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Ecofriendly synthesis of silver nanoparticles from leaves extract of *Phyllanthus niruri* (L.) and their antibacterial properties

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ABSTRACT

In recent times, plant-mediated synthesis of nanoparticles has garnered wide interest owing to its inherent features such as rapidity, simplicity, eco-friendliness and cheaper costs. For the first time, silver nanoparticles were successfully synthesized using *Phyllanthus niruri* leaf extract in the current investigation. The silver nanoparticles were characterized by UV–Vis spectrophotometer and the characteristic surface plasmon resonance peak was identified to be 423 nm. The morphology of the silver nanoparticles was characterized by scanning electron microscopy (SEM). The size of the silver nanoparticles was found to be 10-50 nm, with an average size 15 nm. FTIR analysis was done to identify the functional groups responsible for the synthesis of the AgNPs. The antibacterial potential of synthesized AgNPs was compared with that of aqueous extracts of *P.niruri* by well diffusion method. The AgNPs at 50µl concentration significantly inhibited bacterial growth against *A.hydrophila* **(**16 ± 0.09 mm**)**. Thus AgNPs showed broad spectrum antibacterial activity at lower concentration and may be a good alternative therapeutic approach in future.

Keywords: *Phyllanthus niruri,* AgNps, *Aeromonas hydrophila,* Antibacterial Activity.

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INTRODUCTION

Green nanotechnology is an interdisciplinary field for the production of functional nanoparticles of gold, silver, zinc, etc, [1]. Silver nanoparticles (AgNPs) possessed many noteworthy biological roles in the fields like therapeutics (antimicrobial, anticancer, antiparasitic, antidiabetic and antioxidant activities) [2]. Over the past few decades, the use of plants with different applications in medicine and industry has been growing increasingly in the world. In recent years, many environmentally friendly methods have been employed in the synthesis of nanoparticles [4]. The biological methods for AgNPs synthesis using bacteria, fungi, proteins, polypeptides, nucleic acids and plant extracts are simple, nontoxic, affordable and environmentally friendly. These biological methods can be used to generate nanoparticles with acceptable size and morphology [5].

Phyllanthus niruri Linn. Belongs to Euphorbiaceae family and it is a small herb having wide range of medicinal properties, and it is used widely across the world. In Indian ayurvedic and Unani system it is used for Jaundice, ulcers, skin diseases, diabetes, chest pain and urinary complications. Its taste is bitter and acts as astringent and show laxative effect [6]. The extracts of *P.niruri* have a wide range of pharmacological activities like antimicrobial, antiviral, hepato protective, antioxidant, anticancer, anti-inflammatory, antiplasmodial and diuretic[7].To the best of our knowledge and literature survey, *P.niruri* has not been used for the synthesis of silver nanoparticles. In this study, an attempt was made to the green synthesis of AgNPs from *P.niruri* and was characterized. The same was also used to assess their effect on biological systems.

MATERIALS AND METHODS

Plant material extraction

Well matured fresh, healthy *Phyllanthus niruri* leaves were collected from Saliyamangalam, Thanjavur district, Tamilnadu, India and authenticated by professionals in Department of Botany, St. Joseph's College, Tiruchirappalli, India. The herbarium number of the plant is BA 001.The plant materials was washed three times in tap water and two times in distilled water at room temperature during the process all dust and soils are removed and collected plants are dried at room temperature in open air, shadow dried ten days. Then 100 g of coarse powder was crushed dried plant material by using grinder, the soxhlet apparatus were used to plant extraction of 250 ml aqueous using coarse powder and extracts was used to treat further investigation.

Characterization Techniques

UV-Vis spectra analysis

The silver nanoparticles were confirmed by measuring the wave length of reaction mixture in the UV - Vis spectrum of the PerkinElmer spectrophotometer at a resolution of 1 nm (from 300 to 600 nm) in 2 ml quartz cuvette with 1 cm path length.

SEM analysis

The morphological characterization of the samples was done using JEOL Jsm- 6480 LV for SEM analysis. The samples were dispersed on a slide and then coated with platinum in an auto fine coater. After that the material was subjected to analysis.

FT-IR analysis

The characterization of functional groups on the surface of AgNPs by plant extracts were investigated by FTIR analysis (Shimadzu) and the spectra was scanned in the range of 4000–400 cm−1range at a resolution of 4 cm^{-1}. The samples were prepared by dispersing the AgNPs uniformly in a matrix of dry KBr, compressed to form an almost transparent disc. KBr was used as a standard in analysis of the samples.

