerals, amino acids, and vitamin C. Additionally, the plant includes tannins, phenolic chemicals, phyllembelic acid, phyllembelin, rutin, curcuminoids, andemblicol.[12,12] Every part of the plant has a medical value, but the fruit in particular is known for its powerful rasayana properties in Ayurveda and its usage in traditional medicine to treat inflammation, diarrhea, and jaundice. Additionally, a number of plant parts have demonstrated chemopreventive, hepatoprotective, hypolipidemic, antibacterial, antioxidant, antidiabetic, and antitulcerogenic qualities. These phytochemicals have made P. emblica leaf and fruit extract useful as stabilizing and reducing agents in the environmentally friendly creation of metal nanoparticles, especially silver nanoparticles.[13,14]

3. MATERIALS AND METHODS

The Silver nitrate, Double distilled water, Amoxicillin, Nutrient agar, Nutrient broth, Phyllanthus emblica leaf extract were used. Other instruments used were of laboratory and analytical grade.

3.1 Preparation of Extract from Selected Medicinal Plant

The freshly collected leaves of P. emblica were collected from in and around the campus of Shivaji college Kannad. The leaves were washed under running tap water, dried in shade for 15 days, sliced in to small pieces and grinded to coarse powder. 200ml of double-distilled water (DDW) and 20g of powder were heated for one hour. Whatman filter paper No. 1 was used to filtrate the leaf extract and once it had cooled to room temperature, the filtrate was stored in a refrigerator at 4°C for later use.[15]

3.2 Green Synthesis of Silver Nanoparticles:

The 0.1M of aqueous solution of Silver nitrate was prepared and used for the synthesis of Silver Nanoparticles, in the microwave-assisted synthesis of silver nanoparticles, 10ml of plant extract was added to 190ml of aqueous solution of 0.1M$AgNO_3$ and reaction mixture was taken in a conical flask. It was irradiated in a domestic microwave oven operating at medium power (800W) for 2-5 minutes. The generation of silver nanoparticles will be visualised by the colour change from yellow to brown.[16] Synthesized silver nanoparticles were obtained without any chemical reagent, now the synthesized silver nanoparticles were isolated by centrifugation technique at a speed 10000 rpm for about 20 minutes. For the further settlement of particles, the supernatant material was transferred to a beaker and frequent centrifugation was carried out to purify AgNPs. After being oven-dried, the produced nanoparticle pellet was put away for additional examination.[17]

3.3 Characterization of Silver Nanoparticles

3.3.1 UV-Vis Spectrophotometer

The UV-Vis spectroscopy is a valuable tool for characterising stability, optical qualities, and reaction factors such as temperature, pH, and time. A UV-Vis spectrophotometer (PerkinElmer, Germany) at wavelengths of 300–700 nm was used to characterize the AgNPs using *P. emblica* leaves extract. The absorption peak from 350 to 500 nm[18] indicates the reduction of silver ions. The results show the existence of silver ions and a reduction in the analysed substances.

3.3.2 Fourier Transform Infrared Spectroscopy (FTIR)

The Fourier Transform Infrared Spectroscopy (FTIR)The AgNPs solution was centrifuged at 10,000 rpm for 30 min to perform FTIR measurements. Functional groups in the extract of leaf that may be responsible for the formation of AgNPs were identified with FTIR (PerkinElmer Spectrum Version 10.03.06) and the FTIR spectrum was examined. It might aid in the stabilization, capping, and reduction of AgNPs. The FTIR range of 400–4000 cm-1 was achieved using a spectroscopic array.

3.3.3 Scanning Electron Microscopy (SEM)

The dimensions, shape, and arrangement of the synthesised NPs were examined using scanning electron microscopy. The dried samples were placed on dual conductive tape attached to the sample container and left at room temperature. The samples were coated with a layer of platinum-gold to increase conductivity. Samples were analyzed under a voltage of 80 kV.

