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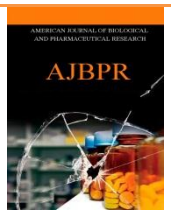

BIOSYNTHESIS OF SILVER NANOPARTICLES USING *CENTELLA ASIATICA* LEAVES EXTRACT AND ITS ANTIBACTERIAL ACTIVITY AGAINST CLINICAL PATHOGENS

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ABSTRACT

In the present investigation, Ag NPs were synthesized using aqueous extract of *Centella asiatica* by simple and eco-friendly route. The aqueous silver ions when exposed to leaves extract of *Centella asiatica* were reduced and resulted in biosynthesis of Ag NPs. The Ag NPs were characterized by UV-visible spectroscopy, Fourier transform infra-red spectroscopy (FTIR), X-ray diffraction (XRD), Scanning electron microscopy–energy-dispersive spectroscopy (SEM-EDS). FTIR analysis was used to identify the functional groups in Ag NPs. The mean particle diameter of silver nanoparticle was calculated from the XRD pattern, according to the line width of the plane, and the refraction peak, using Scherer's equation. SEM images revealed that the particles are spherical in nature. The EDS analysis of the Ag NPs, using an energy range of 2-4 keV, confirmed the presence of elemental silver. The in vitro agar well diffusion method confirmed the potential antibacterial activity of the synthesized Ag NPs against *Klebcella pneumonae* and *Streptococci aurous*.

INTRODUCTION

The field of nanotechnology is one of the most active research areas in modern materials science. Nanoparticles exhibit new or improved properties based on specific characteristics such as size, distribution and morphology .There have been impressive developments in the field of nanotechnology in the recent past years, with numerous methodologies developed to synthesized nanoparticles of particular shape and size depending on specific requirements.

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Nanoparticles have distinguishing properties compared to the bulk form of the same material, thus offering many new developments in the fields of biosensors, biomedicine, and bio nanotechnology. Nanoparticles are also being utilized in medicine for diagnosis, therapeutic drug delivery and the development of treatments for many diseases and disorders. It has been reported that since ancient times silver metal is known to have antimicrobial activities [1] silver nanoparticles (Ag NPs) are of particular interest due to their peculiar properties and wide applications. Silver nanoparticles (Ag NPs), as antibacterial agents, are now used extensively in the fields of medicine [2], drug delivery [3]. Synthesis of nanoparticles is presently an important area of research, searching for an eco-friendly manner and green materials

for current science. A number of AgNPs have been developed by a chemical [4], Langmuir-Blodgett and biological techniques [5]. The latter has emerged as a green alternative, for it is environment-friendly, cost-effective, and easily scaled-up. It has great potential with natural reductions [6], such as plants extract, panchakavya [7], and cow milk.

Plant extracts are more advantageous because using them eliminates the elaborate process of maintaining cell cultures and can be suitably scaled-up for large-scale production under non aseptic environments. Especially, plants that secrete the functional molecules for the reaction, compatible with the green chemistry principles. There are some examples of synthesizing nanomaterial using plants, such as *Helianthus tuberosus Acacia leucophloea* [8], *Polyalthia longifolia*, *Morinda citrifolia* L., *Acalypha indica*, European Rowan (*Sorbus aucuparia*), camphor laurel (*Cinnamomum camphora*) [9].

In the present study an attempt was made to investigate the antimicrobial activity of silver nanoparticles produced by the leaves extract (aqueous extract) of *Centella asiatica*. against the pathogenic clinical micro organisms.

MATERIALS AND METHODS

Preparation of Plant Extracts

Centella asiatica leaves were collected from Jalakandapuram, Salem, Tamil Nadu and shade dried till total moisture is removed from the plant. These, air dried leaves were powdered in an electric grinder. The extraction process was done with the help of Soxhlet apparatus. Water and methanol solvents were used. Extracts were kept in desiccators for the removal of remaining moisture.

Preparation of the aqueous leaves extract

25gm of powdered leaves were subjected to soxhlet extraction with 300ml of water solvent, extraction was carried out for 3hours, 10 cycles and temperature was maintained at 70°C. The aqueous extract was condensed using a rotary vaccum evaporator and the crude extract was obtained (2 g).

Biosynthesis of silver nanoparticles

0.169 gram of $AgNO_3$ (1Mm concentration) was prepared and mixed with 1000ml double distilled water. The extract and the silver nitrate solution was mixed with 1: 10 ratio and incubated in a dark condition for 24 hours [10].

Collection of Synthesized Silver Nanoparticles

The colloidal solutions were separated using centrifugation at 6,000rpm for 10minutes. After the centrifugation process the particles settled down in the bottom of centrifuge tubes were collected and dried using hot air oven at 100 ºC for two hours, whereas the supernatant were discarded.

Characterization of Synthesized Silver Nanoparticles

After the drying process is over, the powders were characterized using different techniques. Following are the list of techniques which are carried out for the characterization of Ag nanoparticles.

UV-Visible Absorbance Analysis

Absorbance spectroscopywas used to determine the optical properties of a solution. A Light was passed through the sample solution and the amount of absorbed light is measured. The absorbance spectrum was various measured at each wavelength. The absorbance can be used to measure the concentration of a solution by using Beer-Lamberts Law. The examination of nanoparticles, the optical properties are much more complicated. These wave lengths arise due to the surface Plasmon resonance of the particle.

The silver nanoparticles were confirmed by measuring the wave length of reaction mixture in the UV-visible spectrum of the PerkinElmer spectrophotometer and sample was taken a 5ml quartz cuvette. The scanning range for the sample is 200-800 nm at a scan speed of 480 nm / minutes. The spectrophotometer was equipped with "UV Win lab" software to record and analyze data. Base line correction of the spectrophotometer was carried out by using a blank reference. The UV-Vis absorption spectra of all the samples were recorded and numerical data were plotted in the "Origin 6.5"[11]

FTIR Analysis

The characterizations of functional groups of Ag NPs were analyzed using Fourier transform infrared (FTIR) spectrophotometer (Spectrum 100; PerkinElmer, Waltham, MA, USA) using potassium bromide (KBr) (analytical grade 95 wt%) pellet method.. The light absorption by the substances at a particular wavelength is a characteristic of the chemical bond. The spectrum was scanned in the range of 4000–400 cm−1 range at a resolution of 4 cm−1. The samples were prepared by dispersing the Ag NPs uniformly in a matrix of dry KBr compressed to form an almost transparent disc. KBr was used as a standard.[12]

X-Ray Diffraction (XRD) Analysis

The phase variety and grain size of synthesized silver nanoparticles were determined by X-ray diffraction spectroscopy (Philips PAN Analytical). The synthesized silver nanoparticles were studied with CUK alpha radition at voltage of 30KV and current of 20 MA with scan rate of 0.03/s. Different phases present in the synthesized samples were determined by X` pert high score software with search and match facility. The particle size of the prepared samples were determined by using Scherrer`s equation as follows

$$D = \frac{\kappa\lambda}{\beta \cos\theta}$$

Where,
D = crystallite size,
λ = wavelength of the radiation,
θ = Bragg's angle and
B = full width at half maximum

Scanning Electron Microscope (SEM) Analysis

The morphological features of synthesized silver nanoparticles produced by *Centella asiatica* plant extract were studied by scanning electron microscope (JSM-6480 LV) After 24 Hours, of the addition of AgNO3 the SEM slides were prepared by making a smear of the solution on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the SEM at an acceleration voltage of the microscope was kept in the range 10-20KV[13].

Antimicrobial activity

The Antimicrobial activity of silver nanoparticles was determined on Muller & Hinton Agar (Hi-Media Pvt. Ltd) using Kirby-Bauer disc diffusion method. Nutrient broth (0.3g beef extract, 0.3g yeast extract 0.5g peptone, 0.5 g NaCl dissolved in 100 ml of double distilled water) was used to cultivate bacterial cultures *Streptococcus aureus, Escherichia coli, pseudomonas aeruginosa,, Klepsiella pneumonie* . Then MHA media was prepared and The media was poured in the Petri dishes in the hand bearable condition and kept for 30 minutes for solidification. After 30 minutes, the fresh overnight cultures of inoculums (100 µl) of four different cultures were spread on to solidified Muller Hinton agar (MHA) plates using for sterile swabs. Sterile wells were made with the help of sterile cork borer aseptic conditions. Samples were added to the wells at aseptic conditions. The samples were mixed with DMSO solution after that test plates were incubated in 37oC for 24 - 48 hours. After period of incubation to form zone of inhibition (in mm diameter) was measured using transparent ruler and tabulated [14].

