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Research Article

BIOREDUCTION OF SILVER NANOPARTICLES FROM AQUEOUS STEM EXTRACT OF *CATHARANTHUS ROSEUS* AND BACTERICIDAL EFFECTS

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ABSTRACT

Objective: Silver nanoparticles (AgNPs) were synthesized using the stem extract of the *Catharanthus roseus* (L.) plant using the green method. The extract from the stem of *C. roseus* plant was obtained using standard dry powder extraction method. The colorless silver nitrate solution was changed into brown color after the addition of stem extract indicates the formation of AgNPs. The chemical compounds present in the stem extract were acting as a reducing agent for the synthesis of AgNPs. **Methods:** The AgNPs were analyzed with the help of UV-visible spectrophotometer for initial confirmation. Fourier transform infra-red spectroscopy (FT-IR) confirmed the presence of various phytochemicals such as carbohydrate, phenolic compounds, flavonoids, saponin. and alkaloids which were responsible for both reduction and stabilizing the action of the silver nanoparticles. **Results:** From the transmission electron microscopy analysis, it was confirmed that Ag-nanoparticles are in nanosize range between 40-50nm and are mono dispersed of particle distribution. **Conclusion:** From the zone of inhibition, it was confirmed that the synthesized Ag NP from the *C. roseus* stem was found to have a very high antimicrobial effect. From this study, it is clear that the AgNp can be used as a potential antimicrobial agent.

Keywords: *Catharanthus roseus*, Silver nanoparticles, Anti-microbial activities.

INTRODUCTION

Nanomaterials are referred as particles with a size up to 100 nm, and they are lying in between micron and atom level [1,2]. Nanoparticles exhibit new properties based on specific characteristics such as size, morphology, and behavior of the particles in different fields such as thermal, mechanical and medical. A nanoparticle has a higher surface to volume ratio as the size of particles decreases. Specific surface area is relevant for catalytic reactivity and other related properties such as antimicrobial activity in silver nanoparticles. As the specific surface area of nanoparticles is increased, their biological effectiveness can also be increased due to the increase in surface energy [1-3]. Nanoparticles can be synthesized using various approaches such as chemical, physical and biological. The chemical method of synthesis is one of the shortest methods of synthesizing a large quantity of nanoparticles. This method requires toxic chemical for the reduction of nanoparticles and non-degradable polymers used as a capping agents for stabilizing the synthesized nanoparticles. Chemicals used toxic compounds lead to non-eco-friendly by-products which lead nanoparticles itself toxic and cannot be utilized for medical application [4,5]. Similarly in physical method, synthesized nanoparticles contain chemical and distribution of nanoparticles vary from others and finally aggregates. The need for environmental friendly protocols for nanoparticle synthesis in biological approaches is free from the use of toxic chemicals as byproducts [6], and the beneficial active biomolecules increases the efficacy of the nanoparticles. Biological methods of synthesis have paved the way for the green synthesis of nanoparticles and proven to be better method due to better stability and slower kinetics [7]. This has motivated an upsurge in research on the synthetic routes that allows better control of shape and size for various nanotechnological applications. The use of environmentally caring materials such as plant extracts [8], bacteria [9], fungi [10], and enzymes [11] for the synthesis of silver nanoparticles offer numerous benefits of eco-friendly, compatibility for pharmaceutical and other medical applications [12].

Recently, more interest towards the medicinal plants with a medical behavior have been used for the synthesis of silver nanoparticles and been successfully utilized in the medical application. The parts of the plants such as leaf, stem, root, fruit, flower, and seed have been separately used for the synthesis of nanoparticles and used in the different application. Depending upon the size, behavior and toxicity effect the synthesized nanoparticles have been employed in required application. Direct implementation of chemically synthesized nanoparticles is not possible in the medical field due to the presence of toxic precursors. By using the biological methods of plants are with medical behavior in nature, directly helps the nanoparticles in drug applications to cure the particular disease without affecting others cells.

Since, India was well-known for its ancient Siddha; a medicinal method using plants was used for curing diseases. Abundant medicinal plants available in India with rich medical characteristic behavior can be utilized for the synthesis of nanoparticles. Previously many plants were utilized for the synthesis of silver and other inorganic nanomaterials. Plants such as *Erythrina indica* [13], *Capparis zeylanica* [14], *Lantana camara* [15] etc., were being used for the synthesis of silver nanoparticles. No research work was previously reported and established for the synthesis of silver nanoparticles from *Catharanthus roseus* (L.).

