Biosynthesis: Physical And Chemical Characterization Of Silver Nanoparticles Using Leaf Extract Of Pergularia Daemia And Its Antimicrobial Activity

K. Sowmiya, J. Thomas Joseph Prakash

Abstract: The recent study deals with the synthesis and characterization of silver nanoparticles bounded to pergularia daemia plant extract silver nanoparticles (PD @ Ag-Nps) was characterized by PXRD, UV-Vis, FESEM and EDX, FTIR, DLS. The highly stable PD @ Ag-NPs was found promising as antibacterial and antifungal agents when tested against human pathogens Escherichia coli, Pseudomonas, Staphylococcus aureus and Candida albicans, Candida vulgaris respectively. Conclusion, the antibacterial and antifungal properties of the synthesized AgNps from pergularia daemia act as major therapeutic drug for microbial infections disease and other health associated disorders.

Index Terms: Antibacterial Activity, Antifungal Activity, Pergularia Daemia, Silver nanoparticles.

1. INTRODUCTION

Medicinal plants have served as a major source of drugs for centuries. India has a rich heritage of knowledge on plant-based drugs both for use in preventive and curative medicine from ancient times with mention of these in Ayurveda, Siddha, Homoeopathy and other reforms. Approximately 88% of the global population uses plant-based drugs or medicines as their first line of defense for maintaining health and combating many diseases [1,2]. The increasing failure of chemotherapeutics and development of bacterial resistance to currently available antibiotics has necessitated the search for novel and effective antimicrobial compounds from natural resources. Plant-derived medicines have gained immense importance in recent years because of their potential efficacy, and no side effects. Plants are a rich source of valuable secondary metabolites such as tannins, terpenes, terpenoids, alkaloids, flavonoids, coumarins, polysaccharides, glycosides, gum and phenols that are used by plants as defense mechanisms against invasion by many microorganisms, insects, and herbivores [3,4]. Plant-derived medicines are believed to be more acceptable to the human body in comparison to modern synthetic drugs[5]. Thus there is a need to derive the maximum benefit from the medicinal plants as well as the traditional system of medicine for providing adequate health services to the people in rural areas. The extracts of medicinal plants have wonderful antibacterial, antifungal, and antioxidant activities [6]. These activities can be improved many folds by a blend of modern day scientific inputs e.g. synthesizing nanoparticles.

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including medicine, pharmacology, sensing devices, micro-electronics and drug delivery etc [7] . Among various nanoparticles, AgNPs are one of the most vital and fascinating nanomaterials that are involved in a range of biomedical applications and have been reported to possess antibacterial, antifungal, anti-viral, anti angiogenic and anti-inflammatory properties(8). Nanoparticles can be synthesized via chemical, physical and photochemical routes, carrying many threats to the environment as well as human beings(9,10). Nanoparticles can be broadly grouped as organic nanoparticles which include carbon nanoparticles (fullerenes), inorganic nanoparticles including magnetic and noble metal nanoparticles (like gold and silver) and semiconductor nanoparticles (e.g. titanium oxide and zinc oxide). Inorganic metal nanoparticles (Gold and silver) are of increasing interest since they provide superior material properties with functional versatility. Due to their advantages over available chemical imaging drug agents besides size features, inorganic particles have been used as potential tools for medical imaging as well as for treating diseases(11). In recent years, green synthesis of nanoparticles using different plant parts/components is receiving considerable attention because of its simplicity, low cost, safety, and eco friendly nature. A number of plant extract mediated green synthesis of AgNPs have been reported in the literature(12). Plant extract may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles. In the present study, AgNPs were synthesized using a leaf extract of pergularia daemia. Pergularia Daemia family Asclepiadaceae is a perennial double herb that grows extensively on the roads of India and tropical and subtropical regions. The whole plant has high medicinal value and has traditionally been used to treat a variety of diseases in humans. Some of the folk used this plant to treat jaundice, anthelmintic, laxative, anti-pyretic, expectorant and pediatric diarrhea [22, 23]. The phytochemical plant has been studied for carbenolides, alkaloids, triterpenes and saponins. The plant has demonstrated many pharmacological functions such as anti-inflammatory, hepatoprotective, anticancer, antidiabetic, antioxidant, anti-bacterial, antifungal, analgesic, antiinflammatory and central nervous system

antidepressants. This review highlights current information on the various pharmacological properties of phytochemistry and pergularia daemia in particular, which may provide an incentive to evaluate the plant as a clinical agent.

2 MATERIAL AND METHOD

2.1 Plant Material Sourse

Plant material leaves of pergularia daemia (figure 1) were collected from trichy, Tamilnadu.

Fig.1 *Pergularia daemia Leaf*

2.2 Preparation of dried extract

The leaves weighing 500 grams were full the dishes were washed with distilled water and dried for 1 month under the shade. Rather than being crushed by it from the pest motor and those 20 g were weighted and crushed into 100 ml of sterility is suppressed the distilled water , boiled for 20 minutes and filtered through Whatman No. 1 filter paper 2 times. The juice was stored at 4°C

2.3 Synthesis of Silver Nanoparticles

In a typical reaction procedure, 6 mL of the pergularia daemia leaf extract was added with 120ML of silver nitrate solution (1 Mm). A change of color absorbed is dark brown to pale yellow (Figure 2) , which shows the formation of silver nanoparticles. The nanoparticles were then centrifuged at 12,000 rpm (Remi 12c). The particles are collected, dried, and washed twice with alcohol to remove impurities. It is then placed in the drying oven. The resulting powder is collected and characterized.

Fig.2 *Colour changed at brown to pale yellow*

2.4 Characterization of silver NPs

The following techniques were applied to characterize the synthesized silver NPs.

UV-Vis Spectra Analysis

A set of silver nanoparticles presented reducing, the respective metal ion solution the juice of the leaves is easily found presented by UV-Vis Spectroscopy. Absorption the amount of spectral extraction of leaves and the metal concentration was measured using a Perkin-Elmer Lamda-45 Spectrophotometer in the range of 200-400 nm.

FTIR Analysis

The functional group was determined using fourier transform infrared spectroscopy .

DLS Analysis

Dynamic light scattering was used to determine the size distribution and average size of the synthesized Ag NPs

PXRD Analysis

The features and size of the manufactured silver NPs were determined using the XRD.

FESEM Analysis

The morphological features of the silver NPs synthesized from the pergularia daemia extract were quickly calculated using FESEM with a voltage of 20 – 25 kv.

EDAX Analysis

Energy Dispersive X-Ray analysis (EDX) was used for the determination of the composition by BRUKER instrument.

Anti bacterial Activity

Antifungal ActivityCultural mediaTo investigate the antibacterial activity of silver nanoparticles, one gram of positive bacterial strains was staphylococcus aureus (MTCC 96). Two gram-negative bacterial strains Escherichia coli (MTCC433) and Pseudomonas aeruginosa (MTCC1688) were prepared as test organisms. All strains were purchased from Microbial Culture and Collection (MTCC), Chandigarh, India. Bacterial strains were cultured at 37ºC and maintained on a gradient of nutrient agar (Difco, USA) for 4ºC. The medium used for the mildew test is Sabou Rot's Dextrose Agar / Broth Hi Media Pvt. Bombay, India. Inoculum

Fungal strains were individually vaccinated for 6 h in Sabou Rat's dextrose broth, and the suspensions were tested to provide approximately 10^5 CFU / ml. Fungal strains are used

Candida albicans (MDCC-3498) and Candida vulgaris (MDCC-227) used in the study were purchased from National Chemical Laboratory (NCL), Pune, Maharashtra, India.

3. Results and Discussion

3.1. UV-vis spectrum Analysis

Silver nitrate was confirmed using UV-Vis spectra analysis. AgNPs are free electrons, leading to a surface plasmon resonance (SPR) absorption band, due to integrated vibration of electrons in metal nanoparticles with resonance the wave of light. Surface plasmon resonance the spectra for AgNPs are obtained at 265 nm (Figure 3) with pale yellow . A quick increase a set of nanoparticles was observed with an increase in reaction time. The

concentration of the juice also plays a role the main role is responsible for it synthesis of symmetric nanoparticles. The can be synthesized by metal nanoparticles reduces metal ions using some chemical molecules, in biosynthesis, it is believed natural substance extract acts agents that reduce the generation of metal nanoparticles.

