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Synthesis and Characterization of silver nanoparticles using Centella asiatica leaves and their antibacterial and antifungal activitie

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Abstract- **Green synthesis of nanoparticle prevents the atmosphere from pollution and its application in various fields has become the favorite pursuit of all researchers. Green synthesis of (AgNPs) was achieved by using the ethanolic extract of Centella asiatica leaf and AgNO3. Reduction of silver ions into silver nanoparticles was observed as a result of the color change from pale yellow to brown. The synthesized nanoparticles have been characterized by UV-Vis spectroscopy and FTIR. UV-Visible spectrophotometer showed absorbance peak in range of 445 nm. Infrared spectrometer (FTIR) analysis was carried out to determine the nature of the capping agents in leaf extracts. The synthesized nanoparticles showed active against bacteria such as P.aeroginosa, H.influenzae, S.aureus, S.pyogenes and fungi like Aspergillus sp., Fusarium sp.**

Keywords: Centella asiatica, Green synthesis, Silver nanoparticles, UV, FTIR etc.

1. INTRODUCTION

The green synthesis NPs is a milestone of nanotechnology. Plant mediated synthesis is purely a green synthetic route and are considered better candidates among the different biological entities as they provide clean, eco friendly, cost effective, safe, conveniently utilizable and beneficial way to the synthesis of metal NPs for the large scale production. A number of biological approaches are explored for the synthesis and stabilization of AgNPs [1-5]. Antimicrobial properties of silver nanoparticles caused the use of these nano-metals in the medical field. The exact mechanism which silver nanoparticles employ to cause antimicrobial effect is not clearly known. The silver nanoparticles exhibits distinct properties such as antibacterial, antifungal, anti-inflammatory, antiviral, anti angiogenesis and antiplatelet activity [6-10].

India is rich in medicinal plant diversity. All known types of agro-climatic, ecologic and edaphic conditions are met within India .The experimental plant Centella asiatica belongs to family Apiaceae (Umbeliferae) is known for its wound healing and memory enhancing properties. It is commonly known as “Mandukparni”. The medicinal value of the plant is reflected by its long back use in the ayurvedic and Chinese system of medicine [11].Several study have shown that the leaves of C. asiatica contain the highest amount of triterpene that this is in response to the need for regulation of the pathways for biosynthesis of terpenoids compounds [12]. The plant is well known for its splendid therapeutic and curative nature. It consists of several phytocompounds such as terpenoids, alkaloids, flavanoids etc. Pytochemicals present in the leaf extract served as effective reducing agent[13].

2. MATERIALS AND METHODS

2.1. Plant Material and Preparation of Dry Biomass

Centella asiatica leaves [Figure-1] were collected from Kanavoor garden, Midalam, Kanyakumari district, belonging to the state of Tamil Nadu, India. The collected leaves were washed twice with distilled water and dried for ten days, and then the leaves were ground to a fine powder.

Figure 1: Centella asiatica plant

2.2. Preparation of Plant Extract

Ten grams of dried powder of Centella asiatica leaves was extracted with 100 mL of ethanol kept on a rotator shaker at 190–220 rpm for 24 h. The contents were filtered through four layers of muslin cloth and the filtrate was centrifuged at 5000 rpm for 15 min. The supernatant extract was concentrated by using reduced pressure vacuum distillation flask. The concentrated extract was sterilized and stored at 4°C till further studies.

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2.3. Silver Nanoparticle Synthesis

The synthesis of silver nanoparticles was done by mixing Centella asiatica leaf extract and 1 mM of aqueous silver nitrate solution (AgNO3) in the ratio 1 : 10 and heated at 80°C until the color of the solution was changed from pale yellow to brown. At this point the solution was cooled to room temperature and centrifuged at 5000 rpm for 10 minutes. The supernatant was discarded and the pellet was air dried in the incubator.

A B

Figure 2: (A) Centella asiatica leaf extract (B) Synthesized Silver nanoparticles

2.4. Characterization of AgNPs

UV-absorption spectra of synthesized AgNPs by using Centella asiatica leaf extract were measured using UV-visible spectrometer (JASCO variant 630 spectrometer). Fourier transform infrared (FTIR) spectral measurements were carried out on the Thermal science-Nicolet Si5, ATR-iD1 spectrometer to identify the potential bimolecular Centella asiatica leaf extract which is responsible for reducing and capping the bio reduced silver nanoparticles.

2.5. Antimicrobial activity of silver nanoparticles

Microorganisms

The bacteria were collected from Department of Microbiology, Kamaraj College, Thoothukudi, Tamilnadu, India. The test organisms used for assay are Pseudomonas aeroginosa, Hemophilus influenzae, Staphylococcus aureus and Streptococcus pyogenes. The fungal cultures such as Aspergillus sp. and Fusarium sp. was used for antifungal activity. The antibacterial activity of the synthesized silver nanoparticle was evaluated by measuring the zone of inhibition.

