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Biosynthesis and Characterization of Silver Nanoparticles by using Leaf Extract of Thalkudi (*Centella asiatica)* and its Antimicrobial Activity

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Abstract

Nanoscience and nanotechnology are the study and application of extremely small particles and can be used across all the field of science. Recently, nano particles have been used in catalysis, electronics, optics and a number of industries including pharmaceutical and agriculture. Green synthesis of silver nanoparticles is consider as simple, cost effective, biocompatible, less toxicity to cell and eco friendlytechnique. In the present study,we have adopted a technique of green synthesis of silver nanoparticles using leaf extracts of a common medicinal plant i.e. Tulsi (*Ocimum sanctum)*. Theleaf extract was act as a bio-reducer for synthesis of silver nanoparticles (AgNPs). The synthesized AgNPs were characterized using UV-Vis absorption spectroscopy and the absorption maximum was recorded at 651nm. The capping actionof bio moleculeson AgNPs was characterized by Fourier Transform Infra-Red Spectroscopy (FTIR). The shape and size of the AgNPs were examined with XRD and scanning electron microscopy (SEM). The XRD analysis indicated the crystalline nature of AgNPs and the SEM photography apparent that the AgNPsweresphericalin shapecoated with biomolecules. The anti-bacterial activity of AgNPs was investigated againsta Gram-negative bacterium (*E. coli)* and a Gram-positive bacterium (*Pseudomonas aeruginosa)* using agar well diffusion method. The synthesized AgNPs were inhibitory effect on both the experimental bacteria. The present work can be concluded that biomolecules present in Tulsi leaf was a potential bio-reducer for synthesis of AgNPs and the synthesized AgNPshad efficacy as bactericidal activity.

Keywords: Biosynthesis; Silver Nanoparticles (AgNPs); FTIR; SEM; Antimicrobialactivity.

Introduction

Nanoparticles are very similar in size having the size of 1-100nm[1]. Nanomaterials are very important and promising area of investigation not only for their structure and properties but also for their wide use in different areas including catalysis, electronics, optics, industries, medicines and agricultures [2, 3, 4]. Some investigators are also reported that novel metal nanoparticles can be used to regulate the biological systems both *in vitro* and *in vivo*[5]. It is observed that only few metals like gold, silver, copper, platinum are utilized for preparation of nanoparticles though a large number of metals are available at the nature. These nanoforms are used in photography, photonics, catalysis, biolabelling, etc [6,7].

Silver nanoparticles (AgNPs) are very interesting for its shape and properties. These nanoparticles are used in clinic, industries and research fields [8,9,10]. Moreover, they are distinguished for their bactericidal activity with low adverse effect to human cell [11]. In this view, AgNPs may be servedas an alternate to the antibiotic especially for against to the multidrug resistance pathogens (MDPs). Silver nanoparticles (AgNPs) are traditionally synthesized by physical and chemical methods [12]. Though the physical method does not utilized chemicals but the demerit

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of the process is utilization of more space and consumption of more energy. The chemical process of synthesis of AgNPs is rapid and economically viable however the pitfall of the process is utilization of chemicals of environmental hazard. The alternate and ecofriendly method of synthesis of AgNPs is green synthesis. The green synthesis processes utilize microorganisms, enzymes, plants extract, fruit extract, for preparation of the nanoparticles [13].

Tulsi (*Ocimum sanctum)*is worshipped, is offered the gods and is grown in Hindu households as a holly plant. The herb has loaded of benefits. *Tulsi* is extremely beneficial for humans. The most common ailments, strengthening immunity, fighting bacterial & viral infections to combating and treating various hair and skin disorders – just a few leaves of tulsi when used regularly can help resolve a host of health and lifestyle related issues.Tulsi assumes utmost importance when it comes to Ayurveda and naturopathy.

Its essential oils are used to treat number of ailments.Thus, by taking into consideration of great medicinal value of the plant leaf extract, we have utilized it for preparation of silver nanoparticles (AgNPs) in aqueous medium. The stable AgNPs were studied their antimicrobial efficiency against two human pathogens, *Pseudomonas aureus* and *Escherichia coli*.

Materials and Methods

Collection of Plant Material

The leaves of Thalkudi (*Centella asiatica)* with good physical conditionswere collected from GIET garden, Gunupur. The plant was further authenticated at department of Botany, Berhampur University, Berhampur. Then the leaves were collected in a sterilized plastic bag and immediately brought to the laboratory for further research work.