3.3.4 Anti-inflammatory activity

The anti-inflammatory potential of AgNPs was studied as described[18] using the protein denaturation method. The potent nonsteroidal anti-inflammatory drug diclofenac sodium is used as the standard. The reaction mixture, consisting of 2 ml of AgNPs, 2.8 ml of phosphate-buffer saline (PBS, pH 6.4), and 2 ml of egg albumin (fresh chicken egg (1 mM)), was incubated at 27°C for 15 minutes. The mixture was heated in a water bath for 10 minutes at 70°C for denaturation. After cooling the sample, absorbance was measured at 660 nm. Each test was performed three times. Percent inhibition of protein denaturation was determined using the following formula:

% inhibition= ((Ac-As)/Ac) × 100

Where, As = Absorbance of sample; Ac = Absorbance of control.

4. RESULTS

Characterization of AgNPs

The aqueous extract of P. emblica leaves was used for the synthesis of AgNPs at a temperature of 70°C. With AgNPs synthesis, the initial colorless reaction mixture gradually turned dark brown after 30 min (Figure 1). Using leaf extract, the reduction of Ag ions to metal was confirmed by UV absorption spectrum value at 440 nm. P. emblicaleaf extract was used to confirm the green synthesis of AgNPs using the surface plasmon resonance band at 440 nm. AgNPs synthesis was observed by UV-visible spectroscopy at different time intervals; the highest reduction and aggregation of AgNPs was observed after 6 h, as shown by the absorption intensity (Figure2). Biomolecules for effective capping and stabilization of metal NPs production by P. emblica leaf extract were determined by FTIR measurements. FTIR spectrum of P. emblica leaf extract is shown in Figure 3. Spectral analysis revealed the number of functional groups acting as limiting agents or stabilizers and responsible for stabilizing the NPs.[19] Extraction mediated AgNPs-based FTIR measurement showed different absorption peaks at 3851, 3741, 3405, 2924, 2345, 2113, 1597, 1384, 1063 and 673 cm−1 with different functional groups O-H, O-H, N-H/O-H, C-H/N-H, O=C=O, N=C=S, N-O, C-H/O-H, S=O and C=C/C-Br respectively.

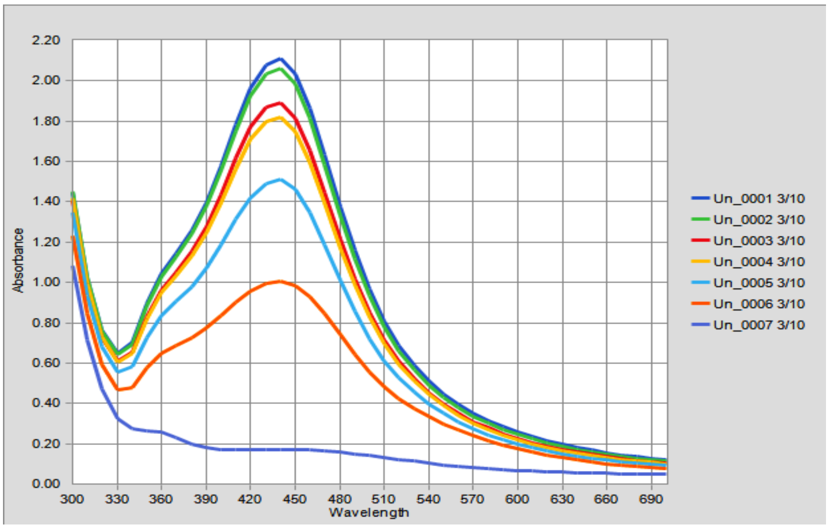

Fig. 2: UV-Visible spectra of P. emblica fresh leaf silver nanoparticles from 0 to 6 hr.

