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Antimicrobial Potential of Green Synthesized Silver Nanoparticles Using *Sida acuta* Leaf Extract

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Abstract

Plants are the natural factories for nanoparticle production as many of their products are being used for metallic nanoparticles production. Silver nanoparticles are being used in a number of consumer products, remediation processes, and medicines due to their antimicrobial and anti-inflammatory and catalytic activities. Present work focuses on a simple, one-step, environmental-friendly biosynthesis of silver nanoparticles using silver nitrate as precursor and leaf extract of herb species (*Sida acuta*); a common wireweed of Malvaceae family, which acts as reducing as well as capping agent. Synthesized nanoparticles were characterized for their morphological description using different techniques like UV-Vis spectroscopy, dynamic light scattering (DLS), transmission electron microscope (TEM) and Fourier transform infra-red (FT-IR) spectroscopy. The antimicrobial activity of these nanoparticles was studied against *Pseudomonas aeruginosa* and *Candida albicans*. The results showed good inhibitory effect against *Pseudomonas aeruginosa* and *Candida albicans* and found to exhibit good antibacterial activity especially at lower concentrations of 4 µg/ml and 8 µg/ml.

Keywords: Silver nanoparticles; Leaf extract; Antifungal effect; Antibacterial activity

Introduction

Nanotechnology has found greener ways for nanoparticles and nanomaterials synthesis through plants and their products [1,2]. Almost all the parts of a plant including roots [3], bark [4], leaves [5], peel [6], tuber [7], seed [8] and fruit [9] extracts are being used for metallic nanoparticle synthesis. Biosynthesis of metallic nanoparticles using plant products such as amino acids, polyphenols, glucose, tannins, sterols, flavonoids, terpenoids as reducing as well as capping agents is an easier, environment friendly, cost-effective approach that does not requires high pressure, temperature, energy and toxic chemicals [10-12]. Metallic nanoparticles has become a mesmerizing field of nanotechnology due to their phenomenal properties and prodigious applications in various fields such as catalysis [13], electronics [14], chemistry, medicine [15] and energy [16]. Among various metallic nanoparticles, silver nanoparticles have been reported to possess antibacterial [17], antifungal [18], antiviral [19], anti-angiogenic [20] and anti-inflammatory [21] properties. The antimicrobial activity of silver nanoparticles is due to their high surface area to volume ratio and unique chemical and physical properties. Their small size allows them to penetrate through bacterial cell wall and disturb its cellular mechanism. Silver nanoparticles are used in cosmetics [22], topical ointments and

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creams for infections, burns, wounds and a number of environmental applications [23]. Silver ions are being used in several consumer products such as; in coatings of medical devices and water filters, air purifying sprays, respirators, socks, wet wipes, soaps, washing machines etc. [24].

There are a number of approaches available for silver nanoparticles synthesis such as chemical reduction [25], sunlight induced synthesis [26], photochemical reactions [27], sonochemical [28], radiation [29] and microwave assisted [30]. Among all, use of plant products and extracts are economically and environmentally benign and easier method for reduction of silver salt into silver nanoparticles. The synthesis of metal nanoparticles using plants is non-toxic, fast, takes place at ambient temperature and low cost.

Zerovalent silver nanoparticles were synthesized using leaf extracts of *Sida acuta* found in hotter regions of India. *Sida acuta* leaf extract has pharmaceutical applications having cryptolepine and quindoline as the major alkaloid of the plant. The *Sida acuta* plant is used in the treatment of malaria, renal inflammation, cold, fever, ulcers, diarrhea and many other diseases [31]. The synthesis mechanism involved simple reaction of organic compounds of leaf extract (reducing and capping agent) with silver nitrate. The synthesized silver nanoparticles were tested for their antibacterial and antifungal activity.

Materials and Methods

Materials

Silver Nitrate ($AgNO_3$, 99.9%) was obtained from Merck Limited, India. All glassware were washed in diluted nitric acid and rinsed thoroughly with double distilled water prior to use and dried in hot air oven. The leaves of *Sida acuta* were collected near the university campus, sector-30, Gandhinagar (Gujarat) India.

Preparation of silver nitrate solution

2 mM silver nitrate was prepared by adding 0.0339 gm of silver nitrate into 100 mL of double distilled water. The solution was mixed thoroughly and stored in brown colored bottle in order to prevent auto-oxidation of silver.

Preparation of leaf extract

Collected leaves were thoroughly washed with distilled water and dried at room temperature. 25 gm leaves were finely chopped and boiled in 100 ml sterile distilled water for 5 minutes in a 250 ml flask. The solution was filtered through Whatman No.1 filter paper. Fresh leaf extracts was used for synthesis of silver nanoparticles.

