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Medicinal Plant Mediated Synthesis of Silver Nanoparticles and their Antibacterial Efficacy

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Abstract : *An environmental friendly approach is employed to synthesize silver nanoparticles. The biomolecules found in plants induce the reduction of Ag+ ions from silver nitrate to silver nanoparticles (AgNPs). In the present study, the extract of dried leaf powder of medicinal plant Phyllanthus niruri is used to synthesize silver nanoparticles. UV-visible spectrum of the aqueous medium containing silver ions demonstrated a peak at 423nm corresponding to the plasmon absorbance of silver nanoparticles. Scanning electron microscope analysis showed different sizes of agglomerated AgNPs. In the colloidal matrix the AgNPs are spherical in shape. AgNPs produced showed good antimicrobial activity against Escherichia coli, Klebsiella pneumoniae Pseudomonas aeruginosa and Staphylococcus aureus.*

Key words: *Phyllanthus niruri, silver nanoparticles, UV-Vis spectroscopy, Scanning electron microscope.*

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Introduction :

Nanotechnology is emerging as a rapidly growing field with its applications in science and technology for the purpose of manufacturing new materials at the nanoscale . (Albrecht *et al.,* 2006) It involves the study of structure at 1 to 100 nanometers (nm) which possess novel properties and functions attributable to their small size. These nanomaterials may provide solution to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine and water treatment (Sharma *et al.*, 2009). Different types of nanomaterials like copper, zinc, titanium, magnesium, gold and silver have come up but silver nanoparticles are currently under exploitation because of their applications as antimicrobials, therapeutics,catalyst and in biomolecular detection. Silver metal nanoparticles can be prepared by both physical and chemical methods (Dhoondia *et al*., 2012). Generally, specific control of shape, size and size distribution is often achieved by varying the synthesis methods, reducing agents and stabilizers (Yeo *et al*., 2003, Chimentau *et al*., 2004). However, most of the techniques are capital intensive and also involve the use of toxic hazardous chemicals. Hence, there is a growing need to adopt the principles of green chemistry to develop an environmentally benevolent biological approach. Recently, the use of biologically mediated silver nanoparticles using green algae (Kumar *et al.,2012*), fungi (Bhainsa *et al.,*2006), bacteria (Dhoondia *et al*.,2012) and plant extracts (Christensen *et al.,*2011) is gaining impetus due to their intrinsic diverse properties like catalysis, optical polarizability, electrol conductivity, antimicrobial activity and Surface Enhanced Raman Scattering (SERS). However, plant mediated synthesis of silver nanoparticles appears to be the best option as the process is simple, efficient, cost effective, safe, and can be suitably scaled up for large scale synthesis. Further, it does not involve harmful chemicals and nanoparticles are more biocompatible and ecofriendly. The synthesis of silver nanoparticles takes place extracellularly and the reaction time has also been reported to be very short as compared to microorganisms. The synthesized silver nanoparticles can be used individually or in combination therapy or synergistic therapy.

The present study investigates medicinal plant mediated biosynthesis of silver nanoparticles using leaf extract of *Phyllanthus niruri*. The herb, *Phyllanthus niruri* is a widespread tropical medicinal plant commonly found in hotter parts of India. The common name is Bhui amla and it belongs to the family Phyllanthaceae. It has many medicinal and health benefits. It acts as protective shield and curative medicine for jaundice, liver cirrhosis and helps to remedy fatty liver. The plant is said to have antifungal, antibacterial, antiviral, antioxidant and hypoglycemic activities.

Materials and methods :

The present research work was conducted in the Department of Industrial Microbiology, Patna Women's College, Patna during the period, July to September, 2014. The plants of *Phyllanthus niruri* (Bhui amla) were collected from College campus in plastic bags and brought to the laboratory for processing. The method given by P Krishnamoorthy and T Jayalaxmi (2012) was followed in this study with modifications. The leaves of Bhui amla were air dried for four days. The dried leaves were ground to a fine powder by using mortar and pestle.

