Fabrication and Characterisation of Silver Nanoparticles with *Illicium Verum* Seed Extract and Evaluation of Its Anti-Bacterial Activity

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Abstract

Nanoparticles are frequently used in a variety of scientific and engineering disciplines worldwide. By using particle size, nanoparticles are used to quickly deliver a material to a particular place. The fabricated silver nanoparticles were verified by UV spectroscopy, DLS, FTIR, and SEM studies. The characterizations confirmed that the fabricated material was nanoparticles, and two bacteria species, S. aureus and E. coli, were used in the tests to determine silver nanoparticles' antibacterial potency. The establishment of anti-bacterial zones demonstrates the ability of the produced nanoparticles to stop the growth of *E. coli* and *S. aureus*. This study concludes that the silver nanoparticles have the ability to inhibit the growth of microorganisms like *E. coli* and *S. aureus*.

Keywords: Silver nanoparticles, antibacterial, SEM, FTIR, DLS, *E. coli*, *S. aureus*.

Introduction

Nanotechnology is the branch of science and engineering that deals with how to control and manipulate Nano scale particles. These nanoparticles range from 1 to 100 nm. The word nano comes from the Greek word "Nanos" meaning very small. They have a size of one billionth of a meter. The Nano-particles are grouped in to two types namely organic and inorganic nanoparticles of carbon and magnetic or semiconductor nanoparticle respectively. Apart from these other Nano materials are the polymeric nanoparticle, solid lipid Nanoparticle, liposomes, Nano crystal, Nanotubes, Dendrimers and many more (Heera & Shanmugam, 2015). Nanoparticles can be synthesized by three methods namely physical, chemical and biological methods. In comparison with other 2 methods, biological method is considered as the rapid, safer and cost-effective alternative to physical and chemical methods.

Nanoparticles are classified as carbon based, metal, ceramics, semiconductor nanoparticles, polymeric and lipid-based nanoparticles. The characterization of nanoparticles can be done by different methods like UV-visible absorption spectroscopy, XRD, XPS, IR, SEM, TEM, DLS and FTIR methods. This characterization method is done to analyse the physiochemical properties of nanoparticle. The synthesized nanoparticles are used in wide variety of applications like drugs, medications, development of printed electronics, sensors and many more (Khan et al., 2019). Other applications include the bio-sensing, drug delivery, bioremediation, water treatment and production of clean energy and many more (Tiquia-Arashiro et al., 2016).

Plants are the nature's treasure. They are the wealth to the human society. Human beings and animals were dependent on plants for their food, shelter, clothing and as well as medicine (Gurib-Fakim, 2006). Different parts of plant have different medicinal properties. Medicines obtained from plants are the herbal medicines. They have no side effects when compared to chemical drugs. There are about 3,50,000 species of plants (Pan et al., 2014). India is one of the richest source of medicinal plants. These medicinal plants are known to cure any types of disease like diabetes, gastrointestinal problem and many more. Plants are used as drugs since 60,000 years ago. Around 50% of drugs are the richest source of medicinal plants. So, the track has changed from synthetic drugs to herbal drugs (Jamshidi- Kia et al., 2018). The aim of the study to determine the anti-microbial activity of silver Nanoparticles synthesized from seed extracts of *Illicium verum* against *E. coli* and *S. aureus*.

Materials and methods

Preparation of seed extract

Illicium Verum seeds were collected and then 1g was taken and dried at room temperature. The seeds were crushed with mortar and pestle into a fine powder. 20 ml of distilled water was taken in a beaker to that 1g of crushed *Illicium Verum* seeds were added and boiled at 60˚ C for 10 minutes until bubbles appeared. The extract was cooled and filtered by using whatman filter paper and stored at 4º C (Sudharsan et al., 2022).

Synthesis of silver nanoparticles

9.04 mg of silver nitrate ($AgNO_3$) was dissolved in 53.23 ml of distilled water from that the solution was divided into half for the addition of extract. *Illicium Verum* seed extract of 0.532 µl of extract solution was added to 26.615 ml of $AgNO_3$ and stirred using magnetic stirrer and it was also subjected to heat. The colour change was observed for every half an hour. The extract solution was then subjected to centrifugation and pellet was obtained, to that add water of 2ml in Eppendorf tube to take OD of the extract (Luna et al., 2015).

UV-visible light spectroscopy

The range of UV absorption were taken between 300 to 700 nm. The deionized water was used as a blank. The OD of star anise seed extract synthesized silver nanoparticles was observed for every one hour. The peak was observed at 437 nm. The OD was observed till 8th day to determine the stability of sample. The eppendorf tube which was centrifuged was also checked for OD (Elamawi et al., 2018).

FT-IR Spectroscopy

Illicium Verum seed extract and Fabricated AgNPs were subjected to FTIR to identified what are all the obtainable functional groups in the nanoparticles. In the 4000-400cm-1 range, a functional group is found to be present. Dried powder were characterized using KBR pellet method (Damayanti et al., 2020).

DLS (Dynamic Light Scattering Spectroscopy)

The term photon-correlation spectroscopy is another name for DLS. It is mostly used to calculate the size distribution of the particles. For the purpose of determining the average particle size of silver nanoparticles, DLS is based on the laser diffraction method with multiple scattering methodology. A Doppler shift happens when a monochromatic beam of light passes through a solution, changing the wavelength of the incoming beam of light by a quantity linked to particle size. (Patra & Baek, 2014).