Synthesis and purification of silver nanoparticles

10 mL of the leaf extract was added to 90 mL of 2 mM aqueous silver nitrate solution (1:9 ratio) and incubated at room temperature. The colour change was observed within ten minutes. This indicated the preliminary confirmation for the formation of plant mediated silver nanoparticles. After 5 hrs. grey colored precipitates of silver nanoparticles saterted to settle down into the bottom. The solution was then centrifuge at 8,000 rpm for 15 minutes. Supernatant was discarded and pellet containing silver nanoparticles was taken. Pellet was washed thrice with distilled water by centrifugation. Finally, the pellet was taken out in petri-plate and kept in oven to dry at 50°C for 4-5 hrs. Grey colored silver nanoparticles thus obtained in powdered form.

Antimicrobial activity of silver nanoparticles and measurement of minimum inhibitory concentration (MIC)

The antimicrobial activity of the biologically synthesized Silver nanoparticles against pathogenic microorganisms Pseudomonas aeruginosa and Candida albicans was measured on Nutrient agar and MGYP plates respectively by using the

disc diffusion method [34,35]. Stock solution (1000 microgram per ml) of each compound was prepared in water. Assay carried out by taking concentration 100 microgram per disk. Hi-media antibiotics discs: Chloramphenicol (10 microgram/disk and Amphotericin-B (100 units/disk) moistened with water were used as standard. The test is performed by applying a microbial inoculum of approximately 1-2×10^8 CFU/mL to the surface of a large Nutrient agar and MGYP plates, and then different concentrations of silver nanoparticles (0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, and 1024 μg/ml) were added to the well present in both the plates. Plates are incubated for 16–24 h at 35°C prior to determination of results. After incubation, the plates were observed for the presence of a zone of inhibition.

Characterization of silver nanoparticles

Silver nanoparticles were subjected to characterization using UV-Vis spectroscopy, DLS, FTIR and TEM in order to obtain the shape, size and purity (attached functional groups) of biologically synthesized silver nanoparticles**.**

UV-VIS Spectroscopy

Reduction of silver ions into silver nanoparticles due to various biomolecules present in the *sida acuta* leaf extract was monitored by measuring the UV-Vis spectrum of the reaction medium after diluting a small aliquot of the sample by distilled water. The scanning range for the samples was 300-700 nm [32].

Dynamic Light Scattering Analysis

The confirmation of particle size, PDI with Zeta Potential is measured by the DLS (Dynamic Light Scattering, Microtrac Zetatrac, U2771, DLS XE-70, Park System equipment) [33].

FT-IR Spectroscopy Analysis

The silver nanoparticles were analyzed by FT-IR Spectrometer (Bruker, Germany) for detection of attached functional groups by showing peaks from the region of 4000 cm-1 to 450 cm-1.

Transmission Electron Microscopic Analysis

TEM shows the shape and size of the particles. The grid for TEM analysis was prepared by placing a drop of the silver nanoparticle suspension on a carbon-coated copper grid and allowing the water to evaporate inside a vacuum dryer. The grid containing silver nanoparticles was scanned by a Transmission Electron Microscope [TECHNAI (fei-optics) 200 Kv].

Results and Discussion

UV-Vis Spectral Analysis

Reduction of silver ions into silver nanoparticles due to various biomolecules present in the *Sidda acuta* leaf extract was confirmed by visible color change from yellow to brown due to surface Plasmon vibrations in the reaction medium. The UV-Vis spectrograph of synthesized silver nanoparticles shown a broad absorption peak at 440 nm due to the surface Plasmon resonance (SPR) of silver nanoparticles FIG.1. 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 silver metal ion reduction occurred rapidly; more than 90% of reduction of Ag^+ ions is complete within 2 hrs. after addition of the silver metal ions to the plant leaf extract.

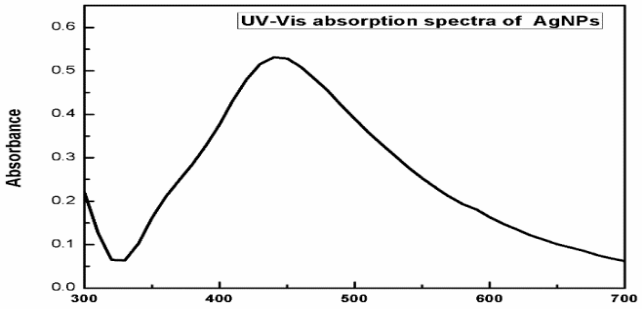

FIG. 1. **Absorption spectrum for silver nanoparticles.**

Dynamic light scattering analysis

The particles size distribution graph obtained from the DLS of silver nanoparticles synthesized by *Sida acuta* plant species is shown in the FIG. 2. The results indicate that obtained size of silver nanoparticles synthesized from *Sida acuta* plants was 222.3 nm. For the confirmation of monodispersity, DLS results indicates 0.388 PDI which depicts that the nanoparticles are well dispersed in the used solvent i.e. water. This PDI value supports well monodispersity of silver nanoparticles and confirms that the nanoparticles are not aggregated; consequently, silver nanoparticles are well dispersed. Measurement of zeta potential ranged from -20.41 mV. TABLE 1 represents the different characteristics of silver nanoparticles characterized by DLS.