Preparation of media

3.8g of Muller Hinton agar medium was weighed correctly and dissolved in 100ml of sterile distilled water, pH was adjusted to 7.2 and was autoclaved at 1210C for 15 minutes. 20ml of molten agar was poured in to the sterile Petri plate and allowed to solidify.

The antibacterial activities of Ag nanoparticles were carried out by agar well diffusion method. Muller Hinton agar plates were prepared, sterilized and solidified. After solidification, 100µl of the suspension containing106 CFU ml−1 of the test microorganisms were swabbed on the Petri plates uniformly. Wells of 6mm in diameter and about 2 cm apart were punctured in the culture media using sterile cork borers to make four uniform wells in each Petri plates. The Ag nanoparticles extract concentration was 100µl, 200 µl, 300 µl and 400 µl for each well respectively and allow diffusing for 45 minutes. The antibacterial activities of Ag nanoparticles extract were determined after 24 hours at 370C incubation in the incubator. The zone of inhibition (in millimeters) produced by the Ag nanoparticles extract against bacterial pathogens were measured. Each sample was used in triplicate for the determination of antibacterial activity.

Antifungal assay was also done by Agar well Diffusion Technique. The fungal cultures were grown on Rose Bengal broth (Hi media). The cultures of 7th day old culture was washed, suspended in normal saline solution and then filtered through glass wool aseptically. The colony forming units (CFU/ml) of 0.1ml suspension of the test fungus was adjusted to 3 ×105 CFU/ml. These conidia were used for antifungal assay tests. Inocula were applied on the surface of the Rose Bengal agar plates and spread by using sterile glass spreader. Wells of 6mm in diameter and about 2 cm apart were punctured in the culture media using sterile cork borers to make four uniform wells in each Petri plates. The Ag nanoparticles extract concentration was 100µl, 200 µl, 300 µl and 400 µl for each well respectively and allow diffusing for 45 minutes. The antifungal activities of Ag nanoparticles extract were determined after 48 hours at 270C incubation in the incubator. The zone of inhibition produced by the Ag nanoparticles extract against fungal pathogens were measured in millimeter.

3. RESULTS AND DISCUSSION:

3.1 UV-Vis Spectrophotometry

UV-Vis spectra is an important technique to ascertain the formation and stability of produced NPs. Reduction of silver ions into silver nanoparticles during exposure to plant extracts was observed as a result of the color change from pale yellow to brown. The color change is due to the Surface Plasmon Resonance phenomenon. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The sharp bands of silver nanoparticles were observed around 445 nm in case of Centella asiatica.

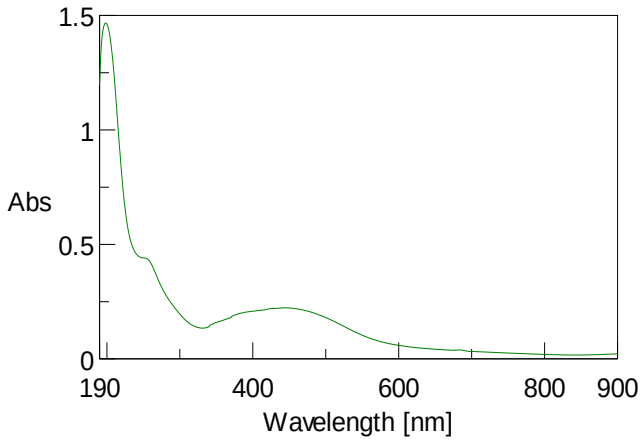

Figure: 3 UV-Visible spectra of AgNPS

3.2 Phytochemical Analysis

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The extracts of Centella asiatica were screened for the presence of phytochemical constituents by following the method of Sofowora (1982) and Kepam (1986) [14] [15]. Preliminary phytochemical analysis revealed the presence of eight compounds such as carbohydrates, tannins, steroids, terpenoids, alkaloids, flavanoids, cardiac glycosides and saponins.

3.3 FTIR

FTIR measurements were carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticles synthesized by Centella asiatica leaf extract.

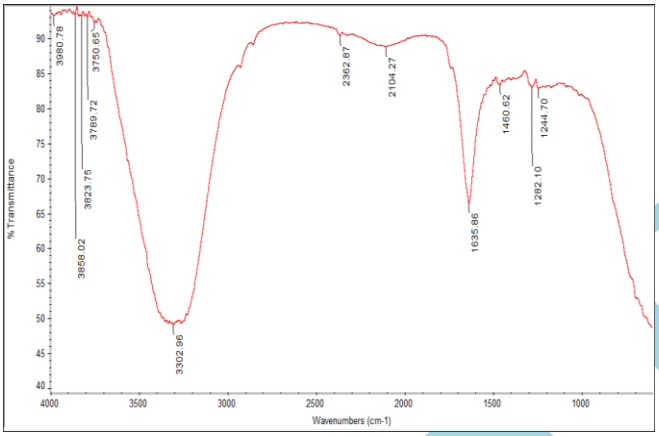

Figure: 4 FTIR spectra of AgNPs

The peak at 3789 and 3750 cm-1 represents the O–H stretching vibration of free alcoholic group. The peak at 3302 cm-1 represents the O–H stretching vibration of carboxylic acid in flavonoids. The peak at 1635 cm–1 is characteristic of carbonyl stretch vibrations from carboxylic acid and phenols, while the stretch at 1460 arises due to the C-O stretching and O-H deformation possibly from the acid groups present in the Centella asiatica leaf extract. The band at 2362cm-1, 2104cm-1, 1282cm-1, and 1244cm-1 assigned to strong stretching vibrations of C-N aromatic and aliphatic amines. The synthesized nanoparticles were surrounded by metabolites such as terpenoids and flavonoids present in the leaf extracts are responsible for the reduction of silver ions and –COO groups participated in the stabilization of nanoparticles.

3.4 Antimicrobial activity of Silver nanoparticles

Statistical Analysis

All the tests were conducted in triplicates. The data were statistically analyzed and expressed as mean ± S.D.

Table: 1 Antibacterial activity of AgNPs

Bacterial pathogens	Concentrtion of SNPs of *Centella asiatica* leaf extract			
	100µl	200 µl	300 µl	400 µl
	Zone of inhibition(mm)			
Pseudomonas aeroginosa	21±1.0	24±0.57	25±0.57	27±0.57
Haemophilus influenza	19±1.0	22±0.53	25±1.0	26±1.0
Staphylococcus aureus	17±1.0	19±1.0	22±1.2	24±1.0
Streptococcus pyogenes	12±1.52	17±1.0	19±1.0	23±1.53

The biologically synthesized silver nanoparticles using Centella asiatica leaf extract were found to be highly inhibition against different pathogenic bacteria and fungi of selected species. The SNPs of Centella asiatica leaf extract of 400µl showed highest antibacterial activity against Pseudomonas aeroginosa (27±0.57 mm) followed by Haemophilus influenzae (26±1.0mm) and Staphylococcus aureus (24±1.0mm) and lowest was Streptococcus pyogenes (23±1.53mm). The SNPs of Centella asiatica leaf extract of 300µl showed moderate antibacterial activity against Pseudomonas aeroginosa (25±0.57mm) followed by Haemophilus influenzae(25±1.0mm) and Staphylococcus aureus(22±1.2mm)and lowest was Streptococcus pyogenes (19±1.0mm). The SNPs of leaf extract of 400µl was observed highest antibacterial activity, followed by moderate activity of 300µl and very lowest activity was observed in 100 µl.

The antifungal activity SNPs of Centella asiatica leaf extract of 400µl against Aspergillus sp. was 12±0.57mm and Fusarium sp. was 10±0.53mm.

Table: 2 Antifungal activity of AgNPs

Fungal pathogens	Concentrtion of SNPs of Centella asiatica leaf extract			
	100 µl	200 µl	300 µl	400 µl
	Zone of inhibition (mm)			
Aspergillus sp.	4±1	6±0.57	10±1	12±0.57
Fusarium sp.	5±0.57	8±1	9±0.53	10±0.53

The silver nanoparticles synthesized via green route are less toxic towards fungal species when compared to bacterial species. Among the four concentrations tested for antimicrobial effect the silver nanoparticles of 400µl effective against all the tested pathogens. The high bactericidal activity is certainly due to big changes in the membrane structure of bacteria as a result of the interaction with silver cations lead to the increased membrane permeability of the bacteria [16]. The ionic silver strongly interacts with thiol group of vital enzymes and inactivate the enzyme activity [17].

CONCLUSION:

In this study silver NPs were synthesized using ethnolic extract of Centella asiatica leaves. Reduction of silver ions into silver nanoparticles was observed as a result of the color

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change from pale yellow to brown. These environmentally benign silver nanoparticles were further confirmed by using UV-Vis and FTIR spectroscopy. Terpenoids and flavonoids were present in the leaves, and they serve as an effective reducing agent. The synthesized AgNPs have promising antimicrobial potential against bacteria such as P.aeroginosa, H.influenzae, S.aureus, S.pyogenes and fungi like A.nigar, Fusarium sp. These biosynthesis silver nanoparticles can potentially be used for different medical applications.

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