Preparation of Plant Leaf Extract

Centella leaf extract is acting as a reducing agent during the synthesis of Silver nanoparticles (AgNPs). The leaves were washed with tap water initially to remove the dirt and impurity present in surface and then shade dried. The fine powder of leaf was prepared by grinding in a mechanical motor and the size of the particle was maintained homogenously.10 gram of leaf powder wastaken in a round bottom flask with 100 ml of double distilled water and soxhelted it for 5 hrs. The leaf extract was cooled and then filtered through whatman No.1 filter paper.The leaf extract was used for preparation of silver nano particles.

Green Synthesis of Silver Nanoparticles

Silver nitrate ($AgNO_3$) was used as raw material for preparation of silver nanoparticles. The commercially available$AgNO_3$ (Hi media Pvt. Ltd. Mumbai, Purity 99%)was used for the experiment. 1 mmol solution of $AgNO_3$ was prepared with double distilled water, considered as stock solution and was stored at low temperature for further use [14]. For production of AgNPs, plant extracts from 1 ml was added to 5 ml of 1 mmol of $AgNO_3$ solution in a screwed test tube and incubated at room temperature (37^0C). The initial color was yellowish brown. The color of the solution starts change up after 10 minutes. The solution attained the stabilization color of deep brown after 5 hrs. This can be attributed to the formation of AgNPs.

Characterization of Silver Nanoparticles

The characterization of synthesized nanoparticles was carried out by using different instrumental methods such as UV-Vis spectro photo meter, FTIR and SEM.

UV-Vis spectroscopy is a useful technique for confirmation of presence of AgNPs. The absorption spectrum of a solution depends on the properties of molecules present in that solution. The synthesized AgNPs was analyzed for their absorbency in a range of 300 nm to 700 nm. A material's absorbance of infrared light at different frequencies produces a unique "spectral fingerprint" based upon the frequencies at which the material absorbs infrared light and the intensity. The surface chemical bonding of the particle was studied by Fourier Transform infrared spectroscopy (FT-IR). The confirmation and size of synthesized AgNPs were analyzed under scanning Electron Microscopy (SEM).

Study of Antibacterial Activity

The anti-bacterial activity of synthesized AgNPs was investigated againsta Gram-negative bacterium (*E. coli)* and a Gram-positive bacterium (*Pseudomonas aeruginosa)* using agar well diffusion method [15].1ml of AgNPs was added to the well cut in the Mueller-Hinton agar plate exposed with microorganism by spread plate method. The zone of inhibition of AgNPs wasobserved after 72 h of incubation at 35°C.

Results and Discussion

Synthesis of AgNPs

Tulsi (*Ocimum sanctum)*is an herbal medicinal plant, used for cure of many diseases of human like

cough and cold, bacterial infection and urinary disorder. The leaves of the plant are also used as aliment of domestic animals. The important bioactive compounds present in leaf are identified asoleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, β-caryophyllene , β-elemene and germacrene D [16] .

The mixture of plant leaf extract and $AgNO_3$ solution was initially yellow brown in colour Fig. 1. After 10 minutes of mixing of plant extract and $AgNO_3$ solution,it was begun to change.This indicates of initiation of reduction solution. The stabilization of color of the solution i.e. deep brown color was observed as after 300 minutes of incubation. The $AgNO_3$ solution and leaf extract are colorless and faint brown color respectively. The mixture solution was at initiation yellowish brown in color. The appearance of color in a solution is due to excitation of surface plasmon vibrations in metal nanoparticles. The change of color of solution is considered as preliminary and significant evidence for synthesis of silver nanoparticles.

UV-Visible Spectrum

The characterization of AgNPs was made further by UV-Vis spectro photometer.The absorption spectra of the synthesized AgNPs are represented in Fig. 2. Absorption maxima were observed at 452 nm. The absorption spectrum clearly indicates that when the concentration of $AgNO_3$ increases, the absorption spectrum decreased indicating presence of high amount of AgNPs [17].

FTIR Analysis

The silver nanoparticles were further characterized by Fourier Transform infrared (FTIR) to identify the presence of biomolecules attached/capped over it. The spectrum shows three shift peaks at3208.3, 1038.1 and 1011.8 respectively (Fig 3). This is possible due to bind of proteins on the AgNPs through the amine groups. It is also indicated the binding of C=O functional group with the silver nanoparticles. The plant leaf extract contain various constituents like carbohydrates, protein, alkaloids of nitrogen compounds, phenolcompounds etc. The cumulative effect of these compounds helps for of formation and retention of AgNPs in longer period [18].