C. roseus is the family of Apocynaceae, which is an important medicinal plant used to treat many of the fatal diseases. *C. roseus* unnaturally contain antioxidant due to which it is mostly used in pharmaceutical industry. *C. roseus* is traditionally used as an oral hypoglycemic agent, and it also contains two terpenoid indole alkaloids such as vinblastine and vincristine, the first natural anticancer agents to be clinically used [19]. Federico ferreres coworkers reported that the methanolic stem extracts of *C. roseus* contains chemical compounds such as, 3-O-Caffeoylquinic acid, kaempferol-3-O-(2,6-di-O-rhamnosyl-galactoside)-7-O-hexoside, 4-O-caffeoylquinic acid, (4)5-O-caffeoylquinic acid, quercetin-3-O-(2,6-di-O-rhamnosyl-galactoside), kaempferol-3-O-(2,6-di-O-rhamnosyl-galactoside), kaempferol-3-O-(2,6-di-O-rhamnosyl-glucoside) and isorhamnetin-3-O-(2,6-di-O-rhamnosyl-galactoside [16]. This plant is found to be ironic in their pharmacological activities that consist of antibacterial, antifungal, antioxidant, anticancer and antiviral activities. Especially, water extracts of this plant are used for various applications, such as bleeding arresting, diabetes, fever or rheumatism [17]. In addition, the leaves of the plants were chewed to suppress the sensations of hunger and fatigue [18].

In this present investigation, the synthesis of silver nanoparticles, using the aqueous extract of *C. roseus* stem was used for the first time. Green synthesized nanoparticles were characterized using ultraviolet (UV), Fourier transform infra-red (FTIR), X-ray powder diffraction (XRD) and transmission electron microscopy (TEM). Further, these biologically synthesized nanoparticles were utilized for the antibacterial and cytotoxicity study against different pathogenic microbes and cancer cells, respectively.

METHODS

Materials

Silver nitrate of AR grade was purchased from Sigma-Aldrich, Mumbai, India and double distilled water was used throughout the process. Whatman No.1 filter paper was used for filtration purpose. All glasswares were washed well and dried using hot air oven before use.

C. roseus

C. roseus plants were collected from the nearby farms of Kovilpatti, Tamil Nadu, India. The collected stem was cleaned using normal running water and then were washed well with double distilled water before use. The stems were cut into smaller pieces with sterilized knife and then dried in normal room temperature for complete removal of moisture present in the stem. Fig. 1 shows the full plant image of *C. roseus* (pink flower). The dried stems were thereafter grinded using mechanical grinders and fine powder were collected which was stored for the further synthesis process.

Plant stem extract preparation

The 5 g of fine powder is mixed with 100 ml of double distilled water then it is boiled at 100°C for 20 minutes. The obtained extract was filtered through normal filter paper to get pure liquid and again filtered using Whatman No.1 filter paper to get clear leaf extract. The filtrated extract is stored at 4°C and used for further synthesis process [31].

Synthesis of silver nanoparticle

100 ml of 0.001 mM aqueous solution of silver nitrate was taken in a flask and 10 ml of stock solution of stem extract was added drop by drop using micropipette with 60°C heating and continuous stirring for 20 minutes. The colorless solution changed into a brown color which confirms the formation of colloid silver nanoparticles. The colloidal solution is then centrifuged at 9000 rpm for 20 minutes then the supernatant is collected and stored for further analysis [31].

Characterization

The synthesized silver nanoparticles were confirmed using UV-visible spectrum on a UV-visible (UV-1800 Shimadzu double beam) spectrophotometer. FT-IR measurements were used to determine biomolecules present in both stem extract and informed Ag NPs using Jasco 5300-FTIR model. X-ray diffraction is used to determine the crystalline structure of the silver nanoparticles using X'Pert PRO MRD from PANalytical X-ray diffractometer. The clear particle mean size were identified by the DLS Malvern instrument and morphology of the silver nanoparticles characterized by TEM analysis (Hitachi2000) finally antimicrobial activity were analyzed against Gram-positive and negative bacteria with disc diffusion method.