Preparation of crude leaf extract: 5g of leaf powder was taken in a sterile conical flask. 50 ml of double-distilled water was added to it. The mixture was kept for incubation at room temperature for 48 hrs. The cooled mixture was centrifuged at 5000 rpm for 10 minutes. The supernatant was collected for further processing

Synthesis of Silver nanoparticles: 10 ml of aqueous extract of leaves was added to 90 ml of silver nitrate solution containing stabilizing agent. The solution was allowed to react at room temperature. The bioreduced component was monitored by using UV-Vis spectrophotometer (Thermo-Fisher Scientific) periodically.

Characterization of silver nanoparticles: The colour change in reaction mixture was recorded through visual observation. Synthesized silver nanoparticles was confirmed by sampling the aqueous component at different time intervals and the absorption maxima was scanned at the wavelength of 417- 440 nm on UV-Vis spectrophotometer by using distilled water as reference. The SEM analysis was carried out at Osmania University, Hyderabad. The SEM analysis is employed to characterize the size, shape, morphology and distribution of synthesized silver nanoparticles.

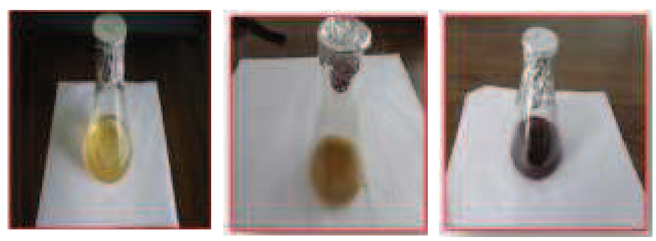
Antibacterial activity : The antibacterial activity of leaf extract mediated silver nanoparticles was performed against pathogenic bacteria *Staphylococcus aureus, Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli* on Nutrient Agar plates using Well diffusion method (Saravanam *et al.,* 2011).

Pure cultures of bacterial pathogens were grown in Nutrient broth at 37ºc for 24 hours. Three wells of 5mm diameter were made in each Agar plate by using a sterile cork-borer. Then 20 µl of the biosynthesized AgNPs solution was loaded in one well and a mixture of 10µl of AgNPs solution + 10µl of antibiotic Tetracycline in the second well using micropipettes. As control, 20 µl of 1 mM $AgNO_3$ solution was poured in the third well. Then the Agar plates were inoculated by spreading the bacterial pathogen to create a confluent lawn of bacterial growth. The plates were incubated at 37ºc for 24 hours. The zone of inhibition in millimeter around each well was measured.

Results and Discussion :

The aqueous extract of *Phyllanthus niruri* was used to synthesized silver nanoparticles. It is well known that silver nanoparticles exhibit yellowish-brown colour in aqueous solution due to excitation of surface plasmon resonance in silver nanoparticles (Mulvaney, 1996). In the present study, the confirmation of the formation of AgNPs and their characterization were done by visual colour change, UV-Vis spectroscopy and SEM studies.

The colour intensity of the synthesized AgNPs increased with the duration of incubation period (Fig 1a-c). A visible color change from Pale yellow to yellow within 10 minutes of addition of leaf broth to the silver nitrate solution indicates the initiation of the formation of silver nanoparticles, which was confirmed by the UV-Vis spectroscopy analysis. The colour changed from yellow to brown within 60 minutes. The reaction was rapid as the dark brown colour appeared within 24 hours. after which there was no significant color change which is evidence for the completion of reduction reaction. Generally, biosynthetic methods are considered as time consuming when compared with chemical methods, such speed in reaction time can be attributed to the secondary metabolites present in the medicinal plant (Chandra, 2013). Similar findings were reported by Ramteke *et al.*, (2012) and Christensen *et al*., (2011).



(a) At 0 hr-Pale yellow (b) At 1 hr- Light brown (c) After 24hrs –Dark brown

Fig. 1 (a-c): Colour change in reaction mixture

UV-Vis spectral analysis: The reaction of silver nitrate solution with medicinal plant *Phyllanthus niruri* leaf extract was optically measured using UV-Vis Spectrophotometer (Thermo-Fisher scientific) in the different wavelength range of 417 nm to 440 nm. The data shows a strong broad peak at 423 nm which corresponds to the absorbance of silver nanoparticles (Fig.2.). According to Mie theory, only a single Surface Plasmon Resonance (SPR) band is expected in the absorption spectra of spherical nanoparticle. In the present study SPR band indicates spherical shape of silver nanoparticle (Rajesh *et.al*., 2009).

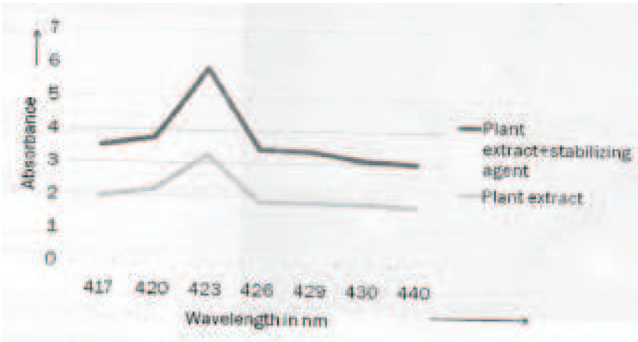


Fig. 2. UV-Vis spectra of silver nanoparticles synthesized by plant mediated method (After 24 hrs).

Scanning Electron Microscopy analysis: SEM micrograph presented in the Fig.3. a & b show the SNPs distributed in the matrix. This embedding of the NPs in matrix could be due to the presence of the primary or the secondary metabolite present in the plant extract . Fig.3.a shows the agglomerated SNPs distributed in the colloidal matrix which probably a protein.Fig.3.b shows different sizes of agglomerated silver nanoparticles.

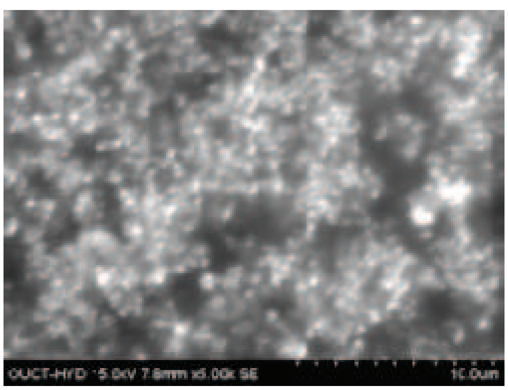


Fig. 3. a. Scanning electron microscopy image of AgNPs derived from leaf of the medicinal plant *Phyllanthus niruri*

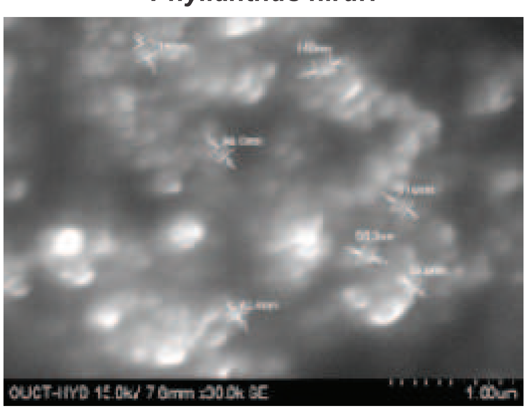
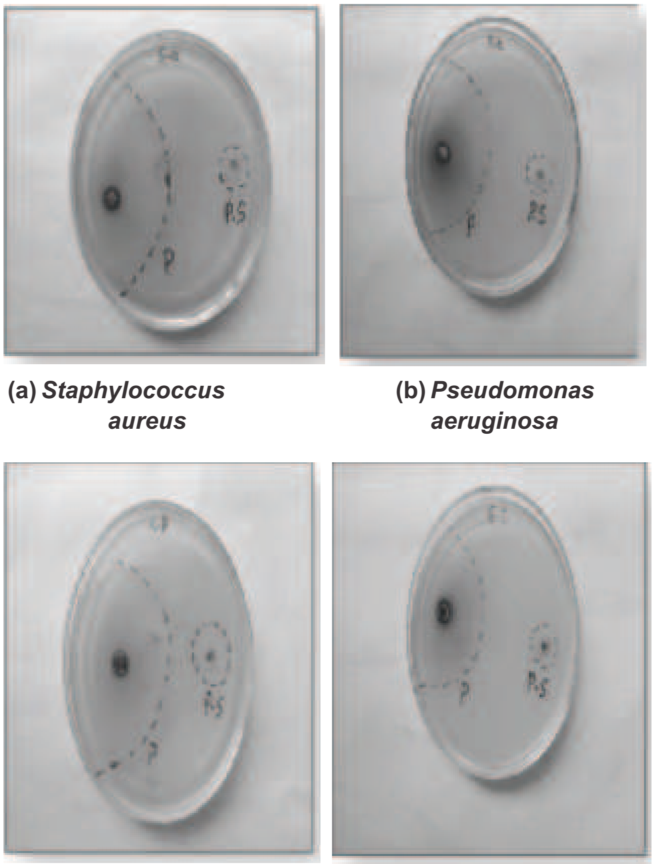