Anti-Bacterial Study

Muller Hinton Agar plates were prepared and the test microorganisms *A.hydrophila* were inoculated by the spread plate method. Filter paper discs approximately 6mm in diameter were soaked with 50μl of the plant extract, $AgNo_3$ and $AgNp_S$ placed in the previously prepared agar plates. Each disc was pressed down to ensure complete contact with the agar surface and distributed evenly so that they are no closer than 24 mm from each other, center to center. The agar plates were 45 then incubated at 37ºC. After 16 to 18 hours of incubation, each plate was examined. The resulting zones of inhibition were uniformly circular with a confluent lawn of growth. The diameters of the zones of complete inhibition were measured [8].

RESULTS AND DISCUSSION

Synthesis of silver nanoparticles

In order to synthesize silver nanoparticles (SNPs), 10 mL of the leaf extract was mixed with 90 mL of 1 mM silver nitrate solution and heated in a water bath, set at 80˚C for 10 min. A color change from yellow to dark brown designates the formation of colloidal SNPs (Figure: 1) [9].

UV-Vis Spectra analysis:

As shown in figure 2, the appearance of brownish color in the reaction mixture indicates the formation of AgNPs. The change in color of the solution was due to the surface plasmon resonance (SPR) and reduction of silver ions by aqueous extract of *P.niruri* In the present result, the Surface Plasmon Resonance (SPR) of AgNPs produced a peak at 423nm, which suggests the dispersal of silver nanoparticles. The SPR bands are influenced by the size, shape, morphology, composition and dielectric environment of the prepared nanoparticles [10]. Previous studies have shown that the spherical AgNPs contribute to the absorption bands at around 400–420 nm in the UV–visible spectra [11]. These absorption bands were assumed to correspond to the AgNP's extra-fine nature, with relatively small size.

Figure 1: Silver nanoparticles visible observation

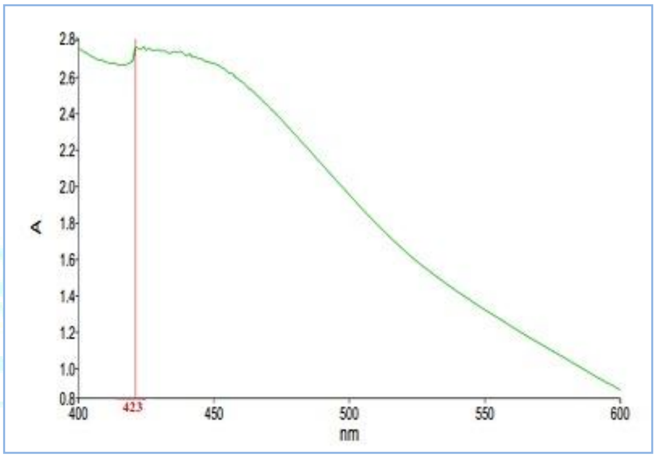

Figure 2: UV-Vis Spectroscopy for silver nanopaticles

SEM analysis of silver nanoparticles

The surface morphology and topography of the SNPs were examined by scanning electron microscopy. Figure 3. Shows high density AgNPs synthesised by the plant extract of *P.niruri* more confirmed the presence of AgNPs. The interactions such as hydrogen bond and electrostatic interactions between the bio-organic capping molecules bond are the reason for synthesis of silver nanoparticles using plant extract [12]. Figure 3 showed that silver nanoparticles are cubical, rectangular, triangular and spherical in shape with uniform distribution. However, on most occasions, agglomeration of the particles was observed probably due to the presence of a weak capping agent which moderately stabilized the nanoparticles [13]. The measured sizes of the agglomerated nanoparticles were in the range 5-50 nm; however, the average size of an individual particle is estimated to be 15 nm. The larger silver particles may be due to the aggregation of the smaller ones.