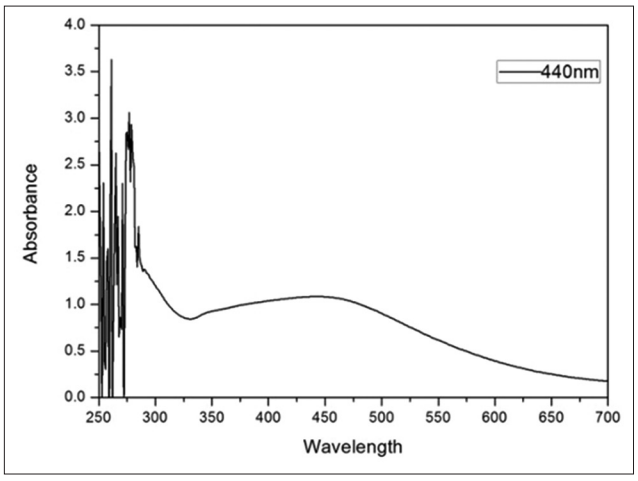

Fig. 1: The ultraviolet-Vis spectrum of silver nanoparticles

Antimicrobial activity

The antimicrobial activity was done on various pathogenic bacteria. The test organisms used were *Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus.* 27 g of Nutrient agar the medium were sterilized by using the autoclave and transferred to petriplate. After solidification inoculated with a fresh growth of test stain, then AgNPs were loaded with different concentrations 25 µg, 50 µg, 75 µg and 100 µg, respectively. The antibacterial assay plates were incubated at 37°C for 24 hrs after the incubation time.

RESULT AND DISCUSSION

UV-vis spectrophotometer

Optical properties of green reduced silver nanoparticles were analyzed by UV-Spectrometry. The silver nanoparticles are intense absorption at 440 nm in visible range due to their surface Plasmon resonance (SPR) effect. SPR effect is nothing, but the combined oscillation of electrons on the surface of the metal nanoparticles. Mie scattering for colloidal silver nanoparticles is varying from 390 to 420 nm [20]. UV-spectroscopy is an influential instrument to interpret the formation of metal nanoparticles. In this reaction, the container contains plant extract, as well as silver salt were read in UV-spectrometry. Fig. 1 shows the peak at 440 nm the formation of silver nanoparticles from silver ions. The peaks consigned to an SPR of so many metal nanoparticles the size ranging from 2 to 100 nm [21]. Based upon the UV-spectrometry physico-chemical and optical properties of the silver nanoparticles were determined, optimized and it were utilized for the high yield synthesis of silver nanoparticles. This UV-spectroscopy one of the effective tool to evaluate the shape and size of the silver nanoparticles in a water medium and spherical shape of the silver nanoparticles absorbed the SPR band near 400-420 nm [22,23]. When the nanoparticles synthesis increases, the SPR intensity also increases. The flavonoids and proteins in the plant extracts act as a reducing and stabilizing agent respectively [24-26], and these capping agents may interrupt the SPR effect after an hour ageing [27].

FTIR analysis of silver nanoparticles

The biomolecules present in the *C. roseus* were characterized by FTIR spectrometry method. In which one can conclude that what are the biological molecules responsible for the synthesis and capping of silver nanoparticles. The FTIR characterization was taken before and after silver nanoparticles synthesis. The FTIR bands of silver nanoparticles were absorbed at 514, 1053, 1521, 1818, 2355, 2891, and 3539 cm^{-1}. In silver nanoparticles synthesis FTIR spectra, the bands intensities were slightly decreases this shows the few biomacromolecules responsible for the capping of silver nanoparticles. The Fig. 2 shows the FTIR spectra of silver nanoparticles. The bands at 3539 and 3542 cm^{-1} due to the presence of alcoholic groups in the plant extracts. Plant extracts were contains a high amount of phenolic compounds which were responsible for the reduction of silver metal ion species into zero valent silver nanoparticles [28]. Amide-I groups were in the 1521 and 1818 cm^{-1} shown the capping agent of silver nanoparticles. The shallow peak at 514 cm^{-1} corresponds to the aromatic C-H vibrations of phenolic compounds such as vinblastin etc., (Krishnan *et al.,* 2006). From the above FTIR stretches and bond, it was revealed that the phenolic compounds in the aqueous plant extract could reduce the silver nitrate into silver nanoparticles.

