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Green Synthesis of Silver Nanoparticles from *Phyllanthus emblica* (Indian Gooseberry) for Potent Antimicrobial Efficacy

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Abstract

Nowadays cost-effective and environmentally friendly technologies for nanomaterial synthesis have gained attention in the biosynthesis of nanoparticles. The present study is focused on synthesizing silver nanoparticles using Phyllanthus emblica for a green approach. UV-Vis Spectrophotometry, XRD, SEM, and FTIR were used to characterise the synthesised silver nanoparticles. It was determined that spherical shaped nanoparticles by SEM examination, which revealed that the average particle size was 20 nm. Antibacterial activity of AgNPs analyzed with the help of Staphylococcus aureus, Vibrio cholerae, Pseudomonas auriginosa, and Bacillus subtilis bacteria, and the result shows that a higher concentration of AgNPs is increasing as well as a zone of inhibition increased.

Keywords: *Phyllanthus emblica Silver nanoparticles, UV-Vis Spectrophotometry, XRD, SEM, FTIR, Antibacterial activity.*

1. Introduction

In nanotechnology, nanoparticles are particles with a diameter between 1-100 nm, and their unique properties have made them highly popular. (Sarsar et al., 2017) Many scientists are interested in nanoparticle synthesis because of its unique properties and wide-ranging applications in areas including catalysis, optics, medicine, and electronics. (Arya et al., 2018) Silver nanoparticles have been widely used because of their numerous applications in a variety of fields, including antibacterial, anticancer, antidiabetic, and antitumor properties, etc. (Raja et al., 2017) There are different ways to create silver nanoparticles, including chemical, physical, radioactive, and biological processes. Chemical reduction, electrochemical processes, sonochemistry, laser ablation, chemical vapor deposition, microwave, hydrothermal, and other processes are among the chemical and physical techniques used to make silver nanoparticles. Many of these techniques demand toxic substances that are harmful to the environment. (Govindarajan et al., 2017) Due to these restrictions, the biological technique for synthesizing silver nanoparticles has received more attention. The production of silver nanoparticles by biological means is inexpensive, quick, and environmentally friendly The biological approach includes the utilisation of plants, bacteria, fungi, algae, etc. Additionally, research reveals that the biomolecules found in the plant extract, such as alkaloids, alcoholic compounds, enzymes, amino acids, proteins, polysaccharides, etc., are in charge of producing and stabilising the silver nanoparticles. (Shah M.,et.al.,2017).

In this perception, *Phyllanthus emblica* L. (Family: Phyllanthaceae) commonly known as emblica exhibits a striking assortment of shapes of development such as herbs and bushes, pachycaulous succulents, climbers, and drifting aquatics. The fruits of Phyllanthus spp. are widely used in the preparation of traditional medicines in Southeast Asia due to unique properties such as rich antioxidant activity, antiaging, antipuretic, and anti-inflammatory (Pientaweeratch et al., 2016; Manikandan et al., 2017). Moreover, this selection was provoked by the opportunity to induce shape control of nanoparticles due to the potential sources of naturally occurring phytochemicals, especially polyphenols, tannins, ascorbic acids, and flavonoids in the fruit extract (Ramesh et al., 2015). The present investigation focused on the effects of the different optical parameters on the silver nanoparticles and investigated of the antibacterial effect of the synthesized AgNPs.

2. Materials And Methods

Preparation of extract from Selected medicinal plants

Extraction of plant extract by the dry fruit. Firstly washed carefully with distilled water to make free from dust particles and dried 24 h at room temperature. After that 2gm dry fruit was soaked in 50 mL of pure distilled water for 24 h. Then synthesized plant extract was filtered and stored for nanoparticle synthesis.