RESULTS AND DISCUSSION

The synthesis of Ag NPs was initially observed by the colour change from light yellow to dark brown colour. The colour change is due to the excitation of surface plasmon resonance vibrations in Ag NPs. Similar results were observed in various plants. Characteristic absorption peaks of Ag NPs can be seen at around 420 nm.

UV-visible spectra analysis

Silver nanoparticles were synthesized using *Centella asiatica* leaves extract. Interestingly, silver nanoparticles were synthesized rapidly within 1hour of incubation period. The aqueous silver nitrate solution was turned to yellowish brown color with in 1hour, with the addition of leaves extract. Intensity of brown color increased in direct proportion to the incubation period. It was due to the excitation of surface Plasmon resonance (SRE) effect and reduction of Ag NO_3. The silver surface Plasmon resonance was observed at 420nm (Fig 1,2) which steadily increases in intensity as a function of time of reaction (ranging from 1Hour to 24 hours) without showing any shift of the maximum wavelength.

FTIR Analysis

The FTIR spectra of *Centella asiatica* leaves extract and bio-synthesized Ag NPs, showed the presence of amino, carboxylic, hydroxyl and carbonyl groups. Display of strong broad O–H stretch carboxylic bands in the region 3412.08 cm^{-1} and carboxylic stretching bands in the region 1564 cm^{-1} was observed. The peaks appearing in the region 1645 cm^{-1} are attributed to the stretching vibration of the NH group that is characteristic of proteins shifted from 1384 cm^{-1} after the synthesis of Ag-NPs. The secondary structure was not affected during reaction with Ag+ Ions or after binding with Ag nanoparticles [15]. These results confirm the presence of possible proteins (Fig 3).

SEM and EDX Analysis

The SEM analysis was used to determine the structure of the reaction products that were formed. SEM image has showed individual silver particles as well as number of aggregates, SEM images of SNPs derived from the leaves extracts of *Centella asiatica* showed the particles are spherical in shape and size ranged from 20 to 35 nm. The morphology of the SNPs was predominantly spherical and they appear to be monodispersed. Further, analysis of the silver particles by energy dispersive spectroscopy confirmed the presence of the signal characteristic of silver (Fig 4,5).

Analysis through Energy dispersive X-ray (EDX) spectrometers confirmed the presence of elemental silver signal of silver nanoparticles .The vertical axis displays the number of X-ray counts whilst the horizontal axis displays energy in K eV. Identification lines for the major emission energies for silver (Ag) are displayed and these correspond with peaks in the spectrum, thus giving confidence that silver has been correctly identified. The SEM image recorded from drop coated films of the silver nanoparticles synthesized with *Centella asiatica* leaves extract. The SEM image showed cubical and relatively uniform shape of nanoparticles formation.

Antimicrobial activity

Antibacterial activity of leaves extracts of C.asiatica and the synthesized Ag NPs were performed against four clinically important pathogens *Escherichia coli, staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* by using agar well diffusion

method. In this activity compared to leaves extracts microorganisms. The antibacterial activity was found maximum against all the pathogens except *P.aeruginosa*.

The results were compared with silver nanoparticles produced by the leaf extracts of *Eclipta alba* which showed highest percentage of bacterial inhibition synthesized silver nanoparticles inhibited the growth of against both gram-positive and gram-negative. Thus, gram-positive bacteria may allow less Ag+ to reach the cytoplasmic membrane than gram-negative bacteria. The AgNPs are also reported to be nontoxic to human and most effective against bacteria [16].

Figure 1. UV-visible spectra of aqueous silver nitrate with *Centella asiatica* extract at different time intravels

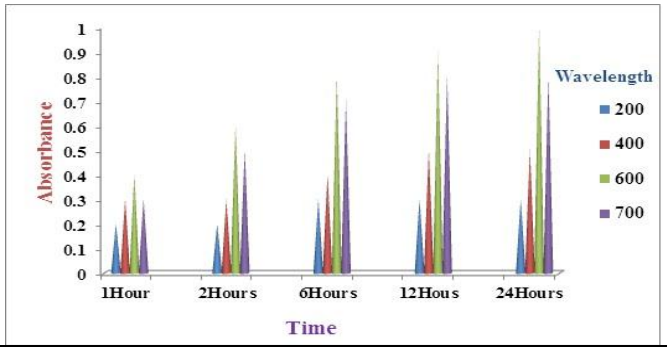

Figure 2. FTIR Spectrum of silver nanoparticles synthesis using *Centella asiatica*

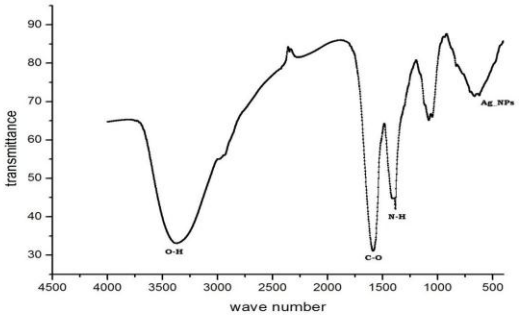

Figure 3. FTIR Analysis

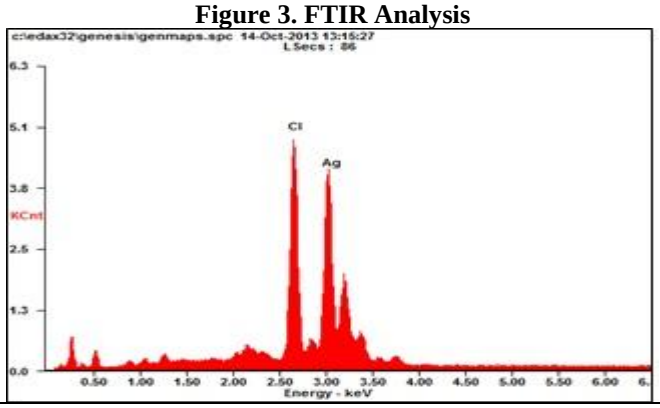

Figure 3. SEM image

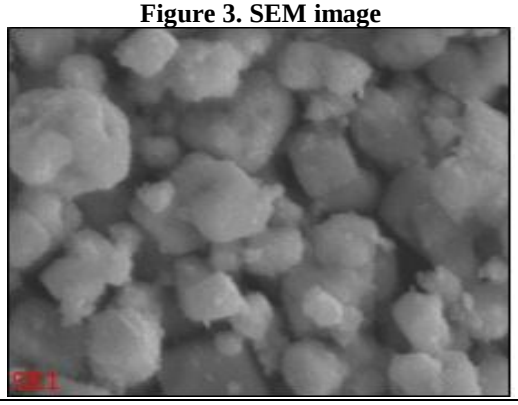

Figure 4. SEM and EDX Analysis of silver nanoparticles synthesis using *Centella asiatica*

Element	Wt%	At%
ClK	25.37	50.84
AgL	74.63	49.16
Matrix	Correction	ZAF

Table 1. Effect of Ag NPs from leaves aqueous extract *Centella asiatica* on Zone of inhibition against clinical pathogenic bacteria

Bioactive compounds	Zone of inhibition (mm)				
	Conc. (µl)	*K.pneumonia*	*E.coli*	*S.aureus*	*P.aeruginosa*
Leaves extract	30	-	-	-	-
	60	-	-	-	-
	90	-	-	-	-
AgNPs	30	5	2	4	-
	60	6	5	7	-
	90	10	6	10	3

CONCLUSION

Biosynthesis of silver nanoparticles using aqueous extract of *Centella asiatica* proved to be one of the potential sources to produce silver nanoparticles with defined scale.UV–Visible spectroscopy showed peaks in the range of 420 nm confirming the formation of Ag NPs. The FTIR studies indicate the capping of certain amide-

containing compounds on the synthesized nanoparticles. The XRD analysis of the powder indicates that the synthesized nanoparticles are crystalline in size (35 nm). SEM-EDS analysis showed that the biosynthesized nanoparticles are spherical in nature and presence of silver compound.

The Ag NPs has great antimicrobial activity against *Klebsiella pneumoniae*, *Staphylococcus aureus* when compared to *E.coli* and *P.aeruginosa*. From the research it's concluded that the aqueous extract of *Centella asiatica* showed potential activity in producing Ag NPs. However, knowing the mechanism of silver nanoparticle would ensure its usage in nanomedicine.

