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Phytosynthesis and antimicrobial studies of silver nano particles using *Ziziphus nummularia* leave extracts

Abstract

Plant mediated biosynthesis of silver nano particles is a rapidly progressing area of Nano biotechnology. In this study silver nano particles were synthesized by aqueous extracts of *Ziziphus nummularia* plant leaves and were characterized by UV–Visible spectrophotometer and transmission electron microscope (TEM). UV–Visible spectroscopy confirmed the formation of silver nano particles because of the formation of particular peak in the region of 400–430. The TEM analysis confirmed spherical and uniform silver nano particles with diameter ranging from 33nm to 89nm. The bactericidal and fungicidal activities of silver nano particles was determined. Eco–friendly synthesized silver nano particles exhibited good antimicrobial activity and these nano particles could be act as an excellent antimicrobial agent in current nano medicine.

Keywords: antimicrobial activity, biological, fungicidal activities, silver nanoparticles, *Ziziphus nummularia*

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Introduction

Nano–biotechnology combines biological principles with physical and chemical approaches to produce nano–sized particles with specific functions. Aluminum, silver, gold, titanium, palladium, iron and copper have been routinely used for the synthesis of nano particles. Synthesis of nano particles using biological process has generated a great interest due to their unusual optical, chemical, electrochemical and electronic properties.[1] Silver nano particles are widely used as an antibacterial agent in every sector of life such as industry, health, food storage, and textile coatings in numeral ecological applications.[2–4] Biological synthesis of silver nano particles using microorganisms and plants such as bacteria, fungi and plant extract has been suggested as possible eco–friendly alternatives to chemical and physical methods.[5] Silver nano particles being extensively synthesized by various plants extracts such as *Ocimum*,[6] *Oleve*,[7] *plectranthusambaoinicus*,[8] *Piper betle*.[9] *Ziziphus nummularia* (Indian jujube or local name, Regu) is a multi–purpose tree mainly grown for its fruits. It has been reported to contain flavonoids, alkaloids, glycosides, pectin, polysaccharides, peptide alkaloids, sterols, tannins, sterols, triterpenoid acids, fatty acids, ziziphin N, O, P, Q and dodecaacetylprodelphinidinB3.[10] The *Ziziphus nummularia* leaves and fruits are used in treatment of cold, diarrhea, dysentery, indigestion, inflammation of gums and tonic.[11] It has is reported to possess antitumor,[12] antibacterial,[13] analgesic and anti–inflammatory,[14] properties. In view of medicinal importance of *Ziziphus nummularia* the present work has been carried out to synthesis and characterization of silver nano particles and to determine their antibacterial and antifungal activities.

Material and methods

Collection of plant leaves and preparation on silver nano particles

Leaves of *Ziziphus nummularia* were collected from the forest area of Sri Venkateswara University, Tirupati, Chittoor District of Andhra Pradesh, India. After collection, leaves were cleaned with distilled water and crushed with motor and pestle. Then five grams of the crushed leaves was taken in conical flask and soaked in 100mL of distilled water. Then the soaked leaves were boiled for fifteen minutes with constant stirring and then filtered through a filter paper (Whatman 1) to get the leaves extract and were stored in the refrigerator till further use. The leaf sample was added separately to the reaction vessel containing silver nitrate ($AgNO_3$) at a concentration 0.1mM and control (without silver nitrate). And observed the color changes from color less to brown color is an indication of formation of silver nano particles in test samples and the sample was analyzed with various sophisticated instruments for presence of silver nano particles.

UV–VIS Spectral analysis

The synthesized silver nano particles were separated by centrifugation at 12000rpm for 10minutes. The supernatant was used for analysis and characterization of silver nano particles. UV–VIS Spectra analysis. The reduction of pure Ag+ ions was monitored by measuring the UV–Vis spectrum of the reaction mixture diluting with a small aliquot of sample into distilled water. The UV–Vis spectral analysis done with UV–VIS spectrophotometer between the range of 300–600nm (Shimadzu, UV–2450).

TEM analysis

Size and shape of silver nano particles were confirmed by transmission electron microscope (TEM). For this plant leaf extract sample was dehydrated using a graded series of acetone. Ultrathin sections were made with microtome and mounted on copper grids. The sections were stained with 2% aqueous uranyl acetate for 10min and triple lead stain for 5min. Micrographs were taken with HITACHI 7500 transmission electron microscope operated at 100kV.

Antibacterial activity

The antibacterial efficacy of silver nano particles was tested against bacterial strains, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Bacillus subtilis*, by agar well diffusion method.[15] For this, 24hours old Luria broth (LB) cultures of tested bacterial strains were spreader on sterile LB

agar plates using sterile spreader followed by placing of silver nano particle solution (100µl/well). Simultaneously Streptomycin standard antibiotic disks were placed as control and the plates were incubated at 37^{0}C for 24hours. After that the formation of zone of inhibition surroundings well was measured.

Antifungal activity

Antifungal activity of silver nano particles was determined, using the agar well diffusion method. For this young fungal cultures were poured on sterile potato dextrose agar (PDA) plates followed by placing of silver nano particle solution (100µl/well).The plates were incubated at room temperature for 4 to 7days. After that the formation of zone of inhibition surroundings well was measured.

Results

Collection of plant leaf material

Ziziphus nummularia plant leaf material was collected from Sri Venkateswara University campus, Tirupati, Chittor District Andhra Pradesh India Figure 1.

Figure 1 *Ziziphus nummularia* leaves.

Phytosynthesis of silver nano particles

Silver nano particle solution was prepared (1mM silver nitrate) and stirred for one minute at room temperature then *Ziziphus nummularia* leaves extract was added. Reaction mixture was kept on shaker for 4hours. After the addition of leaves extract the color of the solution immediately changed from colorless to yellowish brown Figure 2. This change in color indicated the formation of silver nano particles. Formation of silver nano particles by *Ziziphus nummularia* leaves extract was confirmed by peak formation at range 400 to 430 in UV–Visible spectrophotometer Figure 3. The silver nano particles were subjected to transmission electron microscopy (TEM) to understand their topology and size and the results revealed the formation of polydispersed spherical silver nano particles in the range of 33 to 89 Figure 4.

Antibacterial activity of silver nano particles

The silver nano particles exhibited good antibacterial activity against bacterial pathogens, *Bacillus subtitles*, *Pseudomonas aerugionsa*, *Klebsiella pneumonia*, *Escherichia coli* and *Streptococcus aureus*. Table 1 In this study, *Klebsiella pneumonia* showed more sensitive to silver nano particles in terms of formation of maximum zone of inhibition with 1.4cm whereas *Pseudomonas aeruginosa* least with inhibition zone (0.6cm). Remaining bacterial strains were intermediate bactericidal activity.

Figure 2 Bio reduction of silver nitrate.

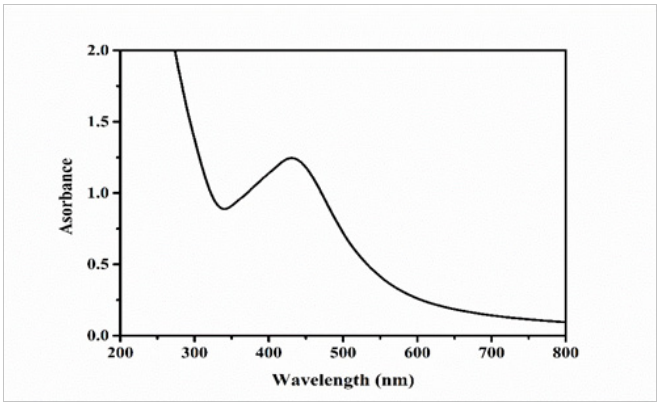

Figure 3 UV–visible spectra of Agnps.

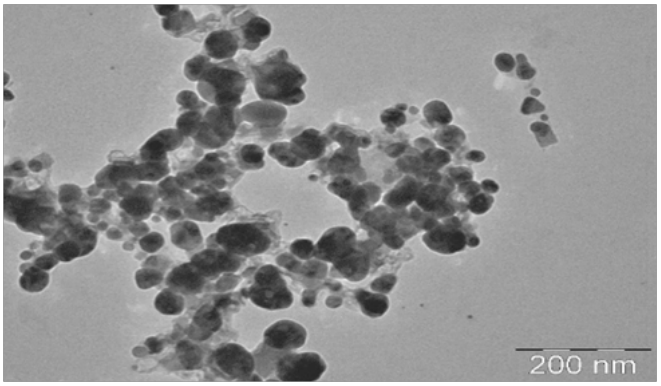

Figure 4 TEM micrograph of Agnps.

Antifungal activity

The data of Table 2 shows that the silver nano particle of *Ziziphus nummularia* against fungal pathogens *Aspergillus niger* and *A flavus*. The *Aspergillus niger* was more sensitive to the nano particles (zone of inhibition 1.6cm) than *A flavas* with zone of inhibition 1.3cm.

Discussion

Various approaches have been used to formation of silver nano particles like physical, chemical and biological methods. In this study, silver nano particles were synthesized by ecofriendly phytosynthesis (Green synthesis) using *Ziziphus nummularia* plant leaf extract.

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Initially, formation of silver nano particles was confirmed by color change from colour less to yellow brown of the reaction mixture Figure 2. Similar findings were made Jaidev and Narasimha.[16] The *Ziziphus nummularia* plant leaves extract acts as a reducing agent in formation of silver nanoparticles and colour changes in mixture is due to excitation of surface plasmon vibrations of silver nanoparticles.[17] The exact mechanism responsible for the synthesis of silver nano particles is yet to be known in detail, however it was hypothesized that the silver ions require NADH–dependent nitrate reductase enzyme for their reduction,[18,19] which was secreted by the plant cell. The reduction of Ag^+ ions was clearly observed in UV spectrum. The size and shape of the silver nano particles reflects the absorbance peak. The size of the nano particles has a linear correlation with the peak intensity. The size of the silver nano particles formed in the process was estimated from the Debye–Scherer equation and the size was confirmed with 33nm by transmission electron microscope (TEM). The reflections observed in this analysis indicates that the presence of silver metal wit very small size.[20] The silver nano particles were more efficient as antimicrobial agent than the most conventional antibiotics today. Several researches have reported the possible inhibitory action of silver nano particles on various gram positive and gram negative bacterial strains.[21] The antibacterial activity of silver nano particles against methicillin resistant *Staphylococcus aureus,*[22] *Escherichia coli,*[23] and *Bacillus subtitles*,[24] has been reported. The silver nano particles synthesized in this study are potential enough to kill bacterial and fungal pathogens a causative agents of various diseases in human beings.

Table 1 Antibacterial activity of silver nano particles

Microorganisms	Zone of inhibition (cm)
Pseudomonas aeruginosa	0.6
Klebsiella pneumonia	1.4
Bacillus subtilis	1.2
Escherichia coli	1.3
Streptococcus aureus	1.1
Standard antibiotic (Streptomycin)	1.2

Table 2 Antifungal activity of silver nano particles

Microorganisms	Zone of inhibition (cm)
Aspergillus niger	1.6
Asperrgillus flavus	1.3
Control (Clotrimazole)	1.2

Conclusion

In this study an antimicrobial agent silver nano particles were synthesized by leaf extract of *Ziziphus nummularia* plant. Formation of silver nano particles was confirmed by UV–Vis spectrophotometer, and size and shape of silver nano particles measured by transmission electron microscope. The synthesized silver nano particles showed good antibacterial and antifungal activities against pathogenic bacteria and fungi. The present study proved that the silver nano particles could be used as antimicrobial agent in current nano medicine.

Acknowledgements

None.

Conflict of interests

The author declares that there is no conflict of interest.

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