

Research Article

Herbal Plant Synthesis of Antibacterial Silver Nanoparticles by *Solanum trilobatum* and Its Characterization

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Received 25 August 2014; Accepted 5 November 2014; Published 30 November 2014

Academic Editor: Hao Wang

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Green synthesis method of nanomaterials is rapidly growing in the nanotechnology field; it replaces the use of toxic chemicals and time consumption. In this present investigation we report the green synthesis of silver nanoparticles (AgNPs) by using the leaf extract of medicinally valuable plant *Solanum trilobatum*. The influence of physical and chemical parameters on the silver nanoparticle fabrication such as incubation time, silver nitrate concentration, pH, and temperature is also studied in this present context. The green synthesized silver nanoparticles were characterized by UV-vis spectroscopy, X-ray diffraction (XRD), scanning electron microscope (SEM), energy dispersive X-ray (EDX), and transmission electron microscope (TEM). The SEM and TEM confirm the synthesis of spherical shape of nanocrystalline particles with the size range of 2–10 nm. FTIR reveals that the carboxyl and amine groups may be involved in the reduction of silver ions to silver nanoparticles. Antibacterial activity of synthesized silver nanoparticles was done by agar well diffusion method against different pathogenic bacteria. The green synthesized silver nanoparticles can be used in the field of medicine, due to their high antibacterial activity.

1. Background

Nanotechnology deals with the synthesis of nanoparticles with controlled size, shape, and dispersity of materials at the nanometer scale length [1, 2]. Nanoparticles possess high surface area to volume ratio. Nanoparticles such as silver, gold, cadmium sulfide, zinc sulfide, and zinc oxide play important role in various fields [3–6]. Recently fabrication of silver nanoparticles has drawn considerable attention due to their physical and chemical properties and application in biomedicine, antiangiogenic activity against bovine retinal endothelial cells, anticancer activity against lung carcinoma cells [7], controlling HIV infection [8], detection of bacterial pathogens [9], and good catalytic activity [10]. Silver nanoparticles are having good history in the field of antimicrobial properties. The silver nanoparticles are vigorously involved in the antimicrobial activity against a lot of disease causing food borne and water borne pathogenic bacteria

and fungus [6]. Some pathogenic microbes killed by silver nanoparticles are *Bacillus subtilis*, *Klebsiella planticola*, *Bacillus* sp., *Pseudomonas* sp. [11, 12], *S. aureus*, *Vibrio cholerae*, *Proteus vulgaris* and *P. aeruginosa* [13], *Shigella dysenteriae* type I, *Staphylococcus aureus*, *Citrobacter* sp., *Escherichia coli*, *Candida albicans*, and *Fusarium oxysporum* [14].

Synthesis of silver nanoparticles has been proved by various biological and green materials such as bacteria both gram positive and gram negative like *Klebsiella pneumonia* and *Bacillus subtilis* [15, 16], *Cladosporium cladosporioides* [17], marine algae *Padina tetrastromatica* and *Turbinaria conoides* [18, 19], the green waste peels of banana fruits [20], carbohydrate molecules like polysaccharide and disaccharides starch, sucrose, and maltose, and monosaccharides like glucose and fructose [21–23]. In the green materials mediated nanoparticles synthesis plant sources have major role in the past ten years. Many plants are used for synthesizing nanoparticles including *Cinnamomum camphora* [24], *Azadirachta*

indica [25], *Nelumbo nucifera* [26], *Garcinia mangostana* [27], pomegranate, and grape fruit extracts [28, 29].

Plants have a lot of phytochemicals in their parts; they are applied in various fields. The biochemicals may play an important role in the nanoparticles synthesis [27]. Some biochemical compounds involved in synthesis of silver nanoparticles are proteins/enzymes and secondary metabolites such as terpenoids [30], some water-soluble polyhydroxy components such as alkaloids, flavonoids, and polysaccharose [31], metabolites (like organic acids and quinones) or metabolic fluxes and other oxidoreductively labile metabolites like ascorbates or catechol/photocatacheuic acid [32], verbascoside, isoverbascoside, luteolin, and chrysoeriol-7-O-diglucuronide [33], quercetin, and other phenolic compounds [34].