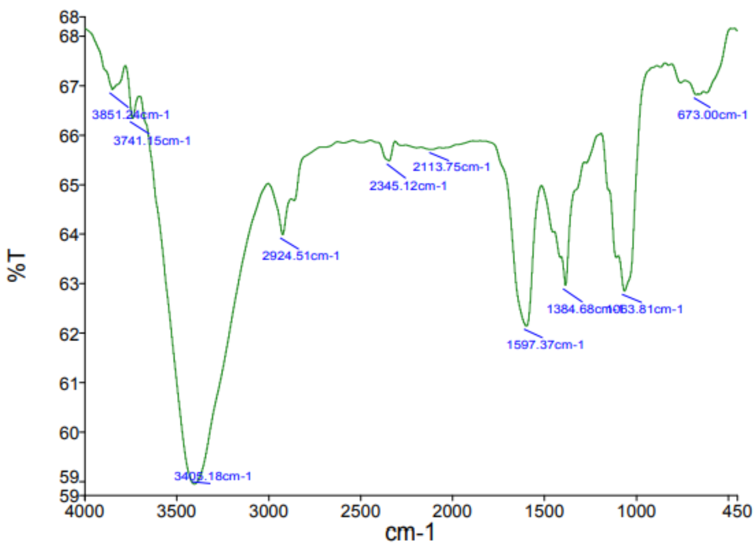

Figure 3: FTIR Spectra of P. emblica fresh leaf silver nanoparticles.

SEM provides visible sample surface morphology. When electrons reflect off the sample surface, an image appears. High-resolution images of NPs surfaces provide us with useful details about their size, shape, topography, composition, conductivity, and other characteristics. The synthesized NPs were visible with FESEM. The shape of the NPs is round and oval. A small number of individual particles, with an average size of 40 to 50 nm, were also identified, but the majority of NPs were aggregated (Figure 5).[20]

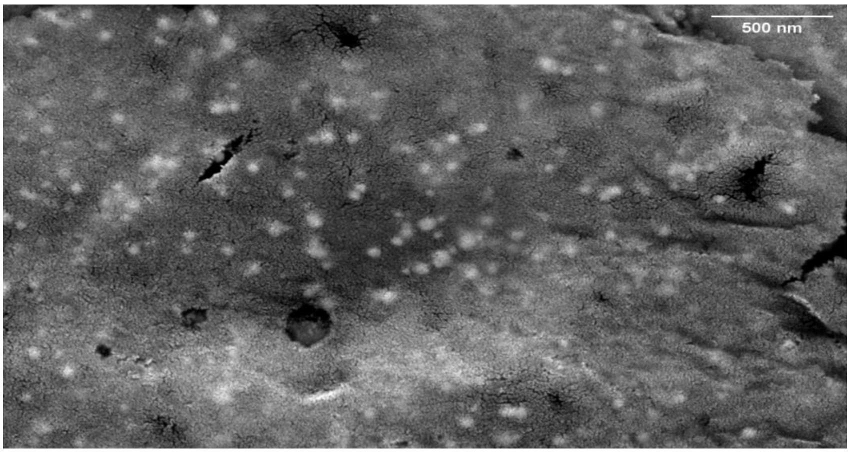

Fig. 5: SEM images of the biosynthesized silver nanoparticles (AgNPs).

The anti-inflammatory potential of silver NPs was evaluated using a protein denaturation assay and compared with the reference drug, Diclofenac sodium. With synthesized AgNPs, protein denaturation was observed (Figure 7). Conventional drugs showed the higher antiinflammatory activity (91.67±1.90%), as compare to silver NPs (71.14± 0.88%).[21]

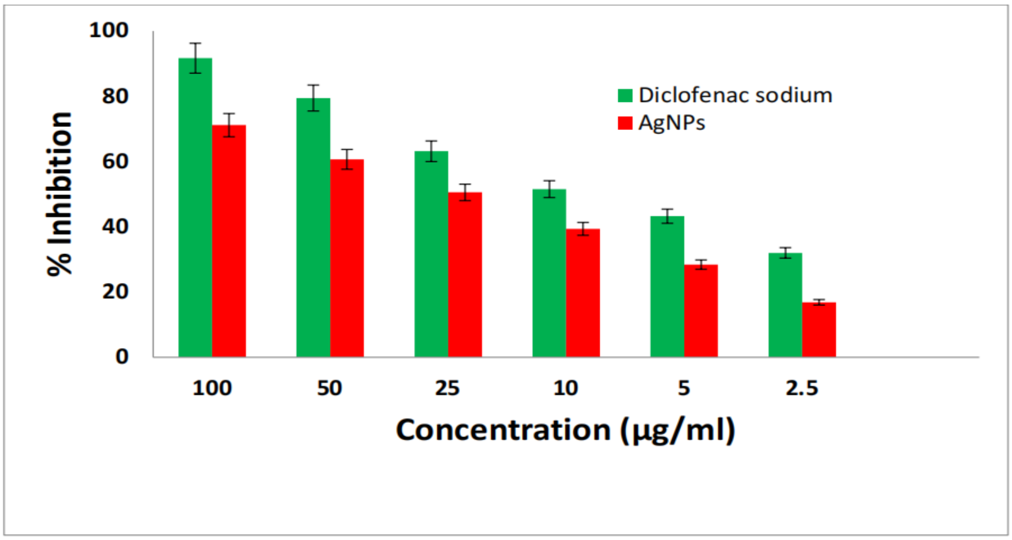

Figure 7: Anti-inflammatory activity of synthesized AgNPs vs Diclofenac sodium.

