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Green Synthesis, Characterization and *in vitro* Antibacterial Activity of Silver Nanoparticles by using Leaf Extract of *Tinospora cordifolia*

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Abstract

Nano-medicine, a fast-emerging area of modern medicine, has intriguing applications. The physical and chemical methods of producing nanoparticles, cause toxicity and disadvantages. Green synthesis of nanoparticles from plant sources circumvents these lacunae, is simple and cost effective. *Tinospora cordifolia* (Giloy), finds immense applications in the field of medicine. This study aimed at the Green Synthesis of Silver Nanoparticles from the reaction between Leaf Extracts of Giloy & aqueous Silver Nitrate solution. The reaction resulted in visual colour change from pale yellow to brown, indicating the formation of Silver Nanoparticles. Surface Plasmon Resonance (SPR) values of these Nanoparticles were measured and obtained at the wavelength of ~462.5nm. Characterization by Scanning Electron Microscopy (SEM) revealed the mean diameter of these Nanoparticles to be 22.75nm. Further, the antibacterial activity of these Nanoparticles was tested against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Eschericia coli* through Agar-Disc method and the efficacy inhibition was observed by Zones of Inhibition (ZOI). It was found that these Nanoparticles were inhibitory to the above bacteria. Hence, this study shows the potential of green synthesis of Silver Nanopartciles from *Tinospora cordifolia* and its subsequent anti-bacterial activity, thus amalgamating the biological benefits of Giloy and Silver Nanoparticles in human health.

Keywords:

Silver Nanoparticles;
Green Synthesis;
Surface Plasmon Resonance;
Scanning electron microscopy;
Antibacterial activity.

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1. Introduction

Nanotechnology is one of the important areas of research in modern material sciences. It deals with controlling and manipulating materials at the nanoscale. Nanoparticles, by virtue of their physical and chemical properties, have applications in fields like biotechnology, biomedical Sceinces, drug delivery, therapautics, nutrition, energy, medicine, physics, chemistry [1-2].

Traditionally, Silver-metal was used to control bodily infections and prevent food spoilage. It has also been used in wound-healing [3]. Current times employ the application of Silver Nanoparticles as antimicrobial agents, wound-dressing materials, in bone and tooth cement.

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There are several methods available for the synthesis of nanoparticles such as physical, chemical, [4-5], enzymatic and biological methods. Drawback of these physical and chemical methods is, their utilisation of high radiation, highly concentrated reductants and stabilising agents, which pose threat to the nature and to human health. Hence biological synthesis which is a single step bioreduction method for the production of nanoparticles has become useful and more research is been focused in this area. Green synthesis of nanoparticles utilises eco-friendly resources like plant extracts [6-15], bacteria, fungi, micro algae like cyanobacteria and also from single cell proteins [16-17].

The biochemical reaction of $AgNO_3$ with the plant broth leads to the formation of AgNPs, by the following mechanism, $Ag^+NO_3^-$ + plant extract → Ag^0 NPs + byproducts.
This reaction between the plant extract components and $AgNO_3$ can be visually observed as a gradual colour change from yellow to dark brown, which is indicative of the production of AgNPs.

The antimicrobial effects of AgNPs are of immense potency, which could make it possible to use these NPs as opposed to the conventional antibiotics. Micro molar doses (1-10µM) of silver ions are sufficient to kill bacteria, while silver can be toxic at high doses to mammals [18].

The biosynthesis of nanoparticles has been studied extensively in the past two decades. Green synthesized nanoparticles could be potential candidates in diverse fields including pharmaceuticals, therapeutics and various other commercial products. Biosynthesis of nanoparticles involves the usage of phytochemicals in the reduction reaction for the synthesis of nanoparticles, and elucidation of their applications in different fields.

Though substantial data available in the area of biosynthetic nanoparticles, more work is required to elucidate more sources. Hence, in the current work, we have concentrated on the plant mediated production of silver nanoparticles (AgNPs), using one of the very important medicinal plants, *T.cordifolia.* AgNPs thus obtained were characterized by measuring the absorption using UV Visible spectrophotometer and SEM. Further, antibacterial activity of the above synthesized silver nanoparticles was assessed on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

2. Research Method

Tinospora cordifolia (Menispermaceae) was collected from Mount Carmel College botanical garden, Bangaore, during June 2016. Silver nitrate ($AgNO_3$, analytical grade), antibiotics, agar and other chemicals were purchased from Nice Chemicals, bacteria for the research work and media were obtained from Himedia.