Solanum trilobatum Linn is an important medicinal plant of the family Solanaceae. The leaves contain rich amount of calcium, iron, phosphorus, carbohydrates, fat, crude fiber, and minerals [35]. This herb is used to treat common cold, cough, and asthma. This plant was used in the Siddha medicine system and treatment of respiratory diseases, chronic febrile infections, tuberculosis, and cardiac and liver diseases [36]. This plant also possesses the antibiotic, antimitotic, antibacterial [37, 38], and anticancer activities [39]. Sobatum, β -solamarine, solaine, solasodine, glycoalkaloid and diosgenin, and tomatidine are the constituents isolated from this plant [40]. Its pivotal action is cardiac, tonic, and carminative [41]. This plant has strong immunostimulatory effect due to the presence of alkaloids and carbohydrates. Due to these antioxidant and antibiotic properties this was used to synthesize silver nanoparticles.

In this present investigation the medically important plant is used for the synthesis of medically valued silver nanoparticles. The morphological, crystalline, and biochemical characters of green synthesized silver nanoparticles were analyzed by UV-vis spectrophotometer, scanning electron microscope, X-ray diffraction assay, transmission electron microscope, and Fourier transform infrared spectroscopy. Finally the medical property of the silver nanoparticle was characterized using antibacterial assay against *Klebsiella planticola*, *Klebsiella pneumonia*, *Bacillus subtilis*, *E. coli*, *Serratia* sp., and *Streptococcus* sp.

2. Materials and Methods

Silver nitrate, Luria Bertani agar, and nutrient broth were purchased from Himedia, Mumbai. Leaves of *Solanum trilobatum* were collected from Sri Paramakalyani Centre for Environmental Sciences, MS University, Alwarkurichi.

2.1. Preparation of Leaf Extract. About 10 g of fresh leaves of *S. trilobatum* was thoroughly washed 2-3 times with distilled water for surface cleaning, and surface sterilized with 0.1% HgCl_2 for 1 min to reduce microbial contamination [42]. The sterile leaves were cut into fine pieces and boiled with 100 mL of double distilled water for 15 min at 60°C and filtered through Whatman number 1 filter paper and stored at 4°C in refrigerator for 2 weeks.

2.2. Synthesis of Silver Nanoparticles. In the typical synthesis of silver nanoparticles, 10 mL of leaf extract was treated with 90 mL of 1 mM silver nitrate solution and kept in room temperature. Subsequently the synthesis of silver nanoparticles was initially identified by brown colour formation and further monitored by measuring UV-vis spectra of the reaction mixture.

To study the effect of parameters such as reaction time, silver nitrate concentration, pH, and temperature on the nanoparticles synthesis the reaction was carried out by the following experiments. Silver nitrate and leaf extract reaction mixture was kept at room temperature and formation of nanoparticles was recorded at different functional times. Influences of silver nitrate concentration (1 to 5 mM, pH: 5.5, temperature: 35°C), pH (3.5, 4.5, 5.5, 7.5, and 9.5, silver nitrate: 1 mM, temperature: 35°C), and temperature (20°C, 35°C, 45°C, and 70°C, silver nitrate: 1 mM, pH: 5.5) were performed to find their effects on nanoparticles synthesis.

2.3. Characterization of Synthesized Silver Nanoparticles. Synthesis of silver nanoparticles was initially characterized by position of SPR band by measuring double beam UV-vis spectroscopy at different wavelengths from 360 to 700 nm. Crystal structure was characterized by XRD at 2θ ranges from 10 to 90° (Philips PW 1830); shape and size were analysed by using SEM (Philip model CM 200) and TEM (JEOL3010). Elemental composition was performed by EDAX (Philips XL-30). FTIR spectrum of silver nanoparticles was obtained on a SHIMADZU instrument with the sample as KBR pellet in the wave number region of 500–4,000 cm^{-1} .