SEM (Scanning Electron Microscopy)

Scanning electron microscopy was used to analyse the morphological characteristics of synthetic silver nanoparticles made from star anise seed extract (JSM-6480 LV). The SEM slides were made by smearing the solutions over slides after adding $AgNO_3$ for 24 hours (Patra & Baek, 2014). The samples were coated with a tiny layer of platinum to make them conductors. They were examined in SEM with a 15 KV voltage.

Anti-microbial activity

For this method 2 micro-organisms are used namely (Gram positive bacteria). 25ml of Luria Bertani broth prepared and were inoculated with pure culture of *E. coli* and *S. aureus* and they were kept in incubator (Sri et al., 2015). 150ml of Muller Hinton agar and agar agar solution was prepared. The agar solution was poured on each petriplate to get solidified. The culture of *E. coli* and *S. aureus* was spread over the agar using cotton swabs. Holes were made with gel puncher and $AgNO_3$ nanoparticles of different concentrations (25, 50, 75, 100µl) was poured in each well and kept in incubator at 36+/-1℃ for 24 hours to observe the zone of inhibition. Streptomycin was used as standard drug. (Salem et al., 2021).

Result and Discussion

Synthesized silver nanoparticle analysis

The Star Anise seed extract was kept under room temperature for 2 days and later colour change was observed. The solution's colour changed from a light, pale yellow to a light brown. The change of colour indicates that silver nanoparticle has been synthesized (Aayasha et al., 2021)

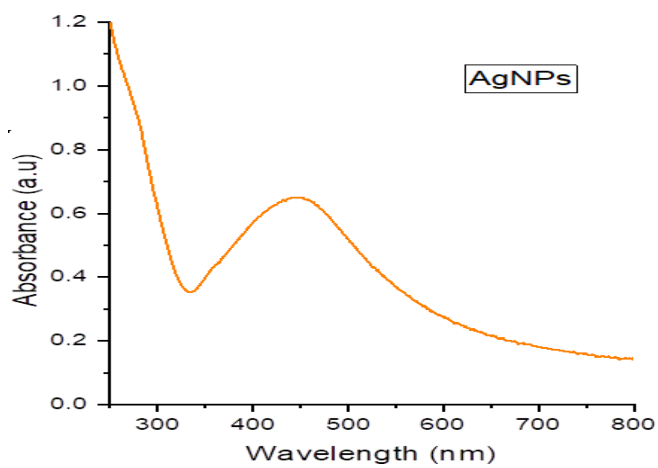
UV-visible spectrophotometer analysis:

Reduction of silver nanoparticles during exposure to seed extracts was observed as a result of the colour change. A visible colour change was observed from pale yellow to mild brown colour within 2 hours. This indicates the formation of silver nanoparticles, after 48 hours, there was a significant colour change from mild to little bit dark brown colour due to increase reaction time, it enhances the growth of silver nanoparticles. It is well known that silver nanoparticles exhibit yellowish brown colour in water due to Surface Plasmon Resonance phenomenon.

Due to the simultaneous vibration of the metal nanoparticles' free electrons in resonance with the light wave, the metal nanoparticles have the free electrons that provide the SPR absorption band. At 437nm, the silver nanoparticles' angular bands were visible. In case of seed extract of star anise, the intensity of absorption peak increases with the increase in time period (Figure 1). The metal nanoparticles were observed o be stable in solution even after 3 weeks of their synthesis. By stability we mean that there was no observable variation in the optical properties of the nanoparticle solutions with time. The intensity of absorption peak at 437nm increases with increasing time period of aqueous component.

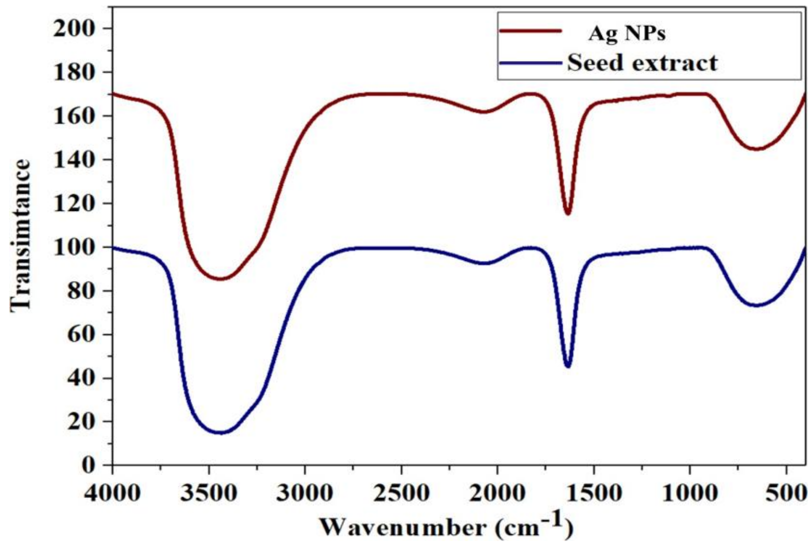
Figure 1: UV peak for synthesized silver nanoparticle

FT-IR Analysis



FT-IR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticle synthesis. The Figure 2 shows the sharp FