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Article

Synthesis of Biologically Active Silver Nanoparticles using *Tinospora cordifolia* Leaf Extract for Antimicrobial Applications

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ABSTRACT

In the present investigation, we report on an eco-friendly and economical way for the synthesis of silver nanoparticles (AgNPs). The AgNPs were synthesized using the leaf extract of *Tinospora cordifolia* plant in the presence of silver nitrate solution. AgNPs were characterized by ultraviolet–visible spectrophotometer, X-ray diffraction, Fourier-transform infrared spectroscopy, and scanning electron microscopy. The antibacterial effect of AgNPs was also tested on several microorganisms by measuring the inhibition zone of minimum inhibitory concentration by disc diffusion method. The results confirmed that biosynthesis of AgNPs can act as an efficient antimicrobial agent against pathogenic bacteria.

Key words: Biosynthesis, Silver nanoparticles, Antimicrobial activity.

1. INTRODUCTION

Nanotechnology is a rapidly growing science of producing and utilizing nano-sized particles. The field of nanotechnology has proved to be one of the most active areas of research. Green nanotechnology is expanding its frontier in the world of science and technology [1]. Synthesis of nanoparticles is increasing exponentially due to its wide range of application in the field of biosensors, bionanotechnology, and biomedicines [2]. Many of the nanoparticle synthesis involve the use of hazardous chemicals. Synthesis of nanoparticles without using toxic chemicals are gaining the importance to develop an eco-friendly process. Hence employing a method of plant extracts which emerged as a simple and an alternative method to chemical synthesis. The green synthesis has been considered as one of the promising methods for the synthesis of nanoparticles due to their biocompatibility, low toxicity, and eco-friendly nature [3] and it provides a single-step technique for the biosynthesis process [4]. Biological approaches using microorganisms and plant extracts for metal nanoparticle synthesis have been suggested as valuable alternatives to chemical methods. The biosynthesis of spherical silver nanoparticles using Tinospora cordifolia plant leaf extract were produced at ambient conditions [5].

Silver is extensively used in nanosystems and employed in various biomedical purposes. AgNPs have excellent medical and nonmedical properties and applications when compared with other metal nanoparticles. The green synthesis approach of nanoparticles possesses reduced or no toxicity. Thus they are used in medicinal drugs as a good antibiotic. Hence, herbal plants extract has been used for the synthesis of nanoparticles. Plant extract contains alkaloids, diterpenoids, lactones, glycosides, steroids, sesquiterpenoid, phenolic, aliphatic compounds, polysaccharides, and number of secondary metabolite which plays a critical role during the nanoparticle synthesis by acting as reducing or capping agents [6-11]. *T. cordifolia* plant is used as a traditional Ayurveda medicine for the treatment of fever, jaundice, cancer, asthma, skin diseases, snake bite, eye disorder, etc. [12]. Studies have shown that AgNPs are highly stable and toxic to bacteria, fungus, and viruses. In the present study, fresh leaves of *T. cordifolia* have been used for the synthesis of AgNPs.

2. MATERIALS AND METHODS

In this study, a simple and economical procedure was adopted for AgNP synthesis. Silver nitrate is used for the synthesis of AgNPs which was purchased from Sigma Aldrich, Mumbai, India. The leaf of *T. cordifolia* plant was used in this work collected from the garden. Synthesized nanoparticles were characterized by ultraviolet (UV) spectroscopy, X-ray diffraction (XRD), Fourier-transform infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM).

2.1. Preparation of Plant Extract

Fresh leaves of *T. cordifolia* were collected locally and rinsed thoroughly with tap water followed by distilled water to remove dust, dirt, and any unwanted visible particles. About 10 g of leaf were weighed, crushed, and transferred into 250 ml beaker containing 100 ml of distilled water and boiled for 15 min. The extract was then filtered using Whatman filter paper 40 to obtain a clear solution which was then refrigerated at 4°C.

2.2. Synthesis and Characterization of AgNPs

For the synthesis of AgNPs, 1 mM silver nitrate solution and leaf extract were taken for the reduction of silver ions.1 ml of 1 mM silver nitrate solution was added dropwise to 5 ml of leaf extract. A distinct color change was observed from pale yellow to brown color at room

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temperature. The formation of AgNPs was confirmed from the UV–visible spectrum of the solution. The nanoparticles were separated out from the mixture by ultracentrifugation.