2.4. Antibacterial Activity of Synthesized Silver Nanoparticles. The antibacterial activity of synthesized silver nanoparticles was performed by agar well diffusion method against pathogenic bacteria, *Klebsiella planticola*, *Klebsiella pneumonia*, *Bacillus subtilis*, *E. coli*, *Serratia* sp., and *Streptococcus* sp. Fresh overnight culture of each strain was swabbed uniformly onto the individuals' plates containing sterile Luria Bertani agar and 5 wells were made with the diameter of 6 mm. Then 25 μL of purified silver nanoparticles, leaf extract, and silver nitrate solution were poured into each well and commercial antibiotic discs are placed as control and incubate for 24 h at 37°C. After incubation the different levels of zonation formed around the well and it was measured. This experiment was repeated for three times.

3. Results and Discussion

3.1. Visual Observation. Silver nanoparticles formation was primarily identified by colour change visually. *S. trilobatum* leaf extract was treated with silver nitrate aqueous solution showed a colour change from yellow to brown within 2 min (Figure 1). The colour change was clear indication for the formation of silver nanoparticles [43]. This brown colour of silver nanoparticles arises due to the surface plasmon vibrations in the aqueous solution [44].

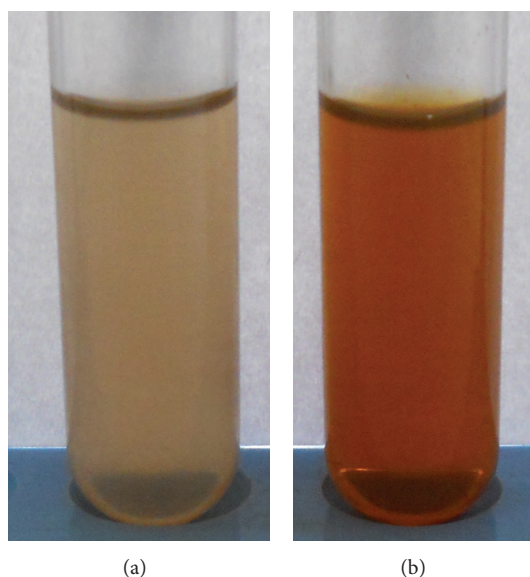


FIGURE 1: Formation of silver nanoparticles was identified by colour change (a) and leaf extract of *S. trilobatum* (b) after the reduction of silver nitrate.

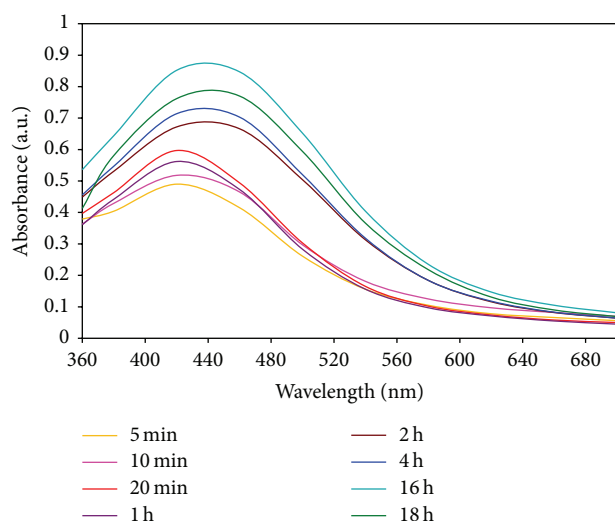


FIGURE 2: Effect of reaction time on silver nanoparticles synthesis by leaves extract of *S. trilobatum*.

3.2. UV-Vis Spectrophotometer

3.2.1. Effect of Reaction Time. Figure 2 shows the time dependant synthesis of silver nanoparticles. The UV-vis spectroscopy method can be used to track the size evolution of silver nanoparticles based on localized surface plasmon resonance band exhibited at different wavelengths. The optical properties of silver nanoparticles are related to excitation of plasmon resonance or interband transmission particularly on the size effect. Figure 2 shows the UV-vis spectra obtained from solution at different reaction times. The spectra show peaks at 420 nm at the time of 20 min. With the increase in reaction time, UV-vis spectra show sharp narrow peak

in 20 min which indicates the formation of disaggregated nanoparticles. This single and strong band indicates that the particles are isotropic in shape and uniform size [44]. After 20 min the peak shifts to 440 nm. By increasing the reaction time the synthesis of nanoparticles also increased by the leaf extract of *S. trilobatum*. Maximum production of nanoparticles was confirmed by maximum absorption which occurs in the UV-vis spectra. The narrow peak and increasing absorbance were observed from 5 min to 4 h without any shift of plasmon resonance band. The absorbance was increased at the incubation time of 16 h and broad band was formed at 460 nm. The reaction is completed at 4 h and is visually identified by appearance of precipitation in the bottom of the flask.

