




Green Synthesis, Characterization and Antimicrobial Activity of Silver Nanoparticles (AgNPs) Using Leaves and Stems Extract of Some Plants

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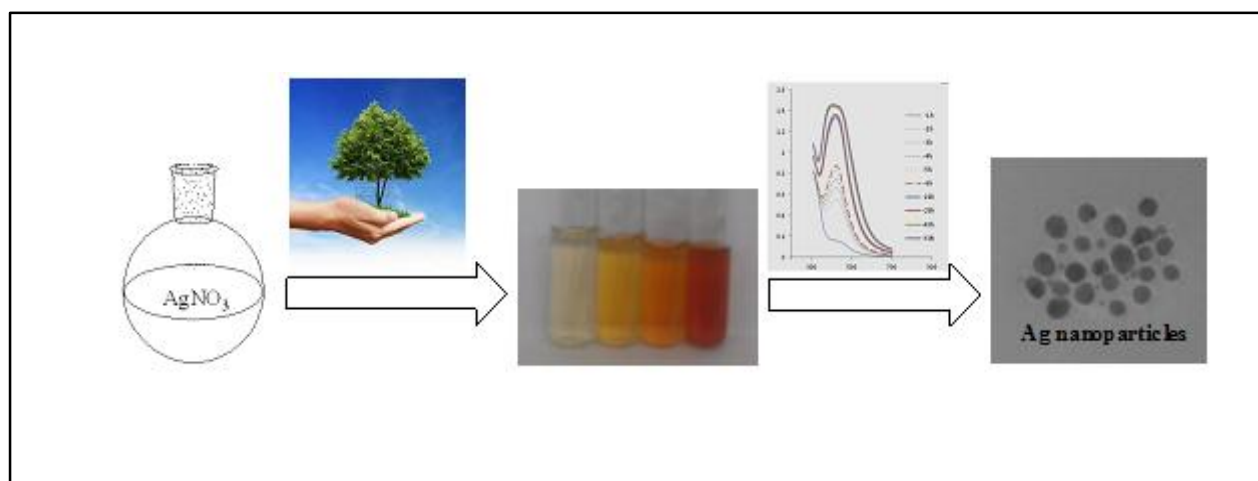
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ABSTRACT

Green synthesis of silver nanoparticles using the aqueous extract of *Ferula gumosa*, *Ferula latisecta*, *Teucrium polium* and *Trachomitum venetum* leaves and stems extract as the reducing and stabilizing agents. This synthesis shows attractive characteristics such as; the use of inexpensive and available plant extracts, non-toxicity, eco-friendly biological materials, and operational simplicity. The extracts incubated with AgNO_3 solution showed gradual change in color of the extracts to yellowish brown, with intensity increasing during the period of incubation. The nanoparticles were characterized by UV-visible absorption spectroscopy, X-ray diffraction (XRD) spectroscopy, Dynamic Light Scattering (DLS) and Transmission Electron Microscope (TEM). The silver nanoparticles synthesized were generally found to be spherical in shape with variable size ranging from 5 to 30 nm, as evident by TEM. The biosynthesized silver nanoparticles (AgNPs) showed good antibacterial activity against clinical strains of two bacteria *Staphylococcus aureus* and *Escherichia coli*.

GRAPHICAL ABSTRACT



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Introduction

In the last decades, the synthesis of nanoparticles and study of them have attracted significant attention of scientists from both fundamental and applied research area. Nanotechnology refers to study and development at the atomic, molecular and macromolecular scale leading to the controlled manipulation and study of structure and devices with length scales in the 1–100 nm range. This definition reveals the fact that quantum mechanical effects are important at this scale; therefore, the definition shifted from a particular technological target to a research category inclusive of all field of research and technologies that deal with the special properties of matter that occur below the given size threshold [1-4].

Metal nanoparticles (MNPs) belong principally to the engineered type of nanoparticles. These nanoparticles (MNPs) such as silver, gold, and copper have received remarkable attraction because of their electronic, catalytic, and unique optical properties making them very attractive in the fields of particularly sensing, bio-conjugation, and surface enhancement Raman spectroscopy. In recent years, a whole bunch of synthetic methods for the preparation of MNPs have been developed: chemical, photochemical, and thermal methods. Among different methods, there is a rising attention to use biological and green techniques to produce a variety of MNPs [5,6]. Among the MNPs, silver nanoparticles play a significant role in the field of biology and medicine. Also, silver nanoparticles are of great interest these days because of their surface Plasmon resonance [7], optical properties [8], catalytic action [9], excellent antimicrobial activity [10], etc. In the past few years, researchers have demonstrated a significant antimicrobial effect of silver nanoparticles to fight against infections and diseases [11-15].