2.3. Characterization of AgNPs

- The synthesized nanoparticles are characterized by UV–visible, FTIR, XRD, and SEM analysis.
- UV-visible spectral analysis for the reduction of silver to AgNPs, the absorption of spectra of the sample was taken from 300 to 500 nm using UV visible spectrophotometer on Shimadzu-1800 UV visible spectrometer (Singapore).
- FTIR – The spectrum was recorded in FTIR spectrum in the range of 400 to 4000 cm^{-1} using Jasco FTIR4100 (Japan) spectrometer.
- SEM – SEM is performed using ZEISS EVO40EP (Germany) microscope and the micrograph images of AgNPs are recorded to investigate the structure and morphology of a sample.
- XRD – The powdered XRD patterns were recorded using Proto Manufacturing INC, XRD benchtop powder diffractometer using Cu-K_α radiation as a source (λ=0.15443 nm). The 2θ angular regions between 10° and 80° are scanned at a scan rate of 2°/min.

3. RESULTS AND DISCUSSION

AgNPs with their unique chemical and physical properties are proving to be an alternative for the development of new bacterial agents. For the synthesis of AgNPs, the aqueous solution of silver nitrate was reacted with *T. cordifolia* leaf extract.

The reaction mixture was initially pale yellow which gradually turns to dark brown indicating the formation of AgNPs. The formation and stability of AgNPs in aqueous solution are confirmed using UV-visible spectrophotometric analysis.

3.1. UV-Visible Spectrophotometric Studies

UV-visible absorption spectrum of AgNPs showed a peak corresponding to surface plasma resonance of AgNPs at around 423 nm (Figure 1). The absorbance reached a maximum strong and broad peak in that spectrum clearly confirm the formation of AgNPs [13]. UV-visible studies indicate the complete reduction of silver ion and formation of AgNPs.

3.2. FTIR Spectral Analysis

IR vibrational spectroscopic studies were used to identify the presence of various functional groups in biomolecules such as alkaloids, carbohydrates, flavonoids, proteins in plant extract of *T. cordifolia*. In the Figure 2, the intense broadband at 3786 cm^{-1} corresponds to stretching vibration of OH groups indicating the presence of water, alcohol, phenols, and amines. The band at 1580 cm^{-1} and 1358 cm^{-1} corresponds to C-C and N-O aromatic stretch present in phytoconstituents. The peak at 1036 cm^{-1} attributed to the stretching vibration of C=O group that is characteristic of proteins shifted from 1036 cm^{-1} after the synthesis of silver nanoparticles. A weak band at 523 cm^{-1} can be assigned to C-X amine stretch. These results clearly indicate that nanoparticles are capped with the organic compound present in leaf extract.

3.3. XRD Studies

The sample of AgNPs could be characterized using XRD analysis to confirm the nature of AgNPs. From Figure 3, the XRD pattern showed four intense peaks which were recorded from 10° to 100° at 2θ angles. The peaks at 38.53, 46.54, 65.01, and 77.94 are indices to be lattice planes with Miller indices (111), (200), (220), and (311), respectively, of the face-centered cubic crystals structure of silver [14]. The average crystal size of the silver was determined as 23.2, 20.5, 14.2, and 12.2 nm with the mean value of the entire peak as 17.58 nm. The Bragg's peak indicates the reduction in grain size. The mean particle

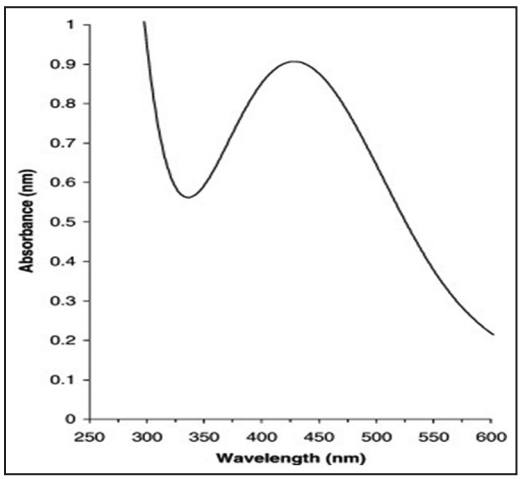

Figure 1: Absorption spectra of silver nanoparticles synthesized using *Tinospora cordifolia* plant leaf extract.

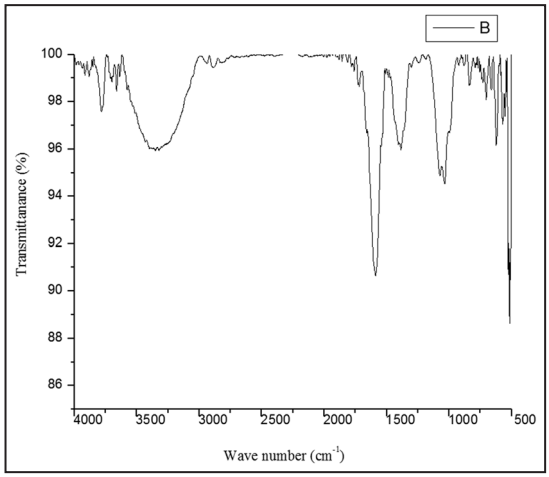

Figure 2: Fourier-transform infrared spectra of silver nanoparticles synthesized using *Tinospora cordifolia* plant leaf extract.