3.2.2. Effect of Silver Nitrate Concentration. In Figure 3 UV-vis spectra show the SPR band at 440 nm in the 1 mM concentration of silver nitrate. The silver nanoparticles were formed at 1 mM silver nitrate solution without aggregation and also it shows monodispersed nanoparticles formation. But the 2–4 mM concentration shows the band at 460 nm with aggregation; 5 mM concentration of silver nitrate solution treated with leaf extract shows the band at 500 nm with broad peak which indicates that the particles are polydispersed. The SPR spectra of silver nanoparticles broadened with the increase of the initial AgNO_3 concentration. Similarly reported by Huang et al. [45], synthesize the silver nanoparticles at different initial concentrations using leaf extract of *Cacumen platycladi*.

3.2.3. Effect of pH. Figure 4 shows the effect of pH on the synthesis of silver nanoparticles. The pH of leaf extract is found to be 6.8; the pH of extract was altered to 5.8, 7.8, and 8.8 to attain the maximum synthesis of silver nanoparticles. The lower pH suppresses the nanoparticles formation due

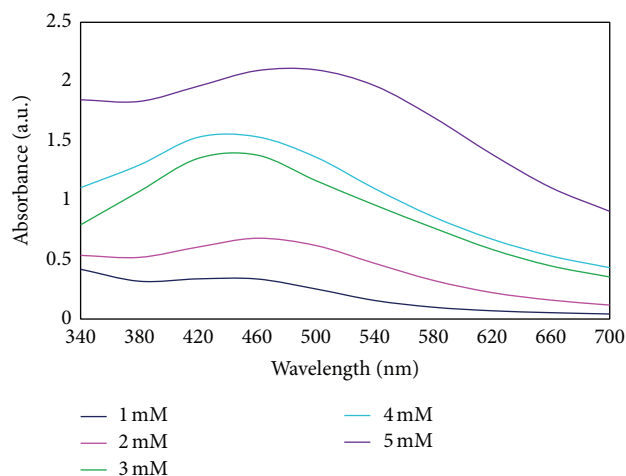


FIGURE 3: Effect of silver nitrate concentration on silver nanoparticles formation.

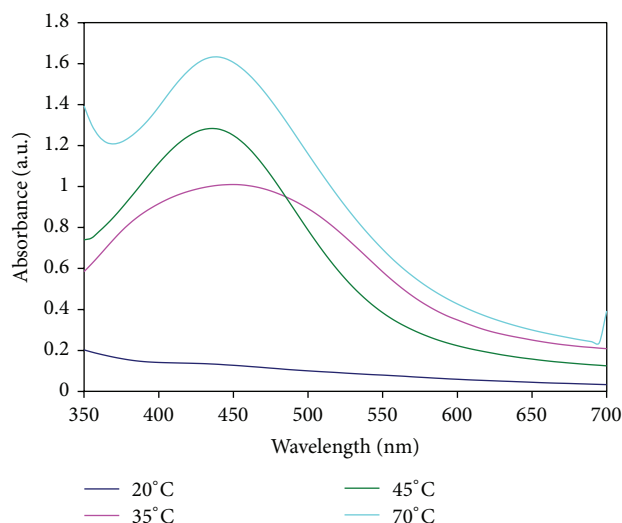


FIGURE 5: Effect of temperature on nanoparticles synthesis.

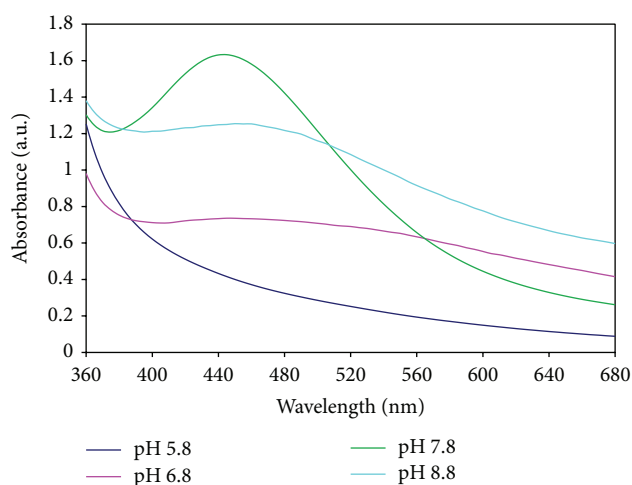


FIGURE 4: Effect of pH on silver nanoparticles synthesis.

to low availability of functional groups of leaf extract. At high pH of 8.8 the absorption band was obtained at 480 nm with broad peak. But the pH of 7.8 shows the narrow peak positioned at 440 nm with maximum production of silver nanoparticles.

3.2.4. Effect of Temperature. UV-vis spectra are obtained from solution at different reaction temperatures (Figure 5). In the low temperature of 20°C there is no SPR band for silver nanoparticles. The spectra show peaks at 440 nm (20°C), 450 nm (45°C), and 460 nm (35°C). These peaks are characteristic Plasmon band for silver nanoparticles. With the increase in reaction temperature UV-vis spectra show sharp narrow peak at lower wavelength regions 440 nm. Maximum production of silver nanoparticles was obtained at high temperature. The SPR peaks of the AgNPs significantly underwent blue shift, suggesting that the same particle shape strongly influences the SPR band in the aqueous solution at higher temperature [46].

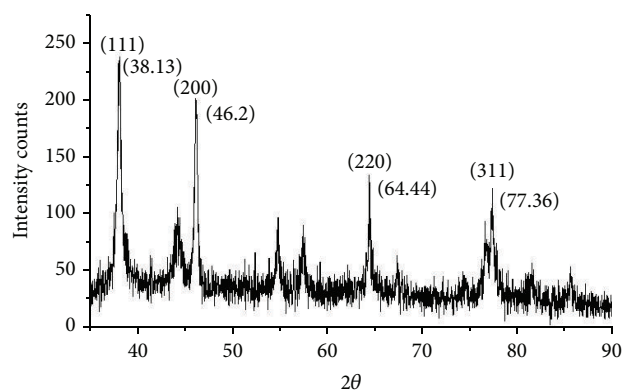


FIGURE 6: XRD analysis of *S. trilobatum* leaf extract mediated synthesized AgNPs.

3.3. X-Ray Diffraction Analysis. Structural and crystalline nature of the silver nanoparticles has been performed using XRD analysis. Figure 6 shows that the biosynthesized silver nanostructure by using *S. trilobatum* leaf extract was demonstrated and confirmed by the four characteristic peaks observed in the XRD image at 2θ values ranging from 30 to 90. The four intense peaks are 38.13°, 46.2°, 64.44°, and 77.36° corresponding to the planes of (111), (200), (220), and (311), respectively. These lattice planes were observed which may be indexed based on the face-centered crystal structure of silver (JCPDS file number 04-0783). The XRD pattern thus clearly showed that the Ag-NPs are crystalline in nature. Similar report was obtained using cell filtrate of *Streptomyces* sp. ERI-3 synthesized extracellularly [40].

3.4. Scanning Electron Microscopy. The SEM image (Figure 7) showing the high density Ag-NPs synthesized by using the leaf extract of *S. trilobatum* further confirmed the development of silver nanostructures. Obtained nanoparticle showed that Ag-NPs are spherical shaped and monodispersed and

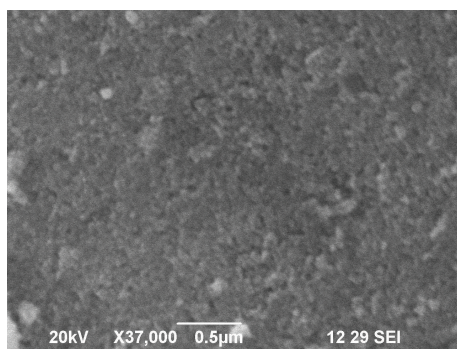


FIGURE 7: Morphology of silver nanoparticles showed by SEM.