2.2 Green Synthesis of Silver Nanoparticles

0.1 M of aqueous solution of silver nitrate was prepared and used for the synthesis of silver nanoparticles. 10 ml of plant extracts was added to vigorously stirred 90 ml of aqueous solution of 0.1M silver and kept at room temperature. The solution turned to bright yellow and to dark brown which confirmed the synthesis of silver nanoparticles. The solution was kept incubated at 200 rpm at about 37 °C under continuous agitation for 48 h (Kasthuri et al., 2009).

2.3. Characterization of Silver Nanoparticles

i) UV-Vis Spectroscopy and Fourier transform infrared spectroscopy analysis

For maximum production of silver nanoparticles, the samples were taken under the absorption spectrum in the range of 200–800 nm using a UV–vis spectrophotometer. UV visible spectroscopy was carried out on JASCO V-750. The duration and progress of the reaction between metal ions and the leaf extract were observed. The reduction of silver ions and formation of silver nanoparticles occurred within an hour. Control was maintained by using AgNO3 and de-ionized water was used as a blank (Logeswari *et al*., 2015).

The characterization of the active functional groups on the surface of silver nanoparticles (AgNPs) synthesized from *Phyllanthus emblica* fruit extract was reported by JASCO FT/IR-4200 Fourier Transmission Infra-Red Spectroscopy. (Joshi 2012)

ii) XRD and SEM Analysis:

The size of the synthesized silver nanoparticles was investigated using an X-ray diffractometer (Model: ULTIMA IV, Rigaku, Japan) operating at a voltage of 40 kV, and a generator current of 40 mA with CuK (α).

The structure and composition of freeze-dried purified silver particles were analyzed by using a scanning electron microscope. Purified AgNPs in suspension were characterized for their size using a Scanning Electron Microscope (JEOL-JSM 6390, Japan). (Joyita Banerjee, and Narendhirakannan R.T.,2011)

iii) Antibacterial activity of *P.emblica* AgNps :

The antibacterial activity of the synthesized silver nanoparticles was tested for their inhibitory against *Staphylococcus aureus, Vibrio cholerae, Pseudomonas auriginosa*, and *Bacillus subtilis.* The antibacterial activity of the biosynthesized silver nanoparticles was evaluated using the disc diffusion method on nutrient agar plates. Both the bacterial cultures were freshly grown overnight at 37ºC in nutrient broth (1 105) CFU/ml. Each culture of bacteria was then spread over the individual nutrient agar plates. The antibiotic Ampicillin was used as a positive control. The cultured plates were then incubated at 37ºC for 24 hours. After incubation for 24 hours, the zone of inhibition was measured. (Muniappan Ayyanar and Pandurangan Subash Babu.(2012)).

3. Results and Discussion

Synthesis of *P.emblica* AgNps :

The AgNPs were successfully synthesized using an aqueous plant extract of *Phyllanthus umbilical* by mixing with silver nitrate solution (1mM). The colour changes from pale yellow to dark brown. This was observed due to the reduction of Ag+ and it indicates the formation of AgNPs. Jain et al., (2009) also observed leaf extracts were mixed with the aqueous solution of the silver ion complex, it was changed into reddish brown color due to excitation of surface plasmon vibrations, which indicated the formation of Ag nanoparticles.

Characterisation of *P.emblica* AgNps

i) UV-vis spectroscopy

The formation of Silver Nanoparticles first, was identified by color change from brown to reddish brown (Figure 2) after the nucleation of the metal ions indicating that phytoconstituents of *Phyllanthus embilica* caused the reduction of Ag into AgNPs in which the phenomenon could be attributed to the surface Plasmon absorption. (Mulvaney P,1996) Similarly, the UV-Vis spectral analysis shows that the highest absorption peaks appeared at 300nm, reflecting the surface plasmon resonance of silver NPs from P.emblica fruit, which is characteristic of Silver Nanoparticles. The results are presented in the figure

ii) FTIR analysis

FTIR measurements were carried out to identify the possible biomolecules responsible for the capping and efficient stabilization of the AgNPs synthesized by the plant extracts. (Raj VD, et.al.,2012).Absorbance bands of P. emblica were observed at 3435.42 cm−1 assigned to O–H (s) stretch, 1637.85 cm−1 assigned to C = C aromatic stretch, 1458.88 cm−1 assigned to C–H bending alkane stretch, and 1048.53 cm−1 assigned to CO-O-CO anhydride stretch, as shown in Figure 2.

iii) XRD Analysis

Figure. 3 shows the XRD patterns of synthesized AgNPs by *Phyllanthus emblica* extract. The peaks observed at an angle of 37.86, 44.06, 64.20, and 77.11 corresponds to the diffraction from (111), (200), (220), and (311) plans, respectively. This nanoparticle holds a good record with the JCPDS No 00-004-0783 directly corresponds with JCPDS 00-004-0783, confirming the successful formation of silver metal Nps. The presence of some addition peaks might have occurred due to the presence of some organic compounds. These are useful in the Debye-Scherrer equation for analyzing average particle size. That means Diffraction peak $D = (k\lambda)/(\beta \cos \theta)$, here λ wavelength of Cukα, D is the crystalline size of the sample powder, β is the full width at half maxima, θ is the Bragg diffraction angle and the K is the constant.

SEM Analysis

The morphological study of synthesized silver nanoparticles using *Phyllanthus emblica* was investigated by SEM. In SEM images of synthesized SEM analysis was done to determine the shape of the nanoparticles. (Fissan et al., 2014). The synthesized *P.emblica* AgNps was found to be spherical (Fig 4).

Antibacterial activity:

The silver nanoparticles synthesized via the green route were highly toxic to multidrug-resistant bacteria and hence has great potential in biomedical application. Because of the enhanced surface area of nanoparticles than atomic size, nanoparticles can easily interact with bacterial cells. The interaction of AgNPs with bacterial cells kills the bacteria by attacking the respiratory chain and cell division as AgNPs get attached to sulfur and phosphorous constituents of the bacterial cells (Rai.M, M. et al.,2009). The antimicrobial activity of silver nanoparticles against *Staphylococcus aureus*, *Vibrio cholerae, Pseudomonas auriginosa,* and *Bacillus subtilis* was investigated and compared with the standard drug Streptomycin. In lower concentrations (100 µg/ml) the maximum inhibition was recorded against *P. aeruginosa* (13 mm) followed by *B. subtilis* and *V. cholera* (10 mm). The highest inhibition activity was recorded against *P. aeruginosa* (16 mm) followed by *V. cholera* (13 mm) and *B. subtilis* (12mm) at 200 µg/ml concentration. In higher concentrations, maximum inhibition was noted in *P. aeruginosa* (20 mm) and minimum inhibition in *B. subtilis* and *V. cholera* (15 mm) respectively. The standard antibiotic streptomycin exhibited excellent anti-bacterial activity. It has been proven that the antibacterial property of *Phyllanthus emblica* AgNps is concentration-dependent.

The antibacterial activity of AgNps synthesized from *Phyllanthus emblica* was investigated against human pathogens and the activity is shown in Table 1. This may be due to the perforation and lysis of AgNPs to the bacterial cell wall followed by the generation of free radicals (Prabhu S and Poulose E,2012) and degradation of DNA (Duran N, et.al.,2015). Concentration of 300µg acquired inhibition against *Pseudomonas sp*. (20 mm), followed by *Bacillus subtilis* (15 mm) and *V. cholera* (15 mm). There is no inhibition in *Staphylococcus aureus* bacteria. The Standard Streptomycin has a higher activity only on *Bacillus subtilis*. The results are presented in Table I and Plate 1.