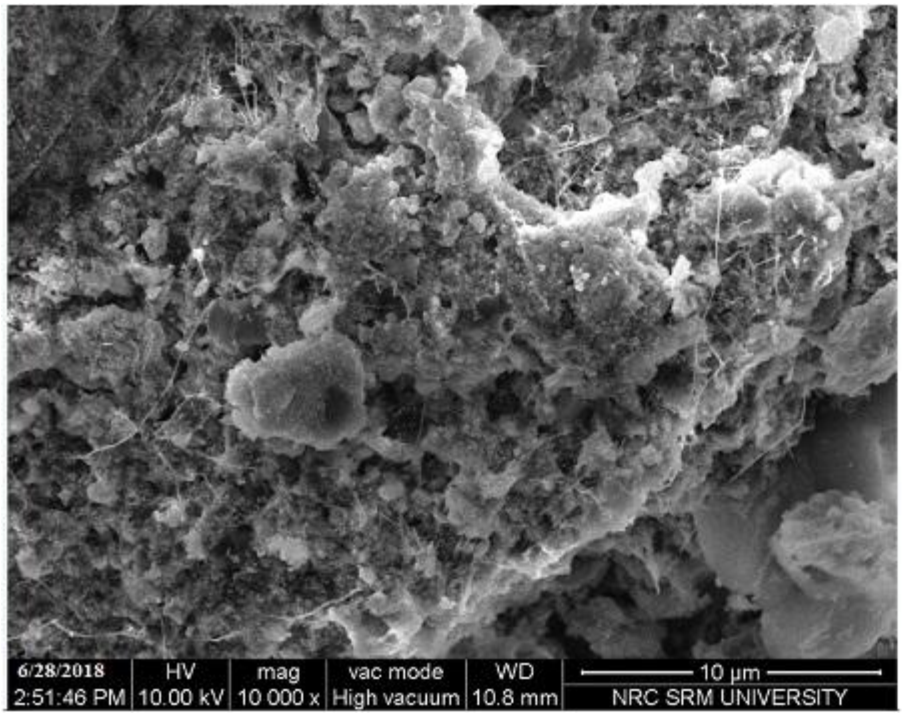

Figure 3: Scanning Electron Microscopy for silver nanopaticles

FTIR analysis

FT-IR spectra taken to detect the major functional groups in plant extract and their possible role in reduction process during the synthesis of AgNPs and stabilization of silver nanoparticles and spectrum peaks were compared with standard values to identify the main biomolecules present in the plant extract. The spectra showed absorption peaks at 3612cm^{-1}, 3498cm^{-1}, 2720cm^{-1}, 1758 cm^{-1}, 1638 cm^{-1},910 cm^{-1} and 610cm^{-1}. The spectra showed broad transmission peak at 3612cm^{-1} and 3498cm^{-1}, which corresponds to hydrogen bonded hydroxyl group (O-H and H stretch) of alcohols and phenols.

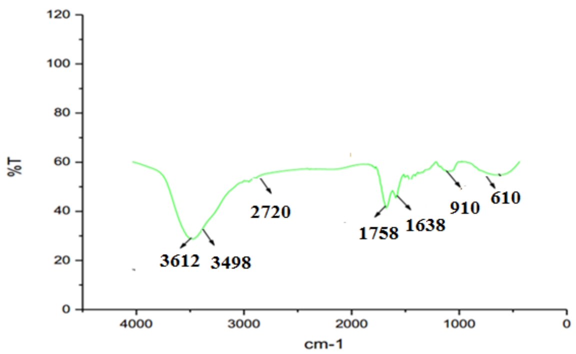

Figure 4: FTIR Analysis for silver nanopaticles

The 2720cm^{-1} peak corresponds to (O–H stretch) carboxylic acids. The 1758 cm^{-1} peak corresponds to (C=O stretch) carboxylic acids. The 1638 peak corresponds to (N–H bend) amines. The 910 cm^{-1} (O–H bend) carboxylic acids. The 610 peak corresponds to (–C≡C–H: C–H bend) alkynes. These bands denote stretching vibrational bands responsible for compounds like flavonoids and terpenoids [14, 15] and so may be held responsible for efficient capping and stabilization of obtained AgNPs. From FT-IR results, it can be concluded that some of the bioorganics compounds from *P.niruri* extract formed a strong coating/capping on the nanoparticles (Figure: 4).

Antibacterial Assay

Antibacterial assay of biosynthesised silver nanoparticles was studied against *A.hydrophila* bacteria using agar disc diffusion method and zone of inhibition is depicted in figure-5 and in Table- 1. The maximum zone of inhibition against *A.hydrophila* (14mm) on normal plant leaves extract. The synthesized silver nanoparticles were tested against selected bacterial pathogens. The maximum zone of inhibition against *A.hydrophila* is 16mm.The silver nanoparticles showed maximum zone of inhibition against fish bacterial pathogen of A.hydrophila than normal plant extract. Silver ions as well as Ag Nps were known to have strong antimicrobial activities [16]. The antibacterial activity of different solutions containing Ag Nps demonstrated that both Gram positive and Gram negative bacteria were inhibited by different solutions with different extents. The results of the antibacterial assay are depicted in Figure.5. These results agreed with previous work carried out by [17-20].