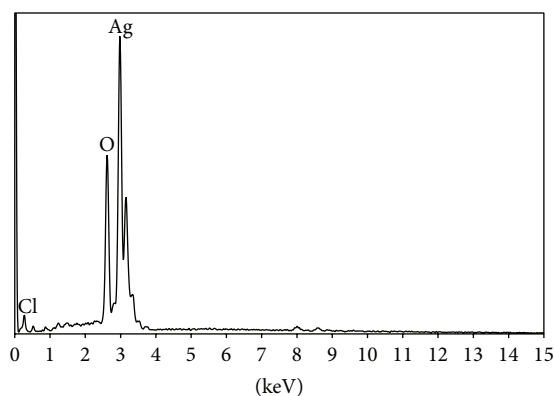


FIGURE 8: Elemental analysis of silver nanoparticles using leaf extract of *S. trilobatum*.

well distributed with aggregation in the size range about 50–70 nm (scale bar 500 nm). Similarly monodispersed silver nanoparticle was reported by using the extract of *Coccinia grandis* leaf extract [43].

3.5. Energy Dispersive X-Ray (EDX) Analysis. Analysis through energy dispersive X-ray (EDX) spectrometers confirmed the presence of elemental silver signal of the silver nanoparticles. The vertical axis displays the number of X-ray counts whilst the horizontal axis displays energy in keV. The EDX spectrum (Figure 8) observed a strong signal from the silver atoms in the nanoparticles at 3 keV and weak signal from “Cl” and “O.” These weak signals are from the plant organic constituents. This analysis revealed that the nanostructures formed were solely of silver.

3.6. Transmission Electron Microscopy. Morphological and size characters of *S. trilobatum* mediated synthesized silver nanoparticles were characterized by TEM (Figure 9). TEM image revealed that the nanoparticles were evenly distributed without agglomeration. Synthesized silver nanoparticles were mostly spherical and their dimensional ranges were from 2 to 10 nm. Thus TEM characterization studies confirm that the synthesized silver nanoparticles were in nanometer size range.

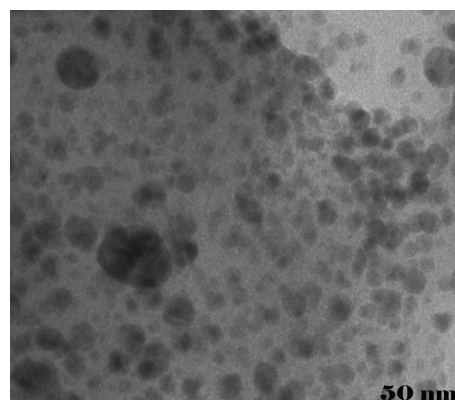


FIGURE 9: TEM analysis of synthesized silver nanoparticles shows well dispersed spherical shape nature.

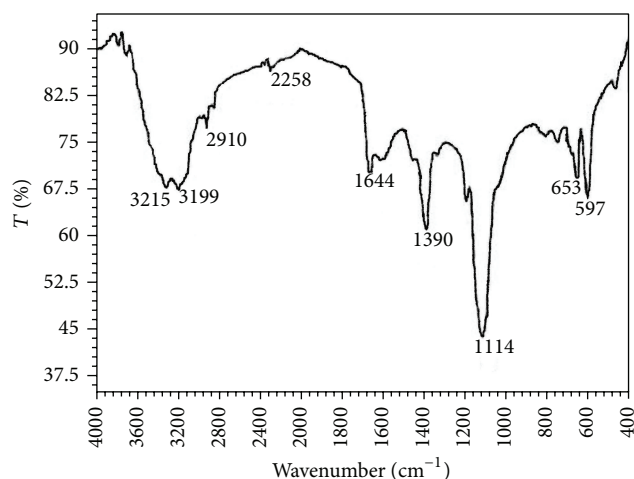


FIGURE 10: FT-IR spectrum of silver nanoparticles synthesized by *S. trilobatum*.