2.1. Preparation of *Tinospora cordifolia* leaf Extracts :

Leaves of *Tinospora cordifolia* were separated, washed thoroughly in running tap water for 10 min. to remove any dust particles and then washed in double distilled water. Extracts were prepared by aqueous method by boiling leaf samples in double distilled/MQ water in a proportion of 1:10 in a 250 ml Erlenmeyer flask for 20min. The cooled extract was filtered through Whatman no. 1 filter paper, the filtrate was spun at 12,000rpm for 30 min. and the supernatant was collected. The supernatant was filtered using 0.2µm filter and the filterate stored in amber colored air tight bottles at 10ºC till the use.

2.2. Synthesis of nanoparticles :

15 mL of the leaf extract was mixed with 85 mL of 1mM silver nitrate and incubated for different time intervals in order to obtain nanoparticles. If required, the mixture is boiled. This mixture was left undisturbed by incubating at room temperature for the reduction reaction.

2.3. Characterization of silver nanoparticles :

2.3.1. Colour change :

Visual colour change of the reaction mixture, if any, from light yellow to dark brown was monitored and photographs were taken at different time intervals and the positive samples were taken for U.V.-Visible Spectrophotometer readings.

2.3.2. UV-visible spectral analysis :

Absorption maxima of Silver Nanoparticles (AgNP) samples from *T.cordifolia* was measured by UV-visible spectrophotometer at the wavelength range of 300-550nm using aqueous $AgNO_3$ as standard.

2.3.3. Scanning Electron Microscopy :

An aliquot of AgNPs obtained from leaf of *T.cordifolia* was coated on copper stub and the size as well as shape of silver nanoparticles was studied using SEM.

2.4. Testing antibacterial activity of Silver nanoparticles using the Agar-Disc method :

For the assessment of antibacterial activity of *T.cordifolia* Leaf AgNPs, three microorganisms were used, including *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The organisms were cultured by inoculating in LB broth and incubating at 37 ºC for 6-8 hours. Cultures were spread on LB agar plates. Penicillin/streptomycin combination of antibiotics was used as positive control, plane distilled water used as negative control. *T.cordifolia* Leaf AgNPs were used in different concentrations. The positive, negative controls and leaf nanoparticles were loaded in the wells created in LB agar plates, incubated for 6-8 hours at 37ºC to study the antibacterial activity of the Silver NPs, through the formation of ZOI by the Agar-Disc diffusion method.

3. Results and Analysis

3.1.Synthesis of Nanoparticles :

Incubation of $AgNO_3$ with *T.cordifolia* leaf extract, resulted in the visual color change indicating the formation of silver nanoparticles. During the Green synthesis of Nanoparticles from the leaf extract of Giloy plant, the phytoconstituents of the leaf extract of *T. cordifolia* helped in the reduction of silver ions and resulted in the formation of silver nanoparticles which is evident in the color change. In the initial 30-60 minutes, the color change was from colorless to pale yellow. Holding time further, enhanced this color change to brown. These results are summarized in Figure 1. Figure 1a indicates the 5 minutes of incubation, in figure 1b the reduction reaction in one hour resulted in pale yellow, further holding time to 48 and 72 hrs resulted in more reduction which is shown by color change to brown and dark brown as indicated in Figure 1c to 1e.