Although a number of methods are being adapted for the synthesis of metal nanoparticles, the most frequently used method is the chemical reduction of metal ions into nanoparticles followed by their stabilization [16–22]. Most of these methods are very expensive and also involve the use of toxic and hazardous chemicals, which may pose potential environmental and biological risks. Hence, material scientists and nanochemists are looking forward to use eco-friendly substances to achieve metal nanoparticles. Nanobiotechnology deals with the synthesis of nanostructures using living organisms. Among the use of living organisms for nanoparticle synthesis, plants have found application particularly in metal nanoparticle synthesis. Use of plants for synthesis of nanoparticles could be advantageous over other environmentally benign biological processes as this eliminates the elaborate process of maintaining cell cultures. Biosynthetic processes for nanoparticles would be more helpful if nanoparticles were produced extracellular using plants or their extracts and in a controlled manner according to their size, dispersity, and shape. Plant use can also be suitably scaled up for large-scale synthesis of nanoparticles [23-27].

Experimental

Materials

Chemicals: Silver nitrate was purchased from Merck and used as received. Distilled deionized water was used throughout the reactions. The plants were collected (*Ferula Gumosa* from Abdanan Ilam Iran, *Ferula Latisecta* from Hezar masjed mountain Khorasan Iran, *Teucrium Polium* from Shahrestanak Tehran Iran, and *Trachomitum Venetum* from Galandoak Tehran Iran) and washed with sterile distilled water and shadow-dried in room temperature in 10 days.

Bacterial strains: The assessment of antibacterial activity was carried out using two different strains. The following microorganisms were used: Standard strains of *Staphylococcus aureus* (ATCC 25923), and *Escherichia coli* (ATCC 9763).

Methods

Preparation of plant aqueous extracts

Aqueous extracts were prepared by mixing 5 g of dried fresh leaves and stems powder of various plants with 100 mL of water with constant stirring on a magnetic stirrer. The mixture was boiled for 15 min before being decanted, and the mixture was cooled and filtered through what man No. 1 filter paper. The boiled extract was refrigerated and used for further experimental procedures.

Synthesis of silver nanoparticles

For all experiments, silver nitrate solution (1 mM) was prepared by adding AgNO₃ in Millipore water. The reaction mixtures containing silver nitrate solution and plant extracts agitated in incubator and synthesis of silver nanoparticles was monitored at different time intervals and the nanoparticles that were formed were characterized further. Extract of two organs of various plants were investigated in this research. The effect of the plant extract was determined by varying the concentration of plant extract in constant silver nitrate solution. To study the effect of temperature on nanoparticle synthesis, reaction mixtures containing plant extract, and 1.0 mM AgNO₃ were incubated at 30, 50, and 70 °C.

Characterization of silver nanoparticles

The synthesis of silver nanoparticles was monitored by sampling the reaction incubated mixture at various times and the absorption maxima was scanned by UV-visible spectra, at the wavelength of 300–700 nm in Perkin

Elmer precisely Lambda 25 spectrophotometer operated at a resolution of 1 nm. The solution mixture was subjected to centrifugation at 12,000 rpm for 20 min; resulting pellet was dissolved in deionized water and filtered through 0.22 μm Millipore filter. An aliquot of this filtrate containing silver nanoparticles was used for XRD, DLS, and TEM analysis. X-ray diffraction (XRD) studies on thin films of the nanoparticle reaction mixtures were carried out using a P analytical X'Pert-Pro instrument in the transmission mode with Cu Kα radiation and with $\lambda = 1.54 \text{ \AA}$. Dynamic light scattering (DLS) was indicated with Zetasizer Nano ZSP, ZEN5600 instrument. Transmission electron microscopic (TEM) was performed with a Jeol JEM 2100UHR, operated at 200 kV that were previously air-dried.

UV-Vis spectrophotometer analysis

The synthesized silver nanoparticles were first characterized by UV-visible spectrophotometer in the range of 300-700 nm using a quartz cuvette with control as reference. The reduction of silver ions was monitored by measuring the UV-Vis spectrum of the reaction medium. The surface Plasmon resonance peaks are found noted to be reliably around 400-450 nm. It is reported that the absorption spectrum of spherical silver nanoparticles presents a maximum between 420 nm and 450 nm.

