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BIOSYNTHESIS OF SILVER NANAOPARTICLES FROM LEAF EXTRACT OF COCHLOSPERMUM RELIGIOSUM AND THEIR ANTIFUNGAL EFFICACY

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ABSTRACT : the biosynthesis of SNPs is not only inexpensive but also environmentally friendly with little or no side effects. In this study, the biosynthesis of silver nanoparticles and their activity against pathogens were carried out. SNPs were rapidly produced using leaf extract of the medicinal plant Cochlospermum religiosum and the formation of nanoparticles was observed within two minutes. The results recorded by UV-Visible spectroscopy and Atomic Force Microscope analysis support the biosynthesis and characterization of silver nanoparticles. The UV-Visible spectrum has a peak at 260 nm and the difference in the size of spherical SNPs varies from 40 to 100 nm. In addition, the synthesized SNP showed effective inhibitory activity against pathogens Aspergillus niger, Aspergillus flavus, Fusarium, Curvularia and Rhizopus. They are very toxic to Rhizopus followed by Aspergillus flavus, Aspergillus niger and Fusarium. Moderately toxic to Curvularia.

KEY WORDS: Antibacterial activity, Cochlospermum religiosum, phytosynthesis, silver nanoparticles.

1. INTRODUCTION :

Plants are the oldest form of medicine known to man. Plants have been used by all cultures throughout history. They are an important part of the development of modern civilization. Ancient man looked to and enjoyed the variety of plants available to him. These plants provided food, clothing, shelter, and medicine. Cochlospermum religiosum (L) Alston is a small sparsely branched tree native to arid regions of India. The flowers are large and bright golden yellow. The seeds are covered with soft hairs and commonly called as Yellow Silk Cotton. A highly beneficial plant used in India since ancient times to treat many ailments. The gum of this tree is used as a sedative, tranquilizer and to treat gonorrhea, syphilis and asthma. Application of stem bark paste can heal the bone fractured areas (1). Herbalists sell the bark under the name Jalajamini as a medicine against diabetes. Nanoparticles in ionic form have been synthesized to evaluate the efficacy of medicinal plants. Nanoparticles obtained from silver, gold, platinum and semiconductors can be successfully used to transport small particles such as drugs. Nanoparticles are highly effective against microbes due to their antiinflammatory properties because their large surface area allows effective interactions (2). Due to the benefits of biomoleculesfound in plants, drugs are more effective when mixed with plant extracts. SNPs can also be prepared by chemical methods, but compared to biosynthetic SNPs they are expensive, pollute the environment, are time consuming and have side effects. AgNPs are believed to have broad-spectrum and high antimicrobial activity, killing many organisms including bacteria, viruses and fungi even in very small amounts (3). Silver is known to be toxic to many small organisms, and therefore silver combinations are widely used for antibacterial purposes (4). Silver nanoparticles are an important innovation in nanotechnology due to their high stability and low chemical reactivity compared to other metals (5).

2. MATERIAL AND METHODS :

The leaves of Cochlospermum religiosum were collected from Tirumala hill and dried in shade for fifteen days. The dried leaves were powdered. Add 1 Mm silver nitrate to the plant extract to obtain 200 ml. of the final solution and centrifuged at 18000 rpm for 25 minutes. The product was stored at -4°C. Heat the supernatants at 50°C to 95°C. A change in the colour of the solution was observed during heating. The reduction of pure Ag^{2+} ions was monitored by measuring the UV-Visible spectrum of the reaction medium using Systronic 118 UV-Visible spectrophotometer after five hours of dilution of a small amount in distilled water. Films were prepared on carbon coated cupper grid and samples were analyzed by AFM. Potato dextrose agar plates were prepared, sterilized, and solidified, and after solidification the fungal culture was steaked on to the plates. Sterile disks were placed in agar plates with silver nanoparticles solution (10 lg/ml) and incubated for 7 days. After 7 days, the zone of inhibition was measured by MIC Scale and the results were tabulated.

3. RESULTS AND DISCUSSION :

Light yellow or yellow aqueous extracts of Cochlospermum religiosum leaves showed to colour change to dark brown within 2 minutes when silver nitrate solution was added **(Fig-01)**. The change in the colour of the extract to dark brown indicates the formation of silver nanoparticles. This property is called surface plasmon resonance of silver (6). The duration of colour change and the thickness of the colour vary from plant to plant (7). The reason may be the large change in SNPs formation or the presence of H^+ ions which decrease the amount. It is known that SNPs appear brown in aqueous solution due to excitation of Surface Plasmon Vibrations in silver nanoparticles (8). Silver nitrate is used as reducing agent because silver has special properties such as good electrical property, catalytic properties and chemical stability. When exposed to herbal extracts, mercury containing silver ions in the solution are reduced, leading to the formation of silver hydrosol. The synthesis of SNPs was confirmed by measuring the UV-Visible spectrum from the reaction medium.

UV-Vis Spectorphotometer analysis: The UV-Visible spectrum of the silver nano colloidal solution synthesized from Cochlospermum religiosum leaves is said to have strong absorbance peaks at 360 nm and 440 nm respectively **(Fig-01)**, and the peaks are broadened ie poly-dispersed. Silver nanoparticles synthesized using Clerodendrum inerm leaf extract showed absorbance between 200-400 nm, Euphorbia hirta showed absorbance peak at 380 nm (9) and Svensonia hyderabadensis showed absorbance peak at 390 nm (10). In many cases like Eucalyptus hybrid (11), Acalypha indica (12), Nelumbo nucifera (13), Solanum torvum (14), Helianthus annus (15) and Cassia auriculata (16) silver nanoparticles synthesized by using leaf extracts showed the absorbance peaks between 400 and 450 nm. The weak absorption peaks at shorter wavelength is due to many organic compounds known to interact with silver ions (17). It is generally believed that UV-Visible spectroscopy could be used to analyze size and shape-controlled nanoparticles in aqueous solutions (18). Silver nanoparticles contain free electrons, which produce SPR absorption band, due to the vibration of electrons and light wave resonance of metal nanoparticles (19). Some metal nanoparticle dispersions exhibit unique signals or peaks due to the excitation of plasma resonances. The width of the peak is a good indicator of the nanoparticle size. As the particle size increases, the peak becomes narrower the band width decreases and the band intensity increases.

Morphological characterization of SNPs: Atomic Force Microscopy (AFM) was used for the morphological characterization of silver nanoparticles. The shape and size of silver nanoparticles were observed in detail using AFM. AFM analysis of Cochlospermum religiosum leaf extract showed the formation of spherical silver nanoparticles with diameters ranging from 40 to 100 nm. The 3D figure shows that the silver nanoparticles had a maximum size of 68.77 nm. **(Fig-02)**. AFM analysis of Citrulus colocynthus leaf extract showed silver nanoparticles with a size of 31 nm (20) and Lemon leaf extract with a size of 50 nm (21). The size of silver nanoparticles synthesized from Glycine max leaf extract varied between 25 and 100 nm (22) and the size silver nanoparticles synthesized from Moringa olifera leaf extract varied between 5 and 80 nm (23). But Parthenium leaf extract silver nanoparticles are irregular in shape with 30 to 80 nm and average size 50 nm (24) (25). Many proteins especially cell wall bound enzymes with amino groups are responsible for the synthesis of silver from various plant species (26).

The stability of silver nanoparticles can be attributed to the production of silver electride which forms a thin layer on the surface of the reaction mixture. The silver electride can convert silver into silver nanoparticles. It is believed that the amount of protein coating the silver nanoparticles allows for controle of size and shape, limiting the aggregation of the particles.

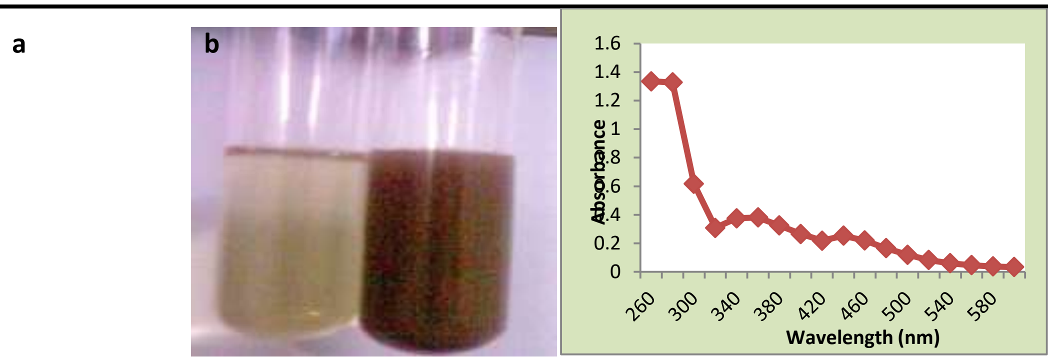


Fig-01: Synthesis of SNPs from leaf

a) Aqueous extract, b) Treated with $Ag(NO_3)_2$, c) UV-Vis Spectra

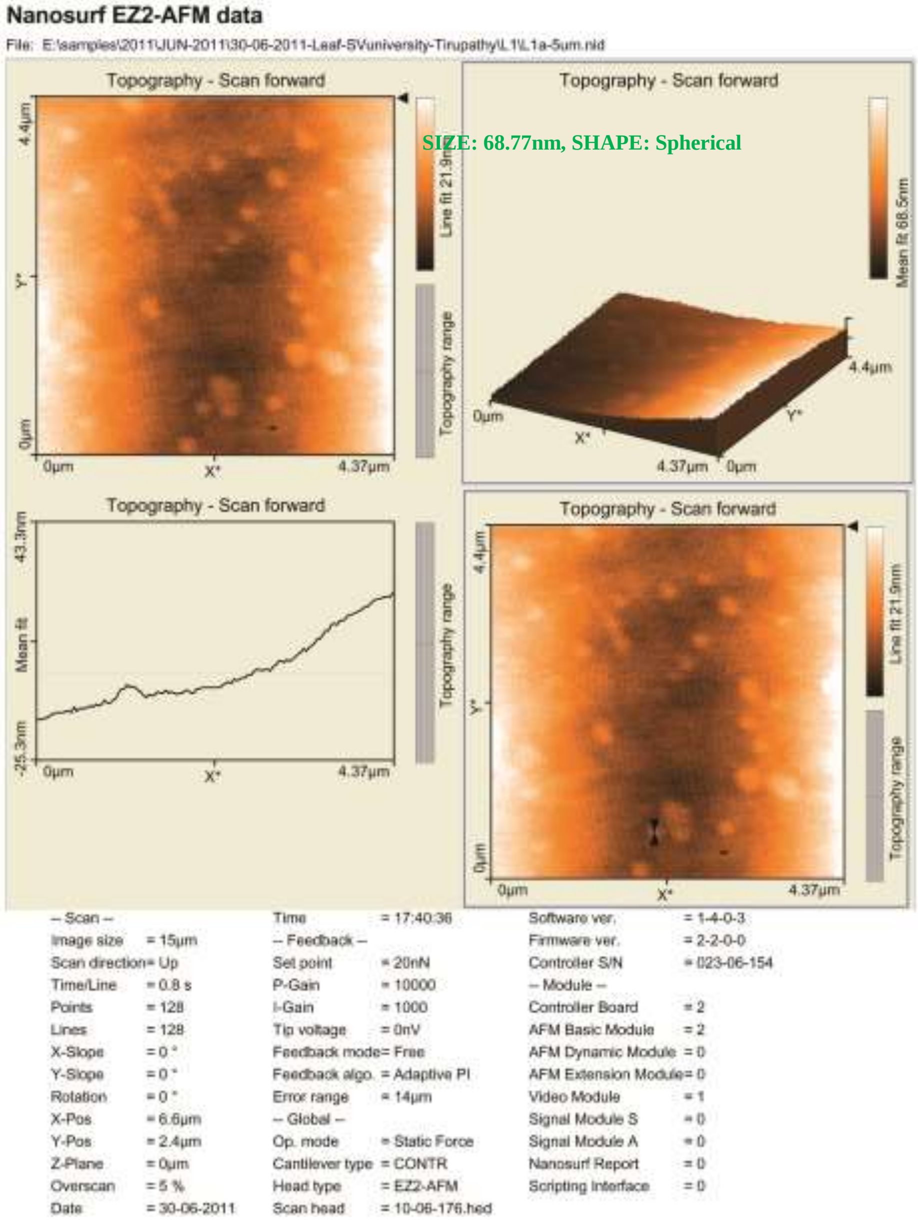


Fig-02: AFM image of leaf SNPS

Antimicrobial activity: this study uses disc diffusion to study the antimicrobial activity of silver nanoparticles against various diseases such as black yeast, yellow yeast, Fusarium, Curvularia and Rhizopus. Aqueous extracts of Cochlospermum religiosum show broad spectrum antimicrobial activity. It represents the diameter of inhibition zone with SNPs around each disc containing 10 µg of SNPs solution **(Table-01)**.

The silver nanoparticles synthesized from the leaf of Cochlospermum religiosum showed effective inhibitory activity against Rhizopus followed by Aspergillus flavus, Aspergillus niger and Fusarium and minimum inhibition zone was observed against Curvularia **(Fig-03).**

In comparison silver nanoparticles are more potent than plant extract. Since the standard is pure chemical and contains active principle its activity is slightly higher than silver nanoparticles **(Graph-01)**.

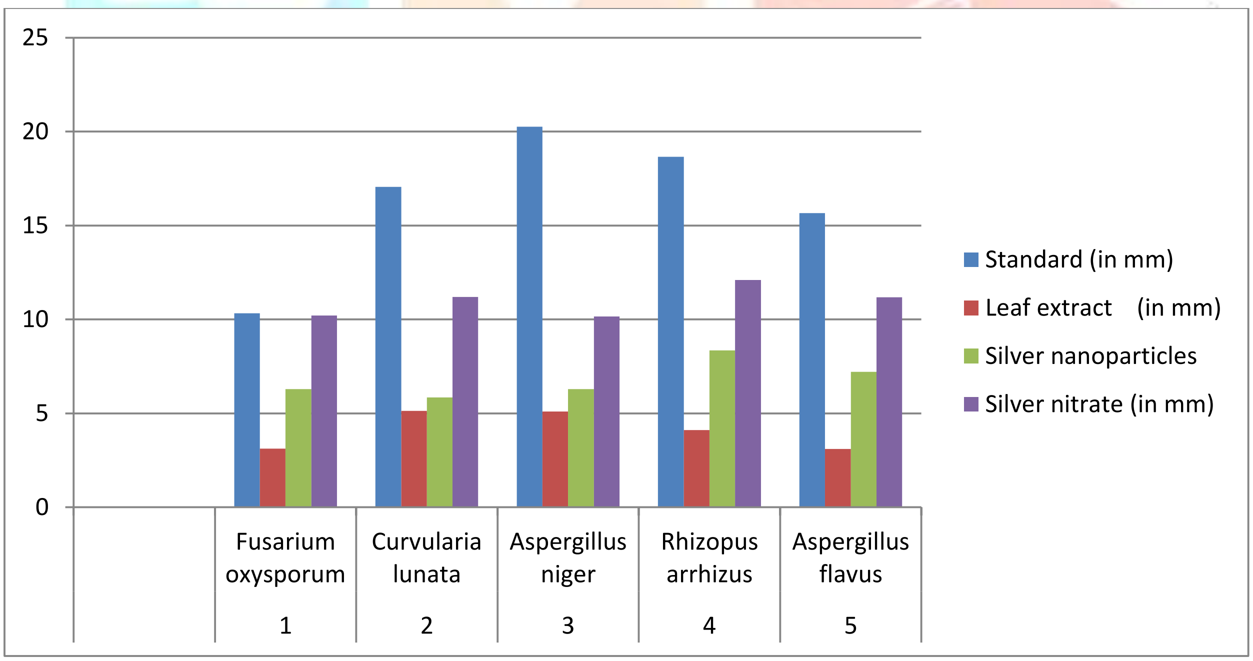
At low concentrations SNPs could prolong the lag phase until the concentration of SNPs was upto 40 µg/ml (27). The inhibitory effect of silver may be different in different systems. It has also been reported to separate respiratory electron transport from oxidative phosphorylation, inhibiting respiratory chain enzymes or affecting the permeability of the membrane to protons and phosphate (28).

SNPs synthesized by plant species are toxic to many antibiotic resistant micro organisms. This suggests that they have great potential for biomedical applications. Similar phenomena have been observed in Allium cepa, Argemone mexicana and Artocarpus heterophyllus (8). Warisnocicharoen et al. (25) found that silver nanoparticles can interact with metabolic processes.

Table-01: Antifungal activity of SNPs synthesized from the leaf of Cochlospermum religiosum

S. No.	Fungal strains	Standard (in mm)	Leaf extract (in mm)	Silver nanoparticles (in mm)	Silver nitrate (in mm)
1	Fusarium oxysporum	10.33	3.12	6.28	10.20
2	Curvularia lunata	17.05	5.12	5.85	11.20
3	Aspergillus niger	20.26	5.09	6.28	10.15
4	Rhizopus arrhizus	18.66	4.10	8.34	12.10
5	Aspergillus flavus	15.66	3.10	7.20	11.18

Graph-01: Antimicrobial activity of SNPs synthesized from the leaf and stembark of Cochlospermum religiosum



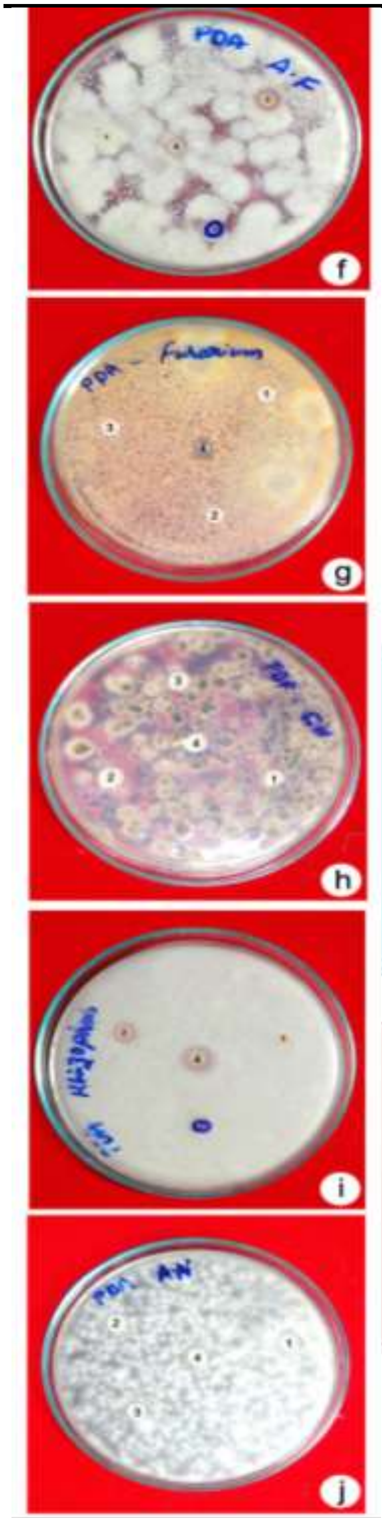


Fig-03: Antimicrobial activity of biologically synthesized SNPs of leaf

f. Aspergillus flavus, g. Fusarium, h. Curvularia lunata, i. Rhizopus, j. Aspergillus niger

1. Leaf extract, 2. Leaf SNPs, 3. Silver nitrate, 4. Nystatin

4. CONCLUSION :

In this study we found that Cochlospermum religiosum is good for the synthesis of silver nanoparticles. The results of this study are an important step towards the use of Cochlospermum religiosum because its nanoparticles show antifungal properties.

In the current situation, bacterial and fungal organisms are developing resistance to traditional drugs and standard drugs. The search for new drugs for the treatement of diseases caused by microorganisms is inevitable. Silver nanoparticles of Cochlospermum religiosum will definitely be used as new drugs for resistant microbial organisms.

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