XRD analysis

XRD analysis of silver nanoparticles was illustrated the crystalline and particles size of the silver nanoparticles. The bio reduced silver nanoparticles reflected the peaks at 27.71, 32.01, 37.93, and 46.11 the

values were corresponds to the (220), (311), (111) and (103) Bragg's angle respectively. These Bragg's angle values matched with JCPDS file No. 01-1167. Fig. 3 shows the XRD pattern of the silver nanoparticles. The noisy peaks at 12° could be adhering of plant biomolecules on the surface of the silver nanoparticles which will enhance the antibacterial and anti-cytotoxicity effect of silver nanoparticles [29]. According to the above interpretation, the synthesized silver nanoparticles have been crystalline, cubic and face centered [30].

Particle size distribution (DLS) and zetapotential of the silver nanoparticles

The size distribution of colloidal silver nanoparticles was confirmed by DLS particles size analyzer. The high proportion of average size silver nanoparticle was absolved at 105 nm (Fig. 4). The broadening of SPR UV-peaks spectra comparatively related to the size range of DLS 25-150 nm of silver nanoparticles. The particles polydispersity shown the 0.264 shows the particles were poly distributed, and this results shown the hydrodynamic size of the silver nanoparticles. The zeta potential value of the silver nanoparticle found to be from −20 and +8.7 mV which were due to surrounding of capping agent on the surface of the silver nanoparticles and the average charge of the particle was −7.56 mV. These biomolecules were responsible for the stability of silver nanoparticles.

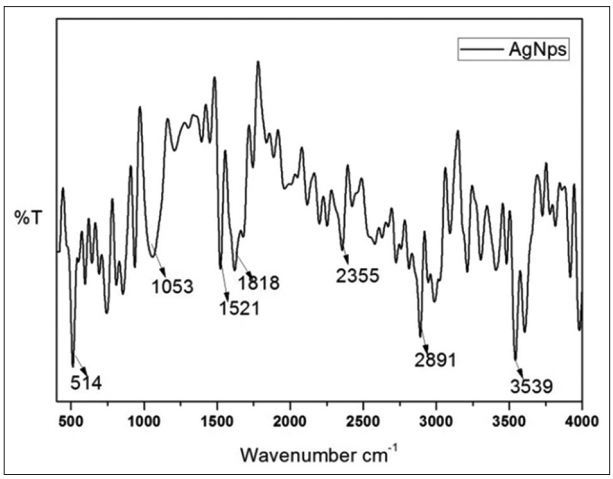

Fig. 2: The Fourier transform infra-red spectrum of isolated silver nanoparticles

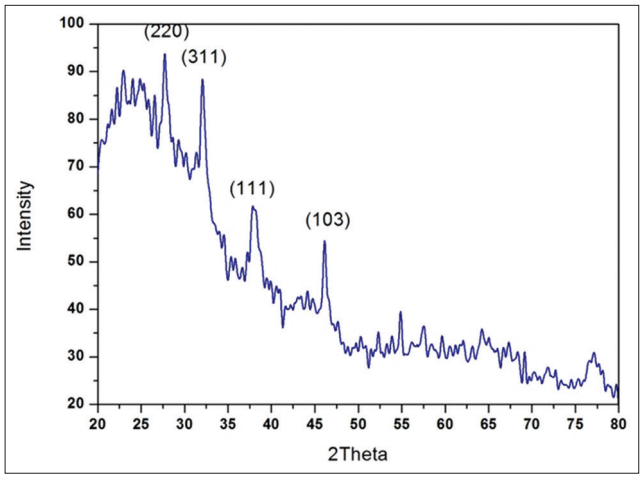

Fig. 3: The X-ray powder diffraction patterns of silver nanoparticles

TEM analysis

Here, the AgNps size was found to be 40-50 nm and the average diameter of the nanoparticles 0.25r and morphology of silver nanoparticles were found to be spherical in size and low polydispersity distribution as shown in DLS results. Fig. 5 showed the TEM image of silver nanoparticles. DLS particles size analyzer showing the hydrodynamic structure of the nanoparticles. Hence, the TEM particle size very less, when compared to the DLS analysis average particle size.