Fig. 3.b Scanning electron microscopy image of the surface morphology and particle distribution in the matrix.

Antibacterial activity of silver nanoparticle: The antibacterial activity of *Phyllanthus niruri* mediated silver nanoparticles was performed against pathogenic bacteria *Staphylococcus aureus* (Fig.5.a), *Pseudomonas aeruginosa* (Fig.5.b). *Klebsiella pneumoniae* (Fig.5.c) and *Escherichia coli* (Fig.5,d) using Well diffusion method. Table 1 shows the zones of inhibition (mm) around the wells with leaf extract mediated synthesized nanoparticles and AgNPs + antibiotic Tetracycline. The clear zone shows the bactericidal effects. However, the bactericidal effect of silver nanoparticle was less in Gram +ve bacteria as compared to Gram –ve bacteria (Table 1). This variation was probably due to the differences in the cell wall composition of the two groups of bacteria. The cell wall of Gram +ve bacteria is made up of thick peptidoglycan layer thus preventing the penetration of NPs. On the other hand, nanoparticle easily penetrated the cell wall of Gram-ve bacteria and affected the permeability of the membrane. No zone of inhibition was observed for $AgNO_3$ solution alone. Coupling of inherent property of *Phyllanthus* with that of SNP has proved to be beneficial. Though AgNPs cannot be compared with antimicrobial activity of antibiotics nonetheless antibiotic resistant microorganisms can easily be killed by AgNPs. Thus, the *Phyllanthus niruri* leaf extract mediated synthesized NPs can be used singly or in combination with antibiotics in combination therapy. This will help to combat the multidrug resistant bacteria (Chanda *et al.*, 2013).

Table 1: Diameter of zone of inhibition of biosynthesized AgNPs and AgNPs + antibiotic against pathogenic Gram +ve and Gram –ve bacteria.

Bacterial strain	Synthesized AgNPs+ ANTIBIOTIC (1)	Synthesized AgNPs (2)
Staphylococcus aureus (Gram+ve)	18	10
Pseudomonas aeruginosa (Gram+ve)	21	12
Klebsiella pneumonia (Gram-ve)	15	10
Escherichia coli (Gram-ve)	15	10



(a) *Staphylococcus aureus*　**(b) *Pseudomonas aeruginosa***

(c) *Klebsiella pneumoniae*　**(d) *Escherichia coli***

Fig.5 (a-d). Antibacterial activity of AgNPs synthesized by leaf extract of *Phyllanthus niruri.*

Conclusion :

The selected plant extract of *Phyllanthus niruri* has potential for the production of nanoparticles and in our study it was found to act as reducing mediator for silver nanoparticles. The synthesis was found to be efficient in terms of reaction time as well. The synthesized silver nanoparticles in combination with antibiotic tetracycline has shown maximum zone of inhibition, therefore, it may be considered in combination therapy.

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