X-ray diffraction (XRD)

The crystalline nature of silver nanoparticles is further confirmed from X-ray diffraction (XRD) analysis. The sample for XRD analysis was prepared by depositing the centrifuged sample on a microscopic glass slide and air-dried overnight. The diffractogram was recorded using a PAnalytical X'Pert-Pro instrument in the transmission mode with Cu Kα radiation and with $\lambda = 1.54 \text{ \AA}$.

Particle size distribution

Particle size distribution of synthesized silver nanoparticles suspension was characterized by Dynamic light scattering (DLS) using a Zetasizer Nano ZSP instrument (ZEN5600, Malvern).

TEM analysis

Size and shape distribution of produced silver nanoparticles were characterized by transmission electron microscopic (TEM) study a few drops of silver nanoparticle solution were dropped onto a TEM grid, and the residue was removed by a filter paper beneath the TEM grid.

Antimicrobial activity

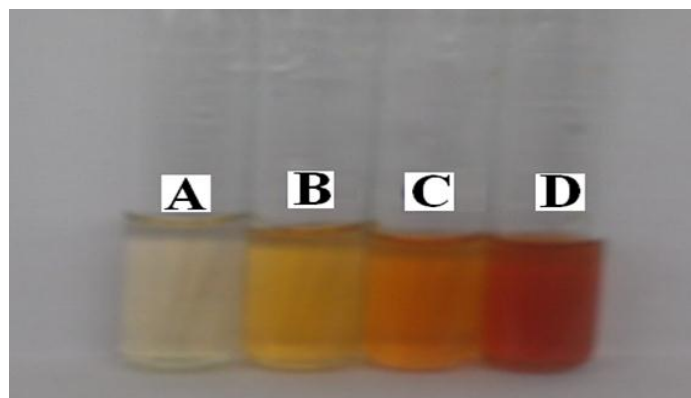
The biologically synthesized silver nanoparticles using medicinal plants were found to be highly toxic against different pathogenic bacteria. Hence, synthesized silver nanoparticles in this research were tested for antimicrobial activity using agar gel diffusion method against pathogenic bacteria, *E. coli*, and *S. aureus*. Approximately 20 mL of molten and nutrient agar media was poured into sterilised petri dishes. The plates were left overnight at room temperature to allow any contamination to appear. The bacterial test organisms were grown in nutrient broth for 24 h. A 100 mL nutrient broth culture of each

bacterial organism was used to prepare bacterial lawns. Silver colloidal solution (40 μ L) was applied on a thick paper disk of 0.5 cm diameter, dried and then applied on the surface of the media plate. The plates containing the bacterial and AgNPs were incubated at 37 °C for 24 h, and then examined for evidence of zones of inhibition, which appear as a clear area around the wells. The diameter of such zones of inhibition was measured using a metre ruler, and the mean value for each organism was recorded and expressed in millimeters. Our characterization results clearly confirmed other related reports regarding the preparation of Ag nanoparticles by various plants 28-30.

Results and discussion

Silver nanoparticles were synthesized using leaves and stems extract of various plants. Surprisingly, silver nanoparticles were synthesized simply within various incubation periods. Reduction of silver ions to silver nanoparticles could be followed by a color change. In our research, the aqueous silver nitrate solution was turned to yellowish brown color within appropriate time, with the addition of plant extract (for example color changes in silver nanoparticle synthesis by leaf extract of *F. Latisecta* in various times is shown in Figure 1.

Figure 1. Aqueous Solution of 1 Mm Agno 3 with *F. Latisecta* Leaf Extracts. After 1h Incubation (A), After 8h Incubation (B), After 20h Incubation (C), After 60h Incubation (D)



One of the most widely used techniques for structural characterization of prepared silver nanoparticles is UV-Vis spectroscopy. Silver nanoparticle formations from silver ions show an absorption peak around 420 nm. It was due to the excitation of surface plasmon resonance (SPR) effect and reduction of AgNO_3 . The silver surface plasmon resonance was observed at 420 nm which steadily increases in intensity as a function of time of reaction (ranging from 1 h to 60 h) without showing any shift of the wavelength maximum. Four plant spices were used in this research. Extracts of leaf and stem organs were used for the synthesis of silver nanoparticles. At first, 0.5 mL of the each plant organ extracts was added to a 20 mL solution of silver nitrate. The samples were incubated for several time intervals at 30 °C for testing silver

nanoparticles preparation. Formation of silver nanoparticle was further checked at different time intervals by UV-Vis spectroscopy. The results are shown in Table 1.