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CONFLICT OF INTEREST: None

REFERENCES

1. Devi LS and Joshi SR. (2012). Antimicrobial and synergistic effects of silver nanoparticles synthesized using soil fungi of high altitudes of eastern Himalaya. *Mycobiology*, 40(1), 27–34.
2. Dar MA, Ingle A and Rai M. (2013). Enhanced antimicrobial activity of silver nanoparticles synthesized by *Cryphonectria sp*. evaluated singly and in combination with antibiotics. *Nanomedicine*, 9(1), 105–110.
3. Meng H, Liong M and Xia T. (2010). Engineered design of mesoporous silica nanoparticles to deliver doxorubicin and P-glycoprotein siRNA to overcome drug resistance in a cancer cell line. *ACS Nano*, 4(8), 4539–4550.
4. Sun Y, Yin Y, Mayers BT, Herricks T and Xia Y. (2002). Uniform form silver nanowires synthesis by reducing AgNO3 with ethylene glycol in presence of seeds and poly (vinyl pyrrolidone). *Chem Mater*, 14, 4736–4745.
5. Naik RR, Stringer SJ, Agarwal G, Jones S and Stone MO. (2002). Biomimetic synthesis and patterning of silver nanoparticles. *Nat Mater*, 1, 169–172.
6. Sivalingam P, Antony JJ, Siva D, Achiraman S and Anbarasu K. Mangrove. (2010). Streptomyces sp. BDUKAS10 as nanofactory for fabrication of bactericidal silver nanoparticles. *Colloids Surf B Biointerfaces*. 98, 12–17.
7. Lee KJ, Park SH, and Govarthanan M. (2013). Synthesis of silver nanoparticles using cow milk and their antifungal activity against phytopathogens. *Mater Lett*, 105, 128–131.
8. Dipankar C and Murugan S. (2012). The green synthesis, characterization, and evaluation of the biological activities of silver nanoparticles synthesized from Iresine herbstii leaf aqueous extracts. *Colloids Surf B Biointerfaces*, 98, 112–119.
9. Prakash P, Gnanaprakasam P, Emmanuel R, Arokiyaraj S and Saravanan M. (2013). Green synthesis of silver nanoparticles from leaf extract of Mimusops elengi, Linn. For enhanced antibacterial activity against multi drug resistant clinical isolates. *Colloids Surf B Biointerfaces*, 108, 255–259.
10. Al-Warthan A, Kholoud MM, El-Nour A, Eftaiha A and Ammar RAA. (2010). Synthesis and applications of silver nanoparticles. *Arabian J Chem*, 3, 135–140.
11. Ajithadas A, Ramraj N, Venkatachalam K and Pandi B. (2014). Synthesis and Characterization of Silver Nanoparticles of Insulin Plant (costus pictus D. Don) Leaves. *Asian Journal of Biomedical and Pharmaceutical Sciences*, 04 (34), 1-6.
12. Garg S. (2012). Rapid biogenic synthesis of silver nano particles using black pepper (piper nigrum) corn extract. *Int J Inno Biol Chem Sci*, 3, 5–10.
13. Vijay Kumara PPN, Pammib SVN, Pratap Kolluc, Satyanarayanad KVV and Shameema U. (2014). Green synthesis and characterization of silver nanoparticles using *Boerhaavia diffusa* plant extract and their antibacterial activity. *Industrial Crops and Products*, 52, 562– 566.
14. Renugadevi. K, Venus and Aswini R (2012). Microwave irradiation assisted synthesis of silver nanoparticle using *Azadirachta indica* leaf extract as a reducing agent and invitro evaluation of its antibacterial and anticancer activity. *International Journal of Nanomaterials Biostructures*, 2(2), 5-10.
15. Geethalakshmi LR and Sarada DVL. (2013). Characterization and antimicrobial activity of gold and silver nanoparticles synthesized using saponin isolated from *Trianthema decandra*. *Industrial Crops and Products*, 51, 107– 115.
16. Yu DG. (2007). Formation of colloidal silver nanoparticles stabilized by Na?–poly (c-glutamic acid)–silver nitrate complex via chemical reduction process. *Colloid Surf B*, 59, 171–178.