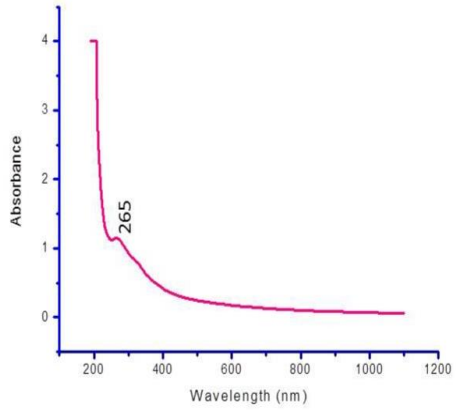


Fig.3 *UV-Vis spectrum of AgNPs Using Pergularia daemia Leaf leaf extract*

3.2. FTIR Analysis

The presence of various functional groups in the molecules responsible for the biological decomposition and stabilization of AgNPs was determined by FT-IR analyzes. To identify functional groups, the observed severe bands were compared with standard values. It has been determined that AGNPs show spectra at wavelengths of 3417, 2959, 2924, 2883, 2427, 1818, 1324, 1077, 826, 723, 597 and 540 cm^{-1} by reducing leaf extract. It was determined that the vibrations at the wavelength of 3417 cm^{-1} originated from the -OH groups in the saponin structure. These bonds may be due to the extension of -OH in proteins, enzymes or polysaccharides in the extracts [24]. The small band at 2959 cm^{-1} was due to the extension of the -CH alkanes. A strong peak at 2924cm^{-1} indicates the bending vibrations of the set amide I group and suggested the possibility of binding AgNPs with the proteins found in the extract. The absorption peak located at 2883 cm^{-1} represents the NH curve. The presence of bands at 2427 and 1818 cm^{-1} is associated with the C – H bond stretching. The band at 1324 and 1077 cm $^{-1}$ caused the C-O stretching vibrations of the carboxylic acid group. A peak of about 826 and 723 cm^{-1} was assigned to the aromatic class. The small peak at 597 cm^{-1} indicates the C-O stretching of the esters or the C-N stretching of the amines found in the extracts. Stress oscillations are observed in the C-O groups and C-C groups at the wavelength of 540 cm^{-1} (Figure 4). Similar scissors and bending vibrations were observed in leaf extracts . It has been suggested that the mechanism of formation of AgNPs by FT-IR analysis may be due to the reduction of Ag + ions associated with the oxidation of phenolic components of polyols [25]. Biological elements bind to these functional groups to metal salts and interfere with the reduction of their nanoparticles [26].

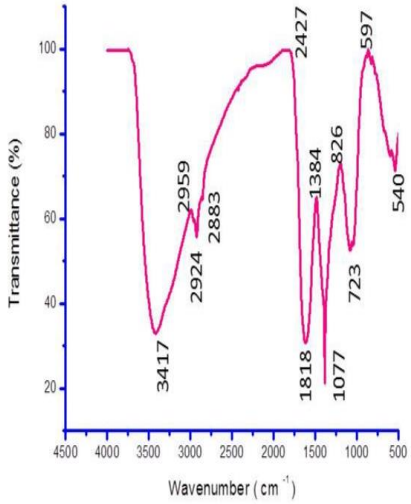


Fig.4 *FT-IR spectrum of AgNPs Using Pergularia daemia leaf extract*

3.3 PXRD Analysis

PXRD Method of Powder Modeling of Phyto Fabricated SNPs the studied plant structure exhibited peaks at 38, 44, 64 and 77. These 2θ values represent 111, 200, 220 and 311 features of silver, respectively confirmed the‘‘face centered cubic’’ (fcc) crystalline structure of metallic silver (fcc) (Figure 5). There are values agreement with JCPDS (Joint Committee on Powder Diversity Standard) File Number 04-078. Diffraction patterns of fcc crystals characterized by the presence of odd or even equal numbers of Miller codes [27]. Besides the peaks due to silver, there are two extra peaks with edge intensity. These peaks may be attributed to proteins covered with SNPs [28]

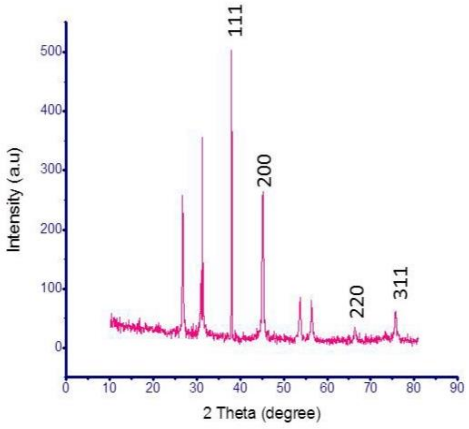


Fig.5 *PXRD pattern of AgNPs synthesized using supernatant of Pergularia daemia leaf*

3.4 DLS Analysis

The particle size of the integrated nanoparticles was determined using the dynamic light scattering (DLS) calibration technique, which is used to determine the particle size by random changes in the intensity of the scattered light from a solution. The DLS diagram of the pergularia daemia Extract revealed that the particle size of

AgNPs was in the range of 10– 100 nm as shown in Figure 6 .