5. DISCUSSION

The synthesis process of “green” NPs, which is more expeditious than traditional chemical synthesis, is remarkable due to its environmentally conscious nature, cost-effectiveness, utility, and extensive range of applications. Biosynthesized NPs are being utilised in various applications such as cancer treatment, drug delivery, DNA analysis, gene therapy, antibacterial agents, biosensing, and response rate augmentation. This field is currently in its early stages of development. Nanoparticles find use in several fields such as electrical engineering, medical, chemistry, biology etc. The morphology and dimensions of colloidal metal particles have a pivotal role in a wide range of applications, such as the fabrication of magnetic and electronic devices, wound healing, the stimulation of antimicrobial genes, and the advancement of biocomposites.

The electromagnetic and optical characteristics of noble metal NPs exhibit variations based on the size and morphology of the particles.[22] In contrast to physical and chemical techniques, the production of NPs using green and environment friendly technologies is characterised by its lack of toxicity, cost-effectiveness, and biocompatibility. Additionally, it necessitates a reduced amount of exertion and time. Algae, bacteria, fungus, and plants are commonly utilised as biological sources for synthesising AgNPs.[23] The leaves of P. emblica in this study were analysed using the phytoreduction process to generate AgNPs.

The process of converting silver into silver NPs involves the utilisation of P. emblica leaf extract as both a capping and reducing agent. A wide range of secondary metabolites, including tannins, glycosides, phenols, terpenoids, saponins, and flavonoids, have been identified in the quest for phytochemicals.[24] Furthermore, it has been found that aqueous extracts of P. emblica leaves possess significant quantities of flavonoids and phenols.[25] The presence of silver NPs was confirmed by the observed alteration in colour following the adding up of concentrated leaf extract to the AgNO3 solution. After duration of 6 hours, the solution undergoes a transformation and assumes a dark brown colour.

An intense peak at 440 nm was found in the UV-Vis spectra, indicating the presence of silver NPs synthesised using green methods. FESEM was used to confirm the shape and surface area of AgNPs. The size of P. emblica AgNPs was evaluated via SEM examination and found to range from 40 to 50 nm. The FTIR spectra reveal the existence of many functional groups that can serve as both reducing and capping agents during the fabrication of AgNPs.

Inflammation has a substantial impact on various diseases. Nonsteroidal anti-inflammatory medicines (NSAIDs) and steroids are the two primary categories of medications employed in the treatment of inflammation. Discovering alternative anti-inflammatory medications with comparable efficiency but without any adverse effects, such as gastrointestinal issues and leukopenia, is crucial.[26] The synthesised AgNPs were assessed for their anti-inflammatory properties using a protein denaturation assay. Both traditional diclofenac sodium and synthesised AgNPs exhibited antiinflammatory effects in vitro, which were depending on the dosage. Based on earlier studies, AgNPs shown a protein denaturation inhibition rate of 71.65%, which is close to 94.24% of diclofenac, a commonly used anti-inflammatory medication.[27]

6. CONCLUSION

Silver nanoparticles were produced utilizing the green synthesis method and Phyllanthus emblica leaf extract. According to the study's findings, the synthesis is a safe method of producing nanoparticles. UV-Vis and FTIR spectroscopy techniques were used to analyze the reduced silver nanoparticles. According to the characterization results, the UV-Vis spectra showed a strong absorption peak at 440nm, demonstrating the creation of silver nanoparticles. FTIR research indicated the existence of several functional groups that are responsible for AgNP reduction and stabilization. The formation of AgNPs reveals the color change. The experimental results concluded that biogenic synthesised AgNPs had significant antiinflammatory activity.

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