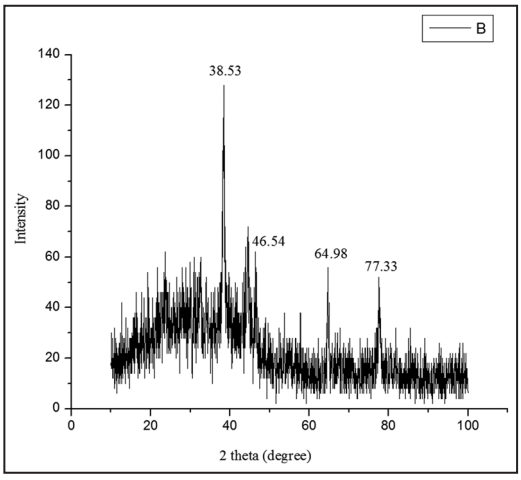

Figure 3: X-ray diffraction pattern of silver nanoparticles synthesized using *Tinospora cordifolia* plant leaf extract.

diameter of AgNPs was calculated from the width of XRD pattern using Scherrer's formula:

$$D = \frac{0.94\lambda}{\beta Cos\theta}$$

Where D is the average crystal domain size and β is full width and half maximum (FWHM). The β is corrected to eliminate the error due to the broadening of peak using FWHM values.

3.4. SEM Analysis

The SEM analysis has provided further insight into the morphology and size details of synthesized nanoparticles. Figure 4 shows the SEM images of AgNPs. The SEM image reveals that sample consists of a large quantity of dispersive nanoparticles in the range of 10–40 nm. However, nanoparticles tend to agglomerate to form still bigger structures.

3.5. Biological Activities

3.5.1. Antimicrobial activity of green synthesized nanoparticles along with their minimum inhibitory concentration (µg/mL)

Resistance to a number of antimicrobial agents among a variety of clinically significant bacteria is becoming increasingly important. There are various problems arising with the use of antimicrobials such as local tissue irritation, interference with wound healing process, hypersensitivity reactions, system toxicity, narrow antimicrobial spectrum, and emergency of resistance. The increasing clinical importance of drug-resistant microbial pathogens has additional urgency in antimicrobiological research. Variety of synthetic compounds is used in medicinal chemistry but is associated with side effects. In this connection, we tried green synthesized nanoparticles as antimicrobial agents.

Antibacterial activity of the green synthesized AgNPs was determined against Gram-positive bacteria (*Streptococcus salivarius*, *Staphylococcus aureus,* and *Micrococcus luteus*) and Gram-negative bacteria (*Pseudomonas fluorescence, Escherichia coli, and Proteus mirabilis*) in dimethylformamide (DMF) by disc diffusion method on nutrient agar medium. The sterile medium (nutrient agar medium, 15 ml) in each Petri plate was uniformly smeared with cultures of Gram-positive and Gram-negative bacteria. Sterile discs of 10-mm diameter (Hi-Media) were placed in the Petri plates, to which different concentrations of drug (20, 40, 80, and 160 µg/disc) of the green synthesized nanoparticles were added. The treatments also included gentamicin as a positive control for comparison. For each treatment, three replicates were maintained. The plates were incubated at 37°C for 24 h and the zone of inhibition was determined.

4. RESULTS

Compound was tested *in vitro* for antimicrobial activity against Gram-positive and two Gram-negative bacterial strains as shown in Table 1. A commercial antibiotic such as gentamicin was used as reference drug. The compound showed broad-spectrum antimicrobial.

Shell-less chorioallantoic membrane (CAM) assay is an angiogenic assay used for validation of angioinhibitory activity of any compound. The CAM assay to note the antiangiogenic activity was performed using the AgNPs. The antiangiogenic effect was studied according to the method of Auerbach *et al*. (Auerbach *et al*., 1974). Briefly, fertilized hens' eggs were surface sterilized using 70% alcohol. The eggs were incubated in fan-assisted humidified incubator at 37°C. On the 4th day, the eggs were cracked out into thin films of the hammock within a laminar flow cabinet and were further incubated. On the day 5th when blood vessels were seen proliferating from the center of the eggs within the hammock, filter paper discs loaded with 100 µg of AgNPs were placed over the proliferating blood vessels and the eggs were returned to the incubator. Results for antiangiogenic effect of the compound were observed after 24 h as shown in Table 2.

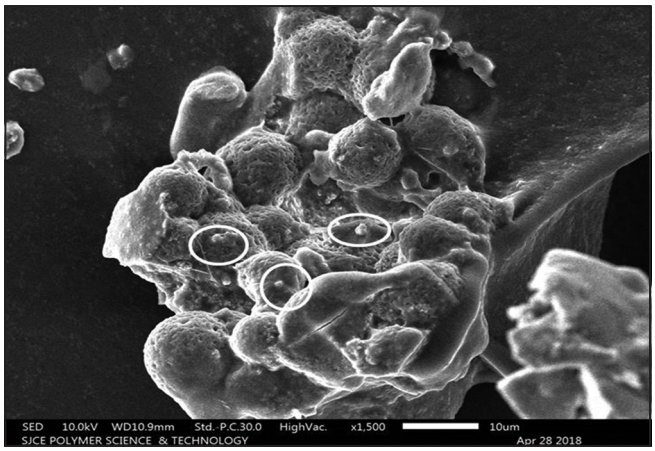

Figure 4: Scanning electron microscopy image of silver nanoparticles synthesized using *Tinospora cordifolia* plant leaf extract.

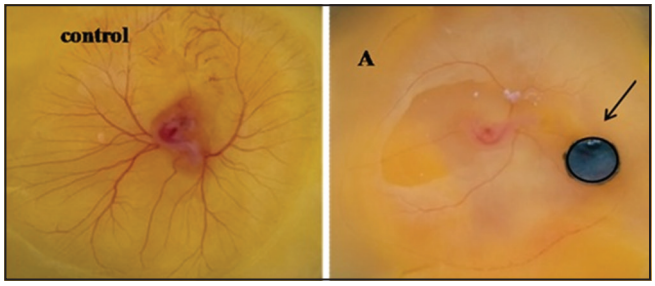

Figure 5: The angioinhibitory activity of silver nanoparticles synthesized using *Tinospora cordifolia* plant leaf extract.

Table 1: Angioinhibitory effect by shell-less chorioallantoic membrane assay.

Concentration of compound (µg)	20	40	80	160
Bacteria/samples	**Zone of inhibition (cm)**			
P. fluorescence (Gram negative)	0.3	0.4	0.6	0.8
E. coli (Gram negative)	0.2	0.4	0.6	0.8
P. mirabilis (Gram negative)	0.1	0.2	0.3	0.4
Streptococcus salivarius (Gram positive)	0.0	0.0	0.1	0.4
S. aureus (Gram positive)	0.2	0.2	0.3	0.5
M. luteus (Gram-positive)	0.1	0.1	0.3	0.4

P. fluorescence: *Pseudomonas fluorescence*, *E. coli*: *Escherichia coli*, *P. mirabilis*: *Proteus mirabilis*, *S. aureus*: *Staphylococcus aureus*, *M. luteus*: *Micrococcus luteus*

The angioinhibitory activity of the compound is shown in Figure 5. The synthesized AgNPs exhibit significant positive results in the shell-less CAM assay model of developing embryos. The investigation of antiangiogenic activity of AgNPs showed a significant reduction of proliferation of capillaries around the zone of application of the discs loaded with the compounds as compared to the site where only the vehicles, DMF, were applied. These results indicate that the green synthesized AgNPs are potent antiangiogenic molecules *in vivo*.

Table 2: Angioinhibitory effect by shell-less chorioallantoic membrane assay.

	MIC values (µg)					
Name of compound	*P. fluorescence* (Gram negative)	*E. coli* (Gram negative)	*P. mirabilis* (Gram negative)	*S. salivarius* (Gram positive)	*S. aureus* (Gram positive)	*M. luteus* (Gram positive)
Silver nanoparticle	15	17	17	40	7	10

P. fluorescence: *Pseudomonas fluorescence*, *E. coli*: *Escherichia coli*, *P. mirabilis*: *Proteus mirabilis*, *S. aureus*: *Staphylococcus aureus*, *M. luteus*: *Micrococcus luteus*

5. CONCLUSION

The synthesis of AgNPs has been reported successfully in the present work. The synthesis is found to be efficient in terms of reaction time as well as stability of AgNPs. Green synthesis of nanoparticles using plant extract is an economical, energy efficient, and cost-effective. Green synthesis of nanoparticles enhances the antibacterial activity against bacteria colony due to enhanced antimicrobial activity in the field of medicine as well as in food industries. The synthesized silver nanoparticles acts as immunomodulator and plays different physiological roles. Their by demonstrating the diverse versatility of the Tinospora cordifolia plant. The further scope of the review remains in exploiting the biochemical and signalizes the pathway of active components of *T. cordifolia* plant. Thus, enabling effective disease targeting truly acts as an incredible source.

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