The biochemical reaction of $AgNO_3$ with the plant broth leads to the formation of AgNPs, by the following proposed mechanism : $Ag^{+}NO_3^{-}$ + plant extract → Ag^{0} NPs + byproducts

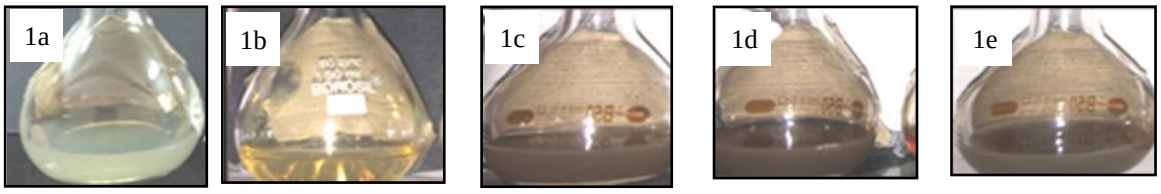

Figure 1 a-e : Shows the visual indication of Green Synthesis of Silver NPs from Leaf extracts of *T. cordifolia.* Gradual color change was observed from pale yellow to brown when $AgNO_3$ was incubated with *T.cordifolia* leaf extract at time intervals. Figure 1a-5min, Figure 1b-60min, Figure Ic-48hrs, Figure 1d- 72 hrs, & Figure 1e-8 days after incubation

3.2.Absorption spectral readings from UV visible spectrophotometer :

Further, to confirm the formation of AgNPs, U.V.-Visible Spectrophotometer analysis was done to measure the SPR values of these AgNPs. The range of wavelength used for the U.V. Visible-Spectrophotometer analysis selected, was 300-600nm. The leaf extracts of Giloy incubated with the $AgNO_3$ solution for different time intervals were sampled to estimate their absorption maxima scanned using a U.V. Visible Spectrophotometer. The samples of 48 hours and 72 hours incubation (Figure 2) gave a maximum absorbance at the wavelength of ~462.5nm, indicative of the SPR phenomenon of Silver NPs. These findings also corresponded to the absorption maxima values indicative of AgNPs, as put forth in literature.

3.3.Scanning Electron Microscopy (SEM) Analysis :

The SEM images of AgNPs obtained from Leaf Extract of *T.cordifolia* is shown in Figure 3. SEM images, indicated the presence of spherically shaped AgNPs with the mean diameter of the AgNPs to be ~22.75nm. While some of the AgNPs seemed to be present as spherical entities of varying diameters, some were found to be agglomerated, which could be explained as a phenomenon arising due to destabilization of electric double layer of the Silver ions. The observations from SEM analyses could confirm that the AgNPs synthesized had an elemental profile indicative of them being AgNPs. In addition to this, the SEM helped to understand the morphology & characteristics of these AgNPs. A vast majority of these particles were found to be spherical in shape, existing as individual entities or in agglomerates.

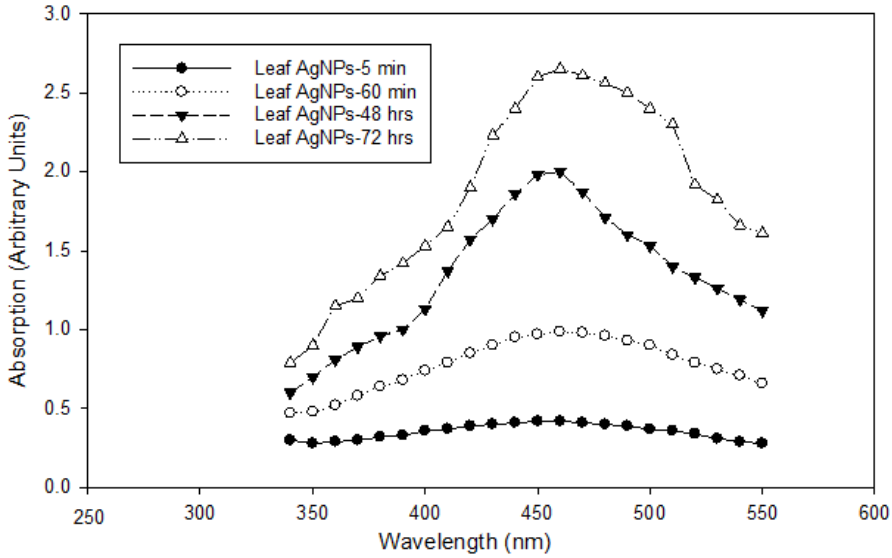

Figure 2: Shows the absorption spectra of *T. Cordifolia* leaf AgNPs at different time-intervals of formation. The absorption of Green Sythesized AgNPs from leaf extracts of *T. Cordifolia* at 5min., 60 min., 48 hrs., 72 hrs.