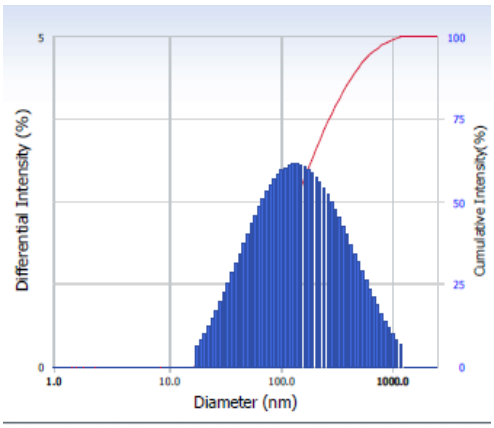


Fig.6 *DLS spectra of silver -Nanoparticles*

3.5 FESEM Analysis

The FESEM image of the synthesized silver nanoparticles is shown in Figure 7. FESEM Micrograms clearly depict that the coordinated silver nanoparticles are spherical and scatter well without any aggregation. Larger particles form a chain like structure and are connected to each other in a synchronized way. The higher magnification of the image shows the upper outer layer of the nanoparticles, which may be due to the phytochemicals and alkaloids of the pergularia daemia surrounding the nanoparticles and acting as the capping agent for the nanoparticles. The presence of these capping agents around the aggregation of nanoparticles has been reduced. FESEM image shows a size distribution of nanoparticles size range 30-45 nm (Figure 7) .

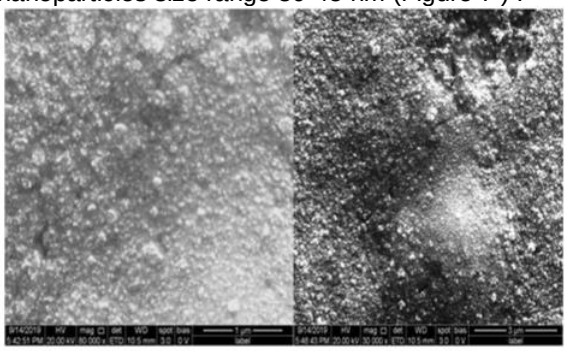


Fig.7 *The typical FESEM image of the silver nanoparticle of Pergularia daemia leaf*

3.6 EDX Spectroscopy Analysis

To better analyze the underlying composition of the phytosensitized SNPs were loaded onto specimens of brass study instead of carbon. EDX analysis of the sample showed the presence basic silver, carbon, oxygen, chlorine, nitrogen . There may be elements like chlorine and nitrogen the sample-made brass. Stock the silver element is phytosensitized SNPs, carbon, and oxygen can be assigned to biomass compounds in the shell. Basic details the composition and their atomic percentages are tabulated interpolation. The EDX analysis showed a peak in the silver region plant structure ensures the formation of silver nanoparticles (Figure 8). The optical absorption peak is found approximately 3 keV, which is common for absorbing metallic silver nano crystallites

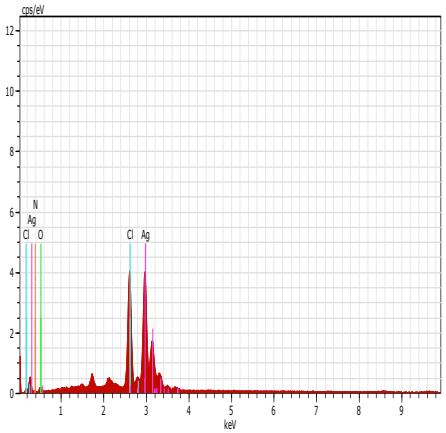


Fig.8 *The EDX of silver nanoparticles of Pergularia daemia leaf*

3.7 Antibacterial activity of silver nanoparticles (disc diffusion method)

The antibacterial activity of silver nanoparticles was determined using the disk diffusion method. Petrideshes (60 mm in diameter) were prepared with Muller Hinton agar and vaccinated with test organisms. A sterile disk of six millimeters wide was inserted into 10 µl of different samples, respectively. The prepared discs were placed on the top layer of agar plates and left for 30 min at room temperature for mixing. Positive control was prepared using 10 µl of amoxicillin fixed antibiotic disc. Foods were incubated at 37ºC for 24 h and the inhibition zone was recorded in millimeters and the test was repeated twice. The results of anti-bacterial activity of various samples by disk diffusion method were tested against pathogens (Table 1). The model showed growth inhibitory activity against D. Escherichia coli (7 mm). All four bacteria in the C sample exhibited antibacterial activity, but it was more susceptible against Pseudomonas aeruginosa (4 mm) and Staphylococcus aureus (5 mm). However, crude extract and synthesized nanoparticles showed excellent preventive action against pathogens (Figure 9).

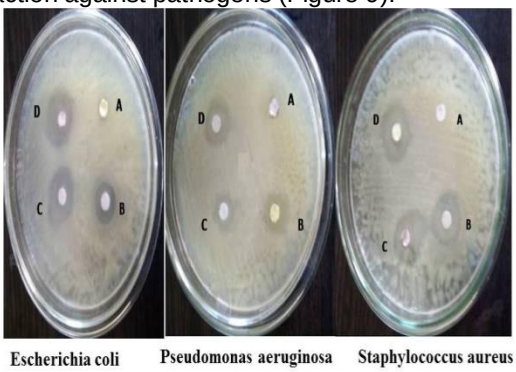


Fig.9 *Antibacterial image of silver nanoparticles*

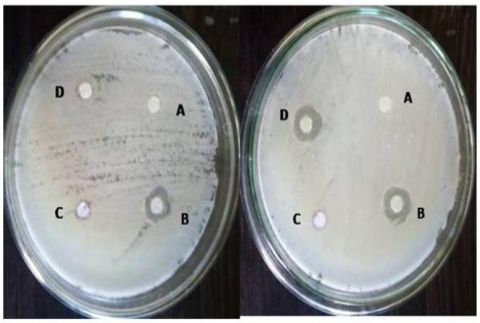
Samples	Concentrations (µl/ml)	Organism / zone of inhibition (mm)		
		Escherichia coli	Pseudo Monas aeruginosa	Staphy Lococcus aureus
A(Amoxicillin)	10	0	0	0
B(Zinc	10	8	8	8

Oxide)				
C (Plant extract)	10	6	3	4
D(Nano particles)	10	7	4	5

Table 1. *Antibacterial results for $AgNO_3$ nanoparticles*

3.8 Antifungal Activity

Determination of the mildew activity of the specimen using the disk diffusion method Petrids (60 mm in diameter) was prepared with Sabou Ratin dextrose agar (SDA) and inoculated with test organisms. 10 µl of six-millimeter-wide sterile disk was inserted into various samples. The prepared discs were placed on the top layer of agar plates and left for 30 min at room temperature for mixing. Positive control was prepared using 10 µl of fluconazole using a standard antibiotic disc. Foods were incubated at 37ºC for 24 h and the inhibition zone was recorded in millimeters (Figure 10). Table 2 shows the results of the fungicide susceptibility test against different samples and test organisms. As a result, the sample D was most effective and demonstrated the highest activity against Candida albicans (2 mm inhibition zone).



Candida albicans Candida vulgaris

Fig.10 *Antifungal image of silver nanoparticles*

Samples	Concentrations (µl/ ml)	Organism / zone of inhibition (mm)	
		Candida albicans	Candida vulgaris
A(Fluconazole)	10 µl	0	0
B (Zinc Oxide)	10 µl	8	8
C(Plant extract)	10 µl	2	2
D(Nanoparticles)	10 µl	2	4

Table 2*.Antifungal results for $AgNO_3$ nanoparticles.*

4 CONCLUSION

Pergularia daemia plant was used for the biodegradation of silver nanoparticles. Biological systems always find a better approach compared to chemicals and physical systems because they are environmentally friendly and economical. Pergularia daemia had the maximum production of nanoparticles in 48 hours by biochemical method. pergularia daemia plant has a maximum of Ag NPs at pH 6.5, AgNO3 concentration of 1 mM. The UV-Visible spectra has conformed the reduction of Ag^+ ions at 265nm.The mean size of Ag NPs was found to be 30–45 nm by FESEM and PXRD peaks showed that the structure of the nanoparticles was FCC in nature. The FTIR method revealed the presence of silver nanoparticles with nitrate reductase enzymes on the surface of silver nanoparticles. EDAX results confirmed the presence of silver nanoparticles. The zone of inhibition experiments demonstrated the presence of an increase in the stability of the nanoparticles.

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