Fig. 1: The mixture of plant leaf extract and $AgNO_3$ solution **i.** At the beginning **ii.** After 5 hrs

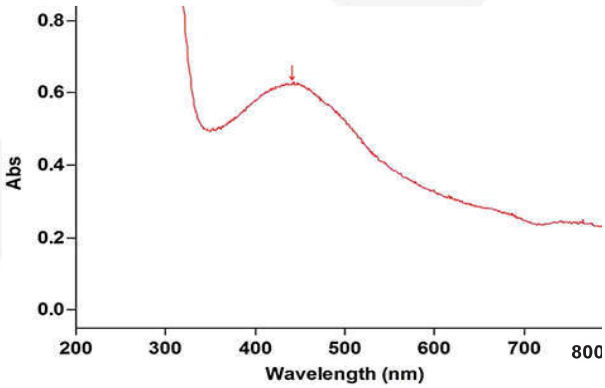

Fig. 2: The absorption spectra of the synthesized AgNPs

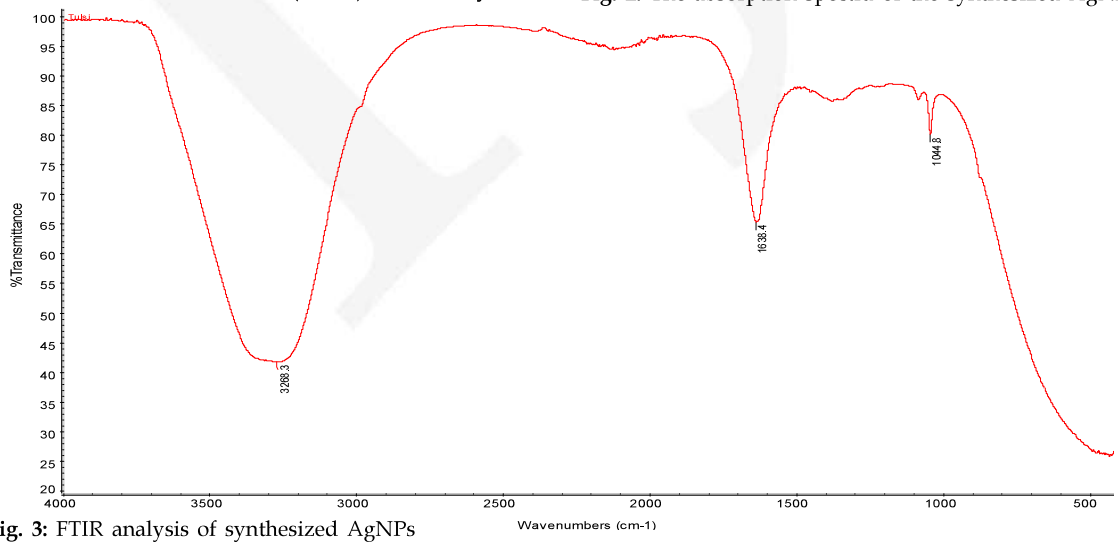

Fig. 3: FTIR analysis of synthesized AgNPs

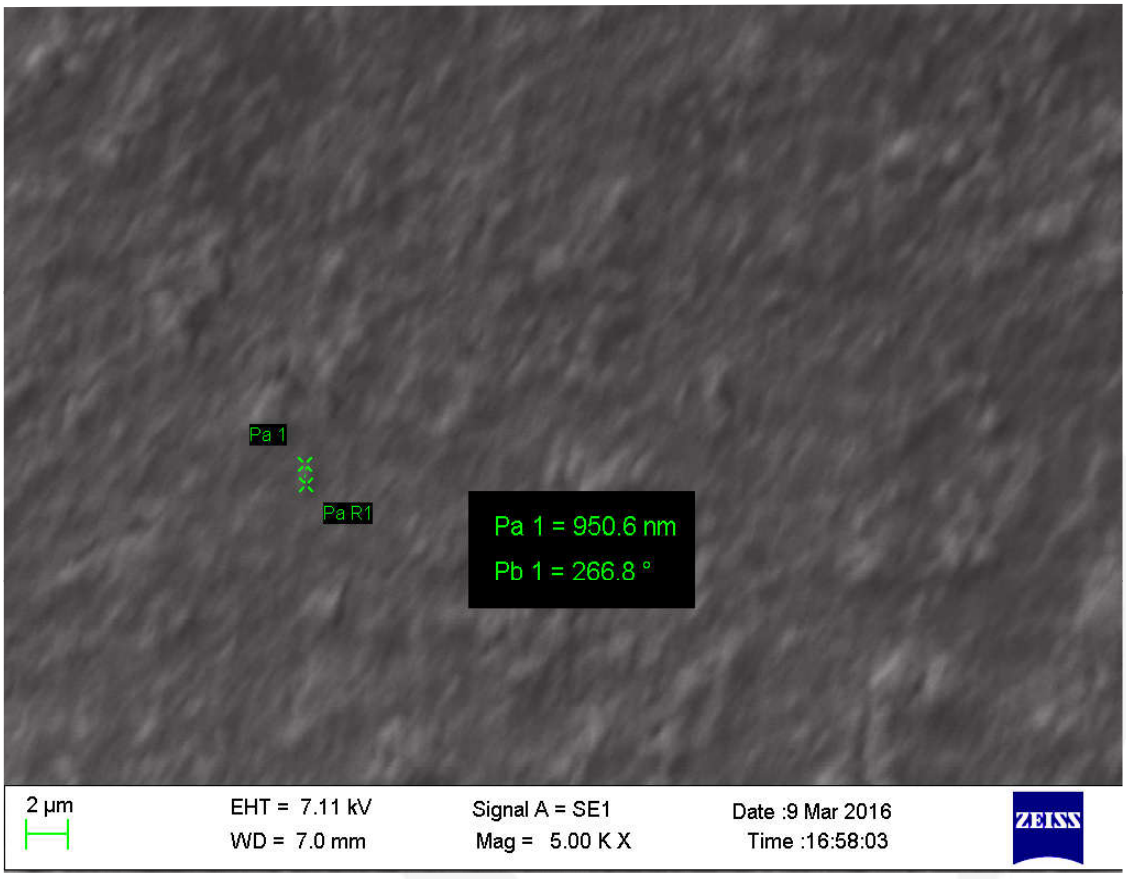

Fig. 4: SEM analysis of synthesized AgNPs

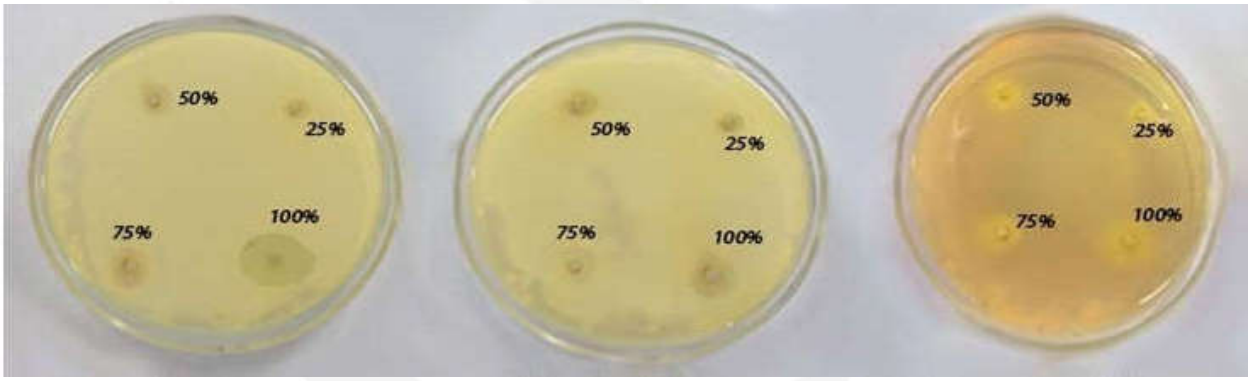

Fig. 5: Zone of inhibition developed by synthesized AgNPs in agar plate

Scanning Electron Microscopy

The surface structure and size of the synthesized AgNPs are further analyzed by Scanning electron microscopy (SEM). It was observed that the surface morphology of AgNPs biosynthesized from leaf extract indicated that they are spherical shape (Fig. 4). It has been also observed that some of AgNPs are aggregated and form the cluster. This leads to formation of various sized nanoparticles. The smallest size of AgNPs was 22 nm.

The AgNPs were capped with plant extract bio-molecules. The molecular composition was determined by EDS detector attached to SEM. This analysis indicated the strong signal in the silver region. Thus, formation of silver nano particles was confirmed. Due to surface Plasmon resonance metallic silver nanoparticles show optical peakand thus confirms the formation of AgNPs.

Antibacterial Activity

The zone of inhibition of AgNPs againsta Gram-negative bacterium (*E. coli)*and a Gram-positive

bacterium (*Pseudomonas aeruginosa)*by the synthesized $AgNO_3$is represented in Fig 5. It has been observed that the synthesized AgNPs had antimicrobial activity against both the studied pathogens.

Conclusion

The green synthesis of silver nanoparticles has quite advantage over physical and chemical methods. The method is simple, easy, non toxic and environmental friendly. The medicinal plants are used by rural people for aliment of several diseases. Even today the molecular assessments of many plants products are not yet revealed. Tulsi (*Ocimum sanctum)*is popular for its medicinal and antioxidant properties. The synthesized AgNPs using leaf extract of tulsi are spherical, stabilized and covered with biomolecules. These are also had antibacterial activity against two studied microorganisms, a Gram-negative bacterium (*E. coli)* and a Gram-positive bacterium (*Pseudomonas aeruginosa).* Therefore, it can be concluded that the bioactive compound present in tulsi can be exploited for green synthesis of AgNPs.

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