3.7. Fourier Transform Infrared Spectroscopy. Figure 10 shows that the FTIR image of *S. trilobatum* leaf mediated synthesized silver nanoparticles indicates presence of biomolecules involved in the reduction process. The minor peak found at 3215 cm⁻¹ represents the –NH stretch of primary and secondary amines or amides. The same peak may be due to the –OH stretch of alcohols and phenols; the peak at 3199 cm⁻¹ is due to the –OH stretch of carboxylic acids; the smaller peak at 2920 cm⁻¹ shows –CH stretch of alkanes and –OH stretch of carboxylic acids, 2258 cm⁻¹ corresponding to –CN stretch of nitriles; 1644 cm⁻¹ indicates –CC– stretch of alkenes and –NH bend of primary amines, 1390 cm⁻¹ for CH₂ and CH₃ deformation, CH₃ deformation, and CH₂ rocking; 1114 cm⁻¹ shows –CN stretch of aliphatic amines; 653 and 597 cm⁻¹ are corresponding to C–Cl and C–Br stretch of alkyl halides and –CH bend of alkynes. FT-IR reveals that carboxyl and amine groups may be involved in the reduction and stabilizing mechanism.

TABLE 1: Zone of inhibition against pathogenic bacteria using green synthesized silver nanoparticles.

Antibacterial agent	Zone inhibition (mm in diameter)					
	<i>B. subtilis</i>	<i>Streptococcus</i> sp.	<i>Serratia</i> sp.	<i>E. coli</i>	<i>K. planticola</i>	<i>K. pneumonia</i>
Leaf extract	6.4 ± 0.23	6.77 ± 0.14	7.00 ± 0.57	6.84 ± 0.12	6.63 ± 0.33	6.71 ± 0.18
Silver nitrate solution	9.0 ± 0.57	12.73 ± 0.14	11.40 ± 0.21	10.43 ± 0.18	10.3 ± 1.2	12.97 ± 0.12
Silver nanoparticles	11.0 ± 0.30	13.13 ± 0.13	12.33 ± 1.20	14.9 ± 0.07	14.1 ± 0.17	13.9 ± 0.09
Commercial antibiotic disc	10.8 ± 0.16	12.57 ± 0.23	11.33 ± 0.66	10.00 ± 0.57	10.3 ± 0.33	11.17 ± 0.60

3.8. Antibacterial Activity of Silver Nanoparticles. Antibacterial activity of synthesized silver nanoparticles was performed against *Streptococcus* sp., *Serratia* sp., *Bacillus subtilis*, *K. pneumonia*, *K. planticola*, and *E. coli* by well diffusion method. The antibacterial activity of synthesized silver nanoparticles was compared with plant extract, silver nitrate, and commercial antibiotic disc. The zone of inhibition was measured and denoted in millimeter (mm) in diameter. The zone of inhibition in diameter was tabulated by performing triplicate experiments (Table 1). Among the four antibacterial agents, silver nanoparticles highly inhibit the growth of pathogenic bacteria. Highest inhibition was noted against *E. coli*, *Streptococcus* sp., and *K. pneumonia*.

4. Conclusion

In this study we successfully demonstrated that *S. trilobatum* leaf extract has the ability to synthesize the nanoparticles. Nanoparticles synthesis initiated within 5 min and gradually increased up to 16 h. At pH 7.8 and temperature 70°C maximum yield of nanoparticles was observed. The size of the silver nanoparticles ranges from 50 to 70 nm, predominantly spherical shapes with crystalline nature. Elemental analysis by EDX shows that strong peak at 3 keV confirms the presence of silver nanoparticles. Carboxyl and amine groups from plant extract may be involved in the bioreduction process of silver ions to nanoparticles and stabilizing mechanism confirmed by FT-IR analysis. The silver nanoparticles show high antibacterial activity when assayed by agar well diffusion method. This green synthesized nanoparticle could be used in the medical field against human diseases due to their high efficiency as antibacterial agent.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

The authors gratefully acknowledge the DST-FIST sponsored programme, DST, New Delhi, India, for funding the research development (reference no. S/FST/ESI-101/2010) to carry out this work.

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