Table 1: Antibacterial activity of $AgNO_3$, AgNPS and leaves extract of *Phyllanthus niruri*

Sample	Concentrations	*A.hydrophila* (mm)
$AgNO_3$	50µl	8 ± 0.03
Plant extract	50µl	14 ± 0.10
AgNPs	50µl	16 ± 0.09

Values were expressed as Mean ± SD. $AgNO_3$ = Silver Nitrate; AgNPs = Silver Nanoparticles

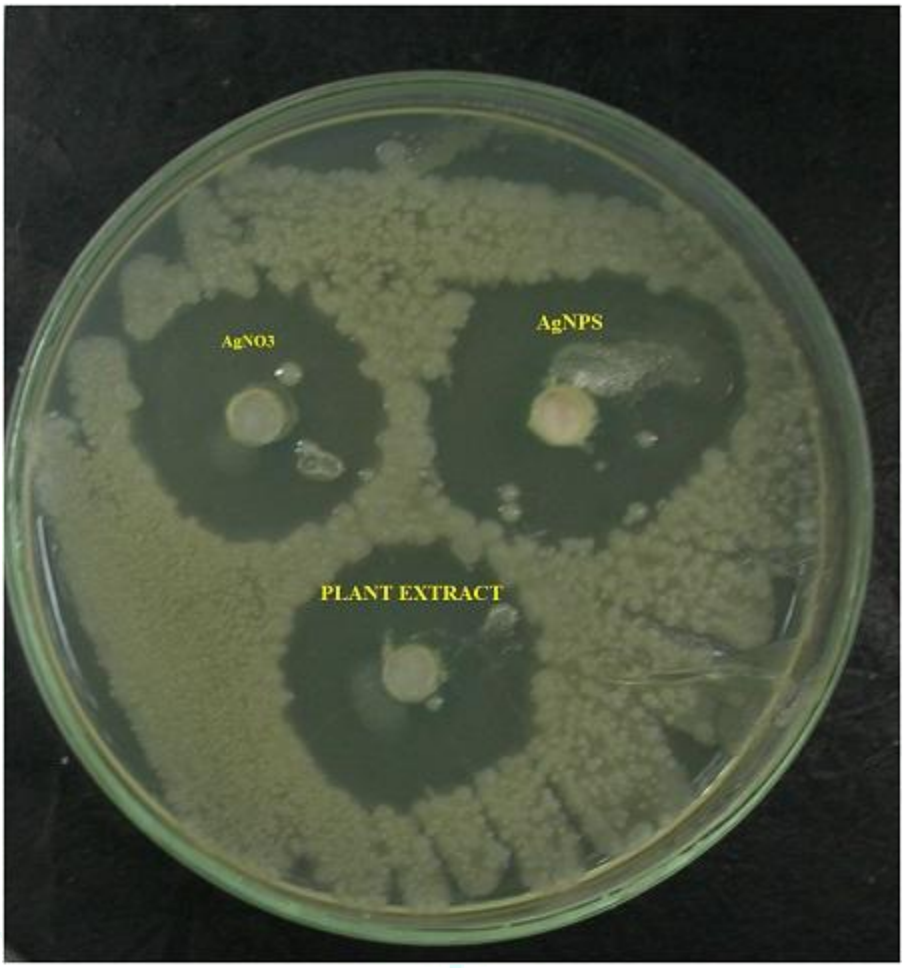

Figure 5: Antibacterial Activity

CONCLUSION

The present study showed a simple rapid and economical rout to synthesize silver nanoparticles. For synthesis of silver nanoparticles through *P.niruri* leaves extract can be effectively used to follow a greener rout. Control over biological synthesis provides particles with good control over size distribution and shape. *P.niruri* leaves extract produced silver nanoparticles have been used in various applications for human being. Further the above silver nanoparticles revealed to possess an effective antibacterial property against *Aeromonas hydrophila*. Silver nanoparticles synthesized via green rout were highly toxic to pathogenic bacteria, hence has a great potential in biomedical application and a potent antibacterial effect too. This green method resulted many advantages such as ecofriendly, low cost and large scale synthesis of silver nanoparticles.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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