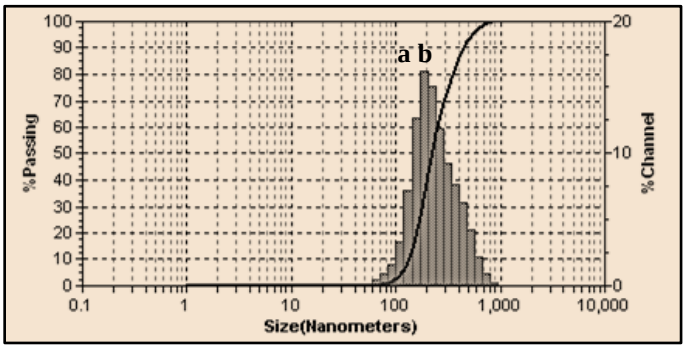

FIG. 2. **DLS graph showing size distribution of silver nanoparticles.**

TABLE 1. **DLS analysis for silver nanoparticles.**

DLS analysis	***Sida acuta***
Particle size (nm)	222.3
Particle width	236.60
PDI	0.388
Zeta potential (mV)	-20.41

FT-IR spectroscopy analysis

FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the silver nanoparticles. FTIR spectrum of silver nanoparticles showed absorption bands at 3433cm^{-1}, 2062.2 cm^{-1} and 1638.3 cm^{-1} in

FIG. 3. The band at 3433 cm^{-1} corresponds to O-H stretching and H-bonded stretching with respective functional groups of alcohols and phenols. The absorption peak at 2062.2 cm^{-1} may be assigned to the aromatic -CH stretching. The bands observed at 1638.3 cm^{-1} may be attributed to the carbonyl groups in the α-helices present in the plant extract. TABLE 2 represents a range of frequencies and their assigned functional groups in FTIR analysis.

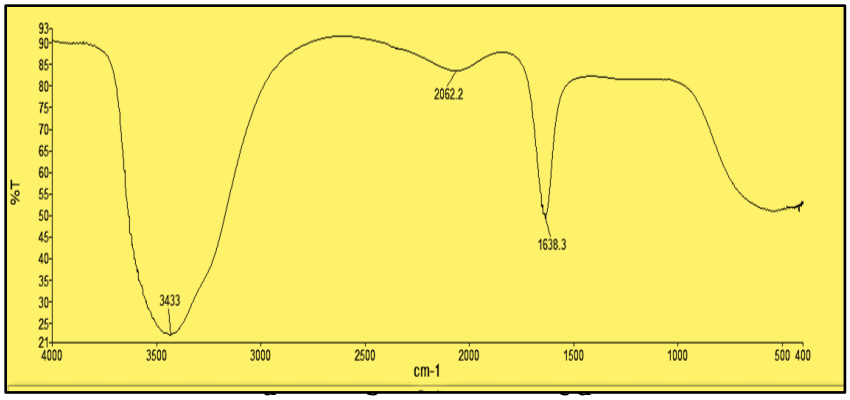

FIG. 3. **FTIR spectrum of silver nanoparticles.**

TABLE 2. **Frequencies obtained from FTIR spectra of silver nanoparticles and their assigned functional.**

Group frequency, wavenumber (cm^{-1})	Functional group/assignment
1633.3-1641.8	Secondary amine, NH bend
2047.4-2069.3	Aromatic -CH stretching
3433-3447.1	Hydroxyl group, H-bonded OH stretch

Transmission electron microscopic analysis

The TEM analysis revealed that the average mean size of silver nanoparticles was found to be 14.24 nm. Nanoparticles were seemed to be spherical in morphology with nano-crystalline structure as shown in the FIG. 4.