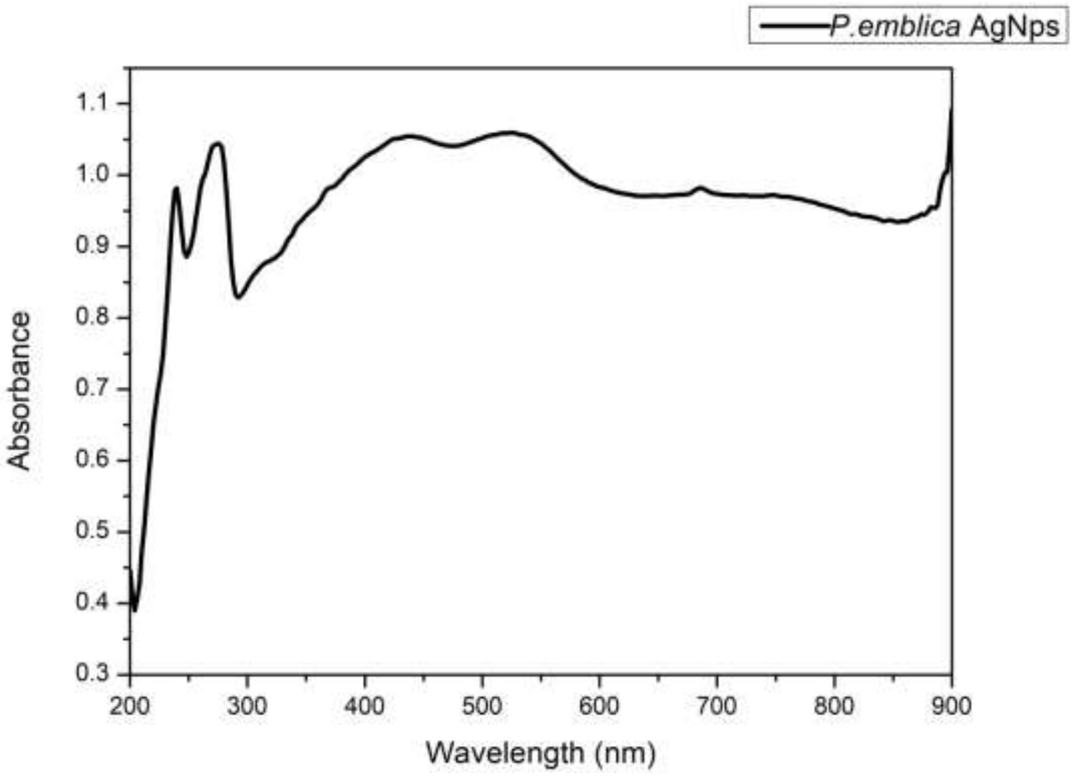

Fig 1: The UV analysis of Ag nanoparticles of *Phyllanthus emblica*

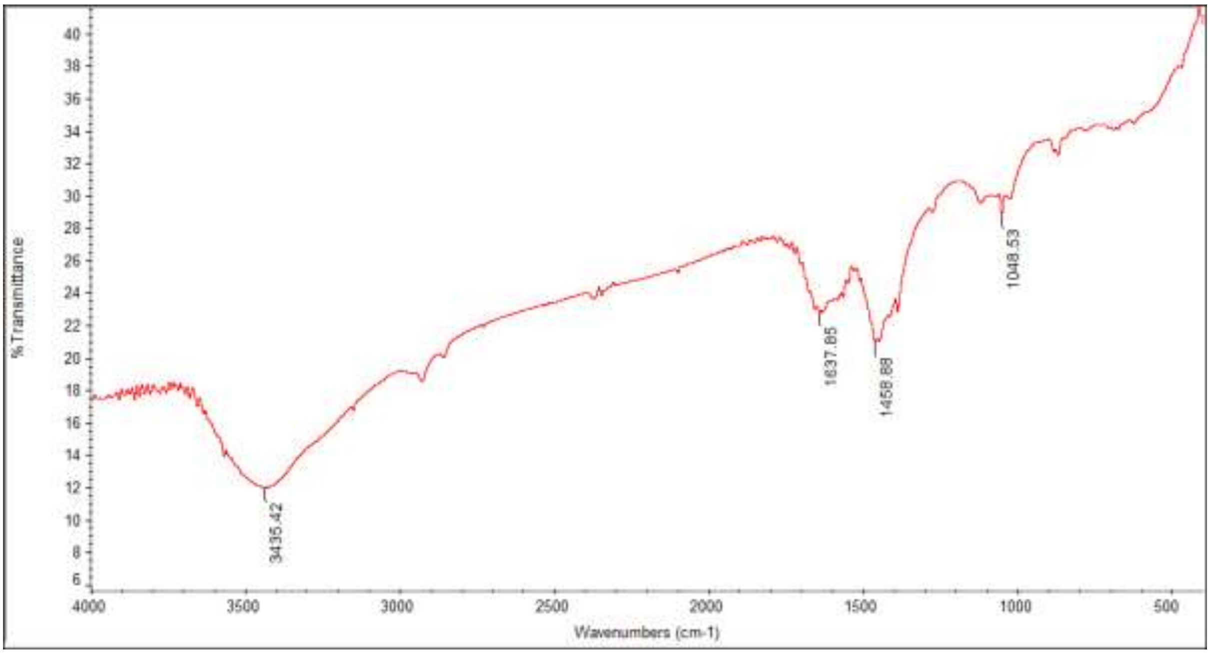

Fig 2: The FTIR analysis of Ag nanoparticles of *Phyllanthus emblica*

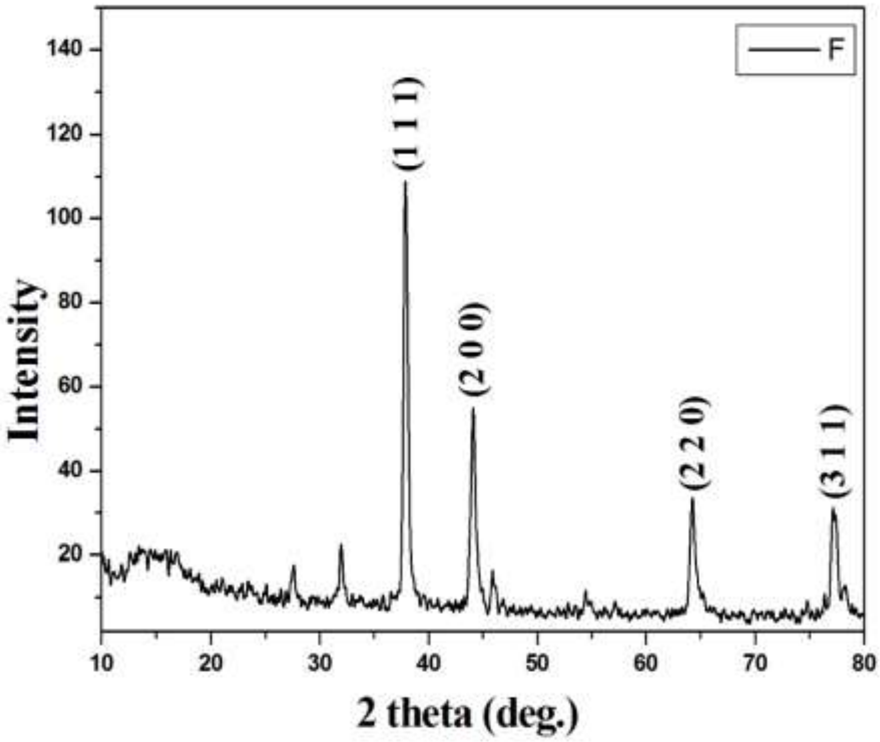

Fig 3: The XRD analysis of Ag nanoparticles of *Phyllanthus emblica*

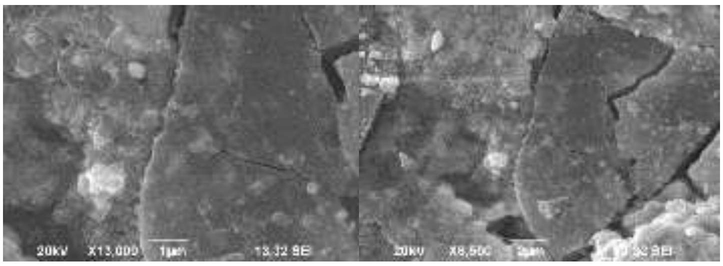

Fig 4: The SEM analysis of Ag nanoparticles of *Phyllanthus emblica*

Table 1: Zone of inhibitory activity of *Phyllanthus umbilical* AgNps against Gram-positive and Gram-negative bacteria.