Antibacterial activity of silver nanoparticles

In this report, silver nanoparticles antimicrobial activity investigated and shown it improved zone of inhibitions (ZOI) against positive and negative pathogens, and silver nanoparticles concentration were fixed in microgram (µg). Even though, silver shown its antimicrobial effects as a dose-dependent, it has shown a good bactericidal effect against Gram-positive bacteria than negative bacteria. The inhibitory mechanism of silver nanoparticles was not clearly known [32]. Even though, numerous mode of action of silver nanoparticles were postulated that, the AgNps could have been bind with disulphide (S-S) groups of the cell membrane, metabolic enzymes, and phosphate groups which is involved in the respiratory reactions from which it might inhibit the microbial growth. Plant extract itself has good properties of antibacterial activity which enhanced the antimicrobial activities of AgNps maximum ZOI was achieved (20 mm) in methicillin resistant *S. aureus* positive strain at 100 µg. ZOI was shown in Table 1. This maximum zone of inhibition due to the negative charged silver nanoparticles might have inhibited the positive bacterial strain growth [13] and AgNps have also shown the relatively good bactericidal effect on negative strains.

CONCLUSION

In this report, an eco-friendly approach for the synthesis of AgNPs using *C. roseus* aqueous stem extract was employed the one step and efficient method to obtain AgNPs without creating any harmful byproducts, toxic chemicals as a reducing agent. The active biomolecules present in the stem extract of *C. roseus* such as terpenoid indole alkaloids, vinblastine and vincristine (polyphenols), carbohydrates and amino acids were responsible for reduction of silver nitrate to zerovalent silver nanoparticle which was revealed from FT-IR studies. Few macromolecules such as proteins and carbohydrate could act as a stabilizer for AgNPs. The bioreduced silver nanoparticles were found to have a relatively good antimicrobial activity against positive and negative strains such as *E. coli, S. aureus, and Pseudomonas aeruginosa*.

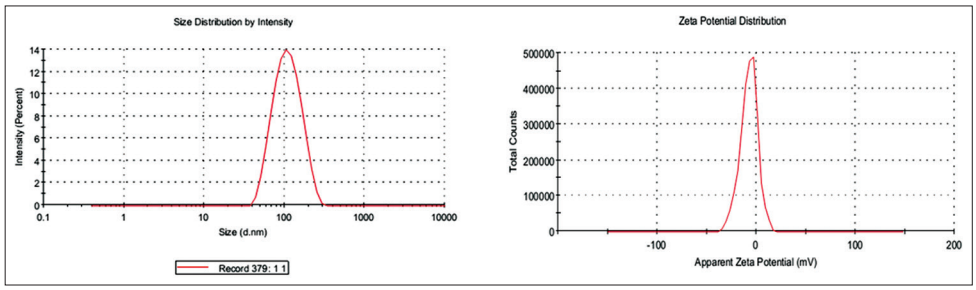

Fig. 4: Hydrodynamic results of silver nanoparticles and surface charge of the silver nanoparticles by DLS.

Table 1: Zone of inhibition for biosynthesized AgNPs from against different bacterial species (mm)

Name of the bacteria's	Control	Gentamicin	Different concentration of AgNPs in (µg)			
			(25 µg)	(50 µg)	(75 µg)	(100 µg)
S. aureus	1	18	7	8	15	20
P. aeruginosa	1	20	8	9	10	15
E. coli	1	19	5	9	19	19.5

E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa, S. aureus: Staphylococcus aureus

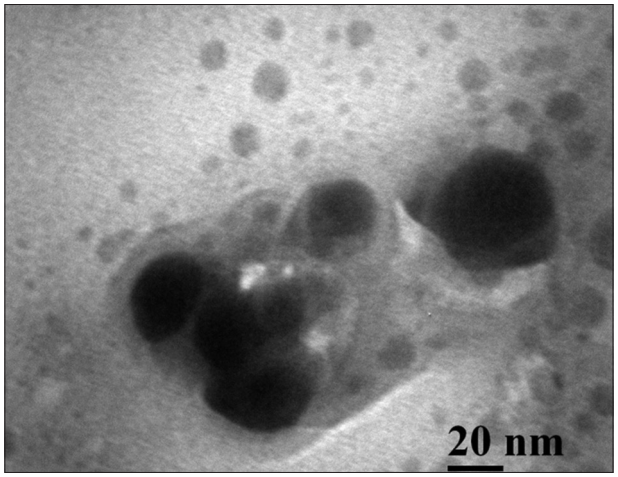

Fig. 5: The transmission electron microscopy image of silver nanoparticles

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