3.4.Assessmei

The Bioassay using AgNPs synthesized from leaf extract of *T. cordifolia* was performed to test the antibacterial activity of AgNPs using the Agar-Disc method, against three test microorganisms, *Escherichia coli*, *Staphylococcus aureus* & *Pseudomonas aeruginosa*. In this method, by the well-diffusion assay, the samples were loaded in the wells punctured on the nutrient agar inoculated with the test organism. The diffusion of the respective samples into the nutrient agar containing the test micro-organism resulted in the bio-reaction between the sample and the

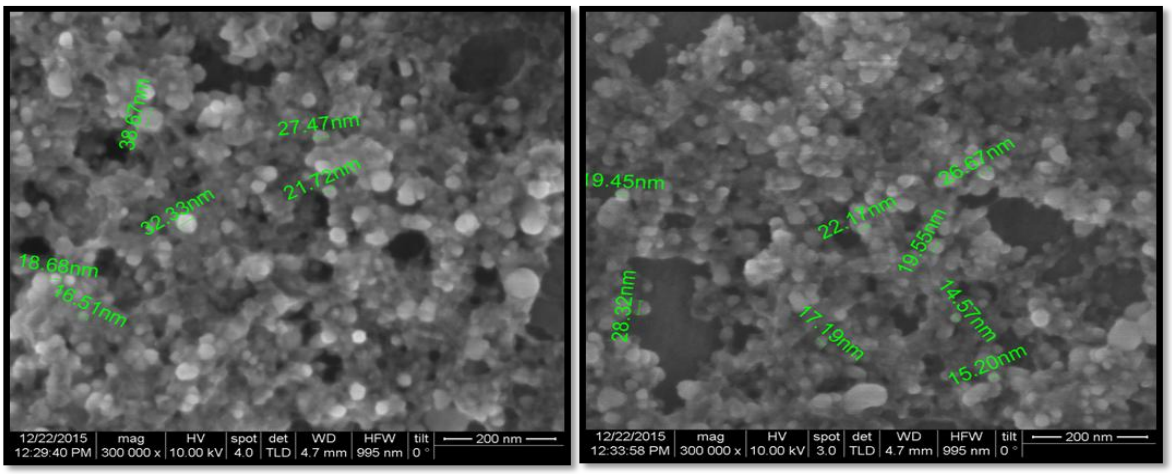

Figure 3: Shows the Scanning Electron Microscopy images of the AgNPs obtained from *T.cordifolia* leaf extracts. Images show spherically shaped nanoparticles with a mean diameter of ~22.75nm

micro-organism which could result in zone of inhibition (ZOI).

As presented in the Figure 4, the leaf AgNPs of *T.cordifolia* were found to be inhibitory to *Staphylococcus aureus* (4A), *Pseudomonas aeruginosa* (4B) and *Escherichia coli* (4C) and it is also evident here, that the increasing concentrations of the AgNPs showed a corresponding increase in the ZOI.

4. Conclusion

In view of the beneficial health values of Silver, this study was aimed at amalgamating the benefits of an immensely valued medicinal plant, *Tinospora cordifloia* (Giloy), along with the metallic Silver Nanoparticles. By achieving a biogenic synthesis of AgNPs using plant derivates/

plant constituents from Giloy, this study also emphasised on the importance of Biological/ Green

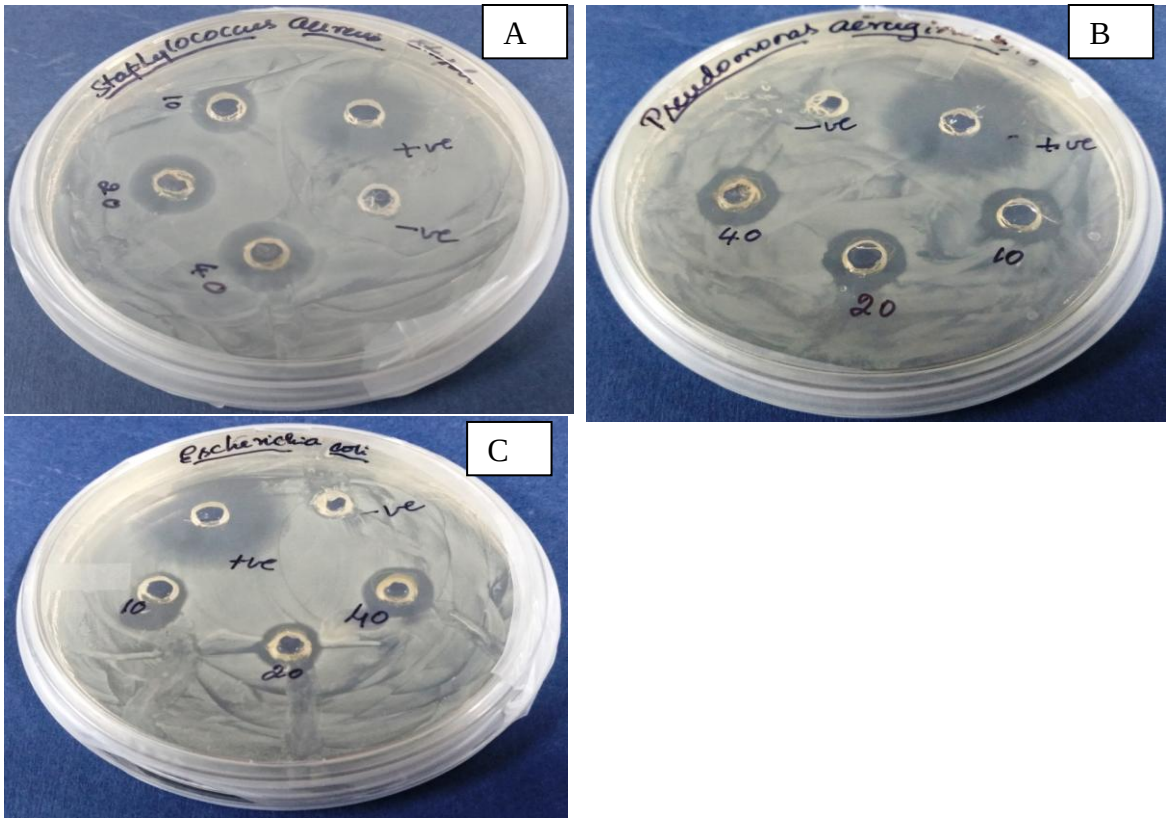

Figure 4: Shows the antibacterial effect of *T.cordifolia* Leaf AgNPs. Figures 4A, B and C show the inhibitory activity of AgNPs on *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*, respectively. Antibiotics- combination of penicillin/streptomycin was used as +ve positive control, distilled water was used as –ve control and 10µl, 20µl and 40µl of *T.cordifolia* Leaf AgNPs were used to assess the antibacterial effect

Synthesis.

Green Synthesis of Silver NPs from leaf extract of *T. cordifolia* was achieved by treating $AgNO_3$ with the aqueous leaf extract of *T. cordifolia*. Reduction of silver by the phytoconstituents helped in the formation of nanoparticles. In the initial 10-15mn., the color change was from colorless to golden-orange. Holding time further, enhanced this color change to orange-brown. Visual color change of the solution from colorless to orangish-brown was indicative of the formation of AgNPs.

Further, the measurement of the SPR values of these AgNPs using U.V.-Visible Spectrophotometer corresponded to the literature, wherein the values obtained in this study fell in the range of SPR of AgNPs.

Going ahead with the next objective of this study, the Bioassay of these AgNPs aimed at testing the antibacterial activity of AgNPs using the Agar-Disc method, against three microorganisms: *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. As presented in the Figure 4, the antibacterial effect of *T.cordifolia* Leaf AgNPs were found to be inhibitory to *Staphylococcus aureus* (4A), *Pseudomonas aeruginosa* (4B) and *Escherichia coli* (4C).

In conclusion, this work aimed at encouraging the use biogenic or environmentally-friendly approaches towards the synthesis of compounds and utilizing them for various applications, including their useage for health benefits.

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