As can be seen in Table 1, after 48 h the largest growth rates of silver nanoparticle synthesis occurred by *F. Latisecta* leaves extract. So, leaves extract of *F. Latisecta* was chosen as appropriate medium for the synthesis of silver nanoparticles.

The effect of substrate concentration is a key parameter that affects the process of nanoparticles synthesis. Hence, the synthesis of silver nanoparticles via leaves extract of *F. Latisecta* was investigated in various concentrations of extract in constant silver nitrate concentrate in 30 °C. The results are shown in Table 2.

Table 1. UV-visible spectroscopy results. Absorption in λ_{max} for various plants extracts ^a

Plant Organs	A: in λ_{max}					
	after 2 h	after 6 h	after 12 h	after 24 h	after 48 h	after 60 h
<i>Ferula Latisecta</i> leaves extract	0.178	0.358	0.411	0.676	1.125	1.127
<i>Ferula Gumosa</i> leaves extract	0.084	0.138	0.324	0.477	0.802	0.901
<i>Teucrium Polium</i> leaves extract	0.045	0.086	0.217	0.305	0.345	0.377
<i>Trachomitum Venetum</i> leaves extract	0.103	0.257	0.304	0.372	0.465	0.488
<i>Ferula Latisecta</i> stems extract	0.128	0.263	0.328	0.567	0.926	0.946
<i>Ferula Gumosa</i> stems extract	0.894	0.104	0.297	0.387	0.761	0.788
<i>Teucrium Polium</i> stems extract	0.028	0.064	0.164	0.208	0.342	0.367
<i>Trachomitum Venetum</i> stems extract	0.986	0.187	0.238	0.311	0.381	0.392

^a Reaction condition: 0.5 mL of the each plant organ extracts in a 20 mL solution of 1 mM silver nitrate at 30 °C

Table 2. Various concentration of *F. Latisecta* leaves extract in constant silver nitrate concentrate in 30 °C^a

Concentration	0.1 mL extract	0.3 mL extract	0.5 mL extract	1 mL extract	2 mL extract
A: in λ_{\max} after 6 h in 30 °C	0.092	0.156	0.358	0.362	0.360

^a All these amounts of extracts were added in 20 mL of 1 mM silver nitrate solution

As can be seen in Table 1, after 48 h the largest growth rates of silver nanoparticle synthesis occurred by *F. Latisecta* leaves extract. So, leaves extract of *F. Latisecta* was chosen as appropriate medium for the synthesis of silver nanoparticles.

The effect of substrate concentration is a key parameter that affects the process of nanoparticles synthesis. Hence, the synthesis of silver nanoparticles *via* leaves extract of *F. Latisecta* was investigated in various concentrations of extract in constant silver nitrate concentrate in 30 °C. The results are shown in Table 2.

As shown in Table 2, 0.5 mL of leaves extract of *F. Latisecta* in 20 mL of 1 mM silver nitrate solution is a suitable concentrate for the synthesis of silver nanoparticles.

In continue, for chose of suitable temperature, synthesis of silver nanoparticles *via* leaves extract of *F. Latisecta* in 0.5 mL extract in 20 mL of 1 mM silver nitrate solution was investigated in three temperatures (30 °C, 50 °C, and 70 °C).

As can be seen in Table 3, in 70 °C the largest growth rates of silver nanoparticle synthesis occurred by *F. Latisecta* leaves extract. So, this temperature was chosen as appropriate temperature for the synthesis of silver nanoparticles.

Encouraged by this achievement, synthesis of large amount of silver nanoparticles was done in appropriate conditions in *F. Latisecta* leaves extract and formation of silver nanoparticle was further checked at different time intervals by UV-visible spectroscopy (Figure 2).

The dry powders of the silver

nanoparticles were used for XRD analysis. The diffracted intensities were recorded from 10° to 80° at 2 theta angles. The diffraction pattern in Figure 3 corresponds to pure silver metal powder. The obtained results illustrate that silver ions had indeed been reduced to Ag⁰ by *F. Latisecta* leaves extract under reaction conditions.

The size distribution histogram of dynamic light scattering (DLS) indicates that the size of these silver nanoparticles is around 31 nm. Some distribution at lower range of particle size indicates that the synthesized particles are also in lower range of particle size. Figure 4 shows the DLS pattern of the suspension of Ag nanoparticles synthesized using plant extract.

To gain further insight into the features of the silver nanoparticles, analysis of the sample was performed using TEM technique. The shape and size of the obtained silver nanoparticles were elucidated with the help of TEM images. Nanoparticles observed from the micrograph majority are spherical with a small percentage of elongated particles and ranged in size of 5–30 nm with an average size of 20 nm in Figure 5.