S. no	pathogens	100mg	200mg	300mg	negative	Streptomycin
1	*Staphylococcus aureus*	-	-	-	-	23
2	*Bacillus subtilis*	10	12	15	--	25
3	*Pseudomonas aeruginosa*	13	16	20	-	23
4	*Vibrio cholerae*	10	13	15	-	20

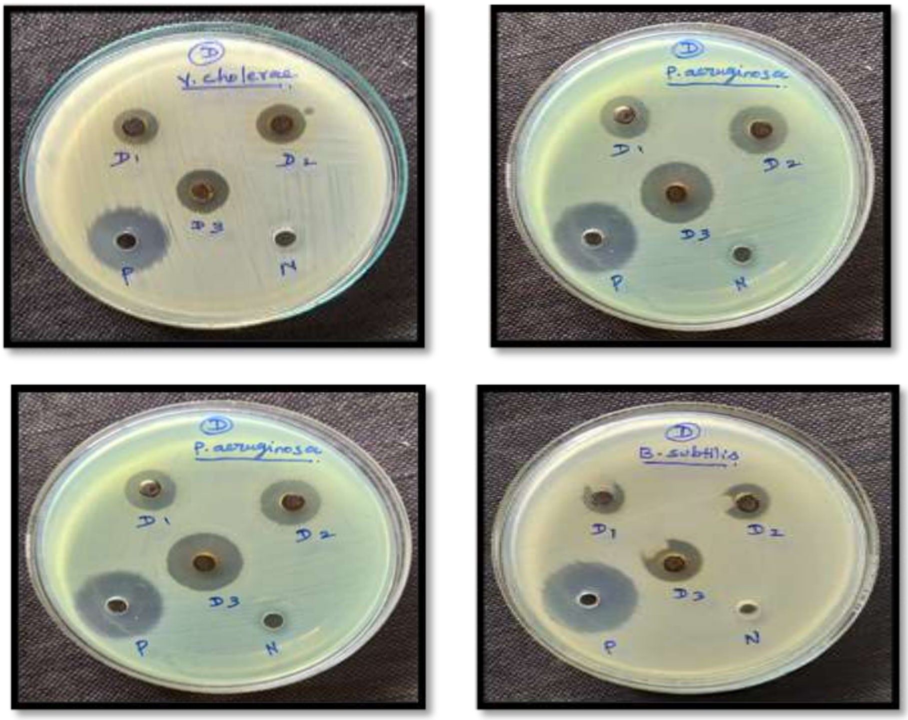

Plate 1: Photos of Inhibition zones obtained in the Antibacterial Assay of *Phyllanthus umbilical* AgNps (B_1-100 µg concentration of AgNps of *P.emblica*, B_2 -200 µg concentration of AgNps of *P.emblica*, B_3-300 µg concentration of AgNps of *P.emblica*, P- Positive control Streptomycin)

4. Conclusion

The silver nanoparticles were synthesized in a biogenic method using *P.emblica* extract. The biosynthesis of silver nanoparticles using *Phyllanthus emblica* provides an environmentally friendly, simple, and efficient route for the synthesis of benign nanoparticles. The reduced silver nanoparticles were characterized using UV-Vis, FTIR, XRD, and SEM techniques. The results proved that the synthesized nanoparticles from the plant *Phyllanthus emblica* fruit extract and aqueous extract possess antibacterial activity. These green synthesized silver nanoparticles can be used as novel therapeutic agents in many biomedical applications.

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