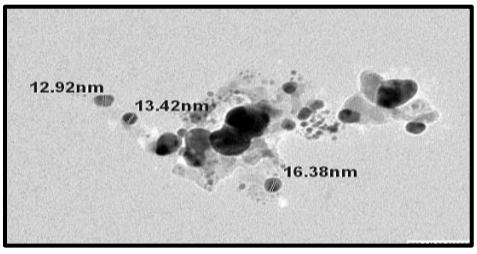

FIG. 4. **TEM image of silver nanoparticles showing average particle size as 14.24 nm.**

Antimicrobial activity of silver nanoparticles and measurement of minimum inhibitory concentration (MIC)

The silver nanoparticles exhibited inhibitory activity towards gram negative bacteria Pseudomonas aeruginosa and fungi

Candida albicans (Figure 5 and 6). The antimicrobial activity of silver nanoparticles was proved from the zone of inhibition [Table 3]. The minimal inhibitory concentration of Silver nanoparticles was found to be 4µg/ml against P. aeruginosa and 8µg/ml against C. albicans [Table 4]. Although the exact mechanism of inhibitory action of silver nanoparticles toward microbial cell is not clear but many researchers have suggested that silver nanoparticles have strong interaction with thiol groups present in the respiratory enzymes in bacteria. Silver nanoparticles adheres to bacterial surface and alters its membrane properties and ultimately causes death of bacterial cell[36]. Fungal cells have ergosterol and numerous gradients between cytoplasmic membranes to keep their membrane potential ability. The silver nanoparticles destroys theses gradients and membrane conformity and causes cell death [37]. Khushboo Singh et al. have reported the antibacterial activity of silver nanoparticles against multidrug-resistant strains of P. aeruginosa [38]. Palaniyandi Velusamy et al. reported that silver nanoparticles can induce nucleic acid DNA damage [39]. Ramamurthy et al. have also reported that silver nanoparticles can penetrate the nucleic acid DNA of gram negative bacteria and exhibits antibacterial potential by damaging the DNA at very low concentrations of silver nanoparticles [40]. In our study we have used a simple, cost effective and eco-friendly method for synthesis of silver nanoparticles. The synthesized nanoparticles showed remarkable antibacterial and antifungal activity against *P. aeruginosa* and *C. albicans.*

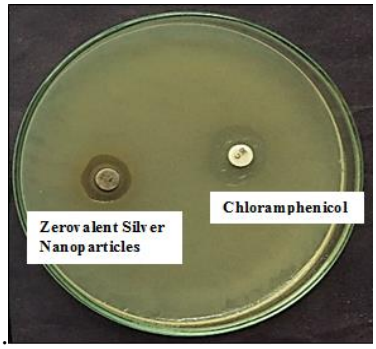

FIG. 5. **Zone of inhibition test for antibacterial activity of silver nanoparticles against *P. aeruginosa*.**

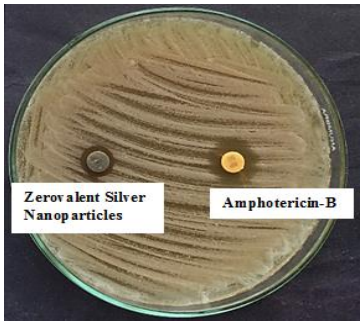

FIG. 6. Zone of inhibition test for antibacterial activity of silver nanoparticles against *C. albicans.*

TABLE 3. **Antimicrobial activity of silver nanoparticles determined by Disc Diffusion method.**

Sr. No.	Samples	Zone of Inhibition (mm)	
		Pseudomonas aeruginosa	***Candida albicans***
1	Silver nanoparticles	12.26	10.33
2	Chloramphenicol	15.13	-
3	Amphotericin B	-	12.10
Diameter in mm calculated by Vernier Caliper '-' means no zone of inhibition			

TABLE. 4. **Microbial growth at different concentrations of silver nanoparticles.**

Sr. No.	Silver nanoparticles (microgram per ml)	***P. aeruginosa***	***C. albicans***
1	1024	-	-
2	512	-	-
3	256	-	-
4	128	-	-
5	64	-	-
6	32	-	-
7	16	-	-
8	8	-	-
9	4	-	+
10	2	+	+
11	1	+	+
12	0.5	+	+
13	0.25	+	+
14	0.125	+	+
	MIC	4 µg/ml	8 µg/ml

Conclusion

Biosynthesis of silver nanoparticles using leaf extract of *Sida acuta* and their characterization was done by TEM, DLS, FTIR and UV spectroscopy and results showed that synthesized silver nanoparticles has strong antibacterial and antifungal activity against *Pseudomonas aeruginosa* and *Candida albicans*. The antimicrobial tests were carried out using disc diffusion method. The growth of inhibition ring of *Pseudomonas aeruginosa* and *Candida albicans* treated by silver nanoparticles were 12.26 and 10.33 mm, respectively. The antimicrobial activity of silver nanoparticles against of *Pseudomonas aeruginosa* and *Candida albicans* might be due to their adsorption on microbial surface, inhibition of intracellular enzyme activity or by disruption of cell wall. Results showed that silver nanoparticles, require a lower concentration to inhibit development of the *Pseudomonas aeruginosa* and *Candida albicans*.

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