Finally, antimicrobial activity of the above synthesized silver colloid solution was determined on pathogenic gram negative bacteria like *E. coli* and gram positive bacteria like *S. aureus* by disc diffusion method (Table 4). This test was performed on nutrient agar plates spread with microbial culture. Silver colloidal solution (40 µL) was applied on a thick paper disk of 0.5 cm diameter, dried and then applied on the surface of the media plate. These plates were incubated to 24 h at 37 °C.

Table 3. Temperature effect on synthesis of silver nanoparticles in *F. Latisecta* leaf extract^a

Temperature	30 °C	50 °C	70 °C
A: in λ_{\max} after 6 h	0.358	0.791	0.945
A: in λ_{\max} after 24h	0.676	1.062	1.332
A: in λ_{\max} after 48h	1.125	1.331	1.432
A: in λ_{\max} after 60h	1.132	1.343	1.441

^a 0.5 mL of leaf extract in 20 mL of 1 mM silver nitrate solution

Figure 2. UV-visible spectrum of aqueous 1 Mm silver nitrate with *F. Latisecta* leaf extract at different time intervals in 70 °C with 0.5/20 concentration

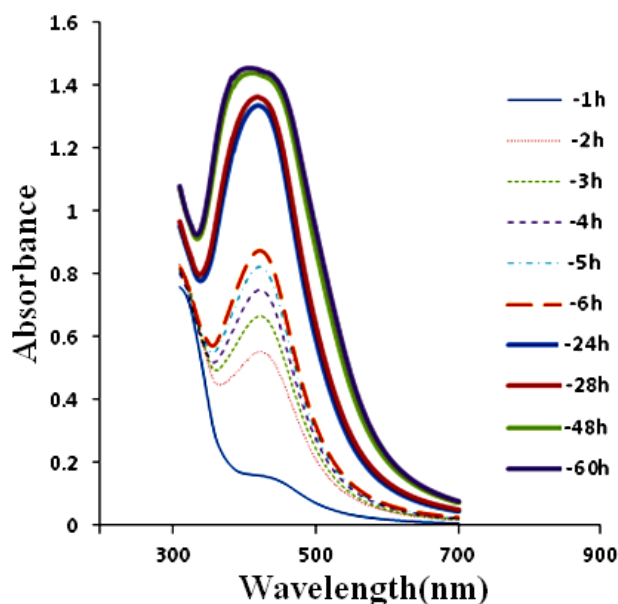
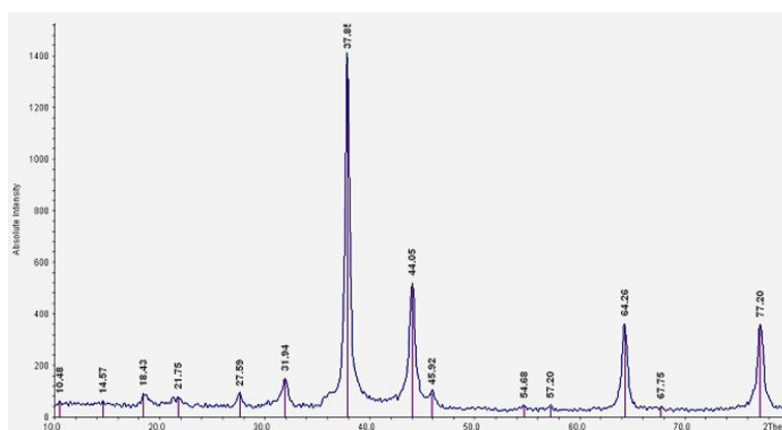


Figure 3. The X-ray diffraction patterns of biosynthesized silver nanoparticles



The susceptibility of test organisms was determined after 24 h by measuring inhibition zone around each disk in mm. The results of antimicrobial activity were compared with four types of antibiotics as control. We also checked for antimicrobial activity of 1 mM AgNO₃ solution which did not show the visible zone. The great inhibitory effect was

observed against *Staphylococcus aureus* with a zone of inhibition of 12 mm diameter followed by *Escherichia coli* with a zone of inhibition of 9 mm diameter. The results are shown in Table 4 and Figure 6 using Graph Pad Prism. ®. 6.0 Software.

As can be seen in Table 4, the synthesized silver nanoparticles by *F. Latisecta* leaves

extract have good antibacterial activity against *Staphylococcus aureus* and

Escherichia coli that it is comparable with some antibiotics.

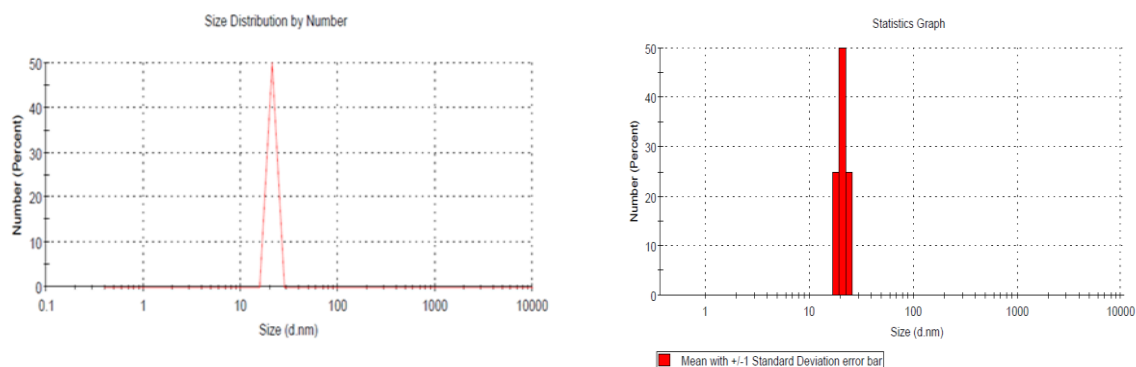


Figure 4. DLS histogram and tem image of synthesized silver nanoparticles

Figure 5. The TEM image of biosynthesized silver nanoparticles

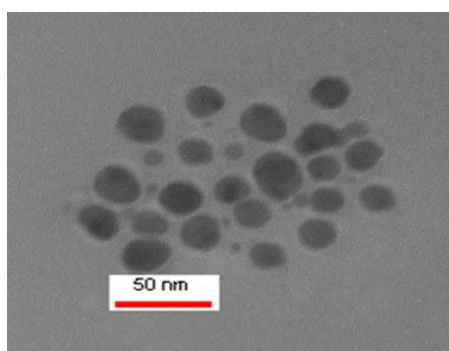
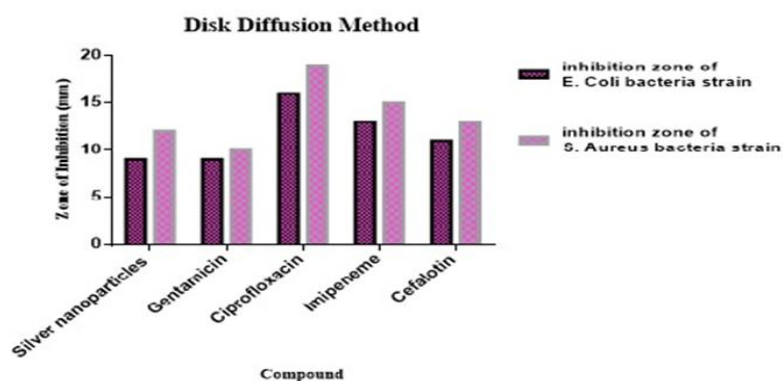


Table 4. Antimicrobial activity of biologically synthesized silver nanoparticles by disc diffusion method

Bacteria strains	Silver nanoparticles	Gentamicin	Ciprofloxacin	Imipeneme	Cefalotin
<i>E. Coli</i>	9 mm	9 mm	16 mm	13 mm	11 mm
<i>S. Aureus</i>	12 mm	10 mm	19 mm	15 mm	13 mm

Figure 6. Antimicrobial activity of silver nanoparticles (1 mm) compared to four types of antibiotics by disc-diffusion method



Conclusion

In Conclusions, different organs of plants extracts can be efficiently used in the synthesis of silver nanoparticles as a green method. In the present study we found that *F. Latisecta* can be good source for simple synthesis of silver nanoparticles. This production through plant, will give a positive message that nanoparticles synthesized through greener routes are much safer for human use. Control over the shape and size of nanoparticles using plant extract seems to be very easy. The silver nanoparticle content was characterized by UV-Vis spectroscopy, XRD pattern, DLS graph, and TEM image. This approach toward the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability, simple, ecological, cheap, and very fast. It can also be suitably scaled up for large-scale synthesis of nanoparticles. This is the first report on the synthesis of silver nanoparticles using *F. Latisecta* extract and their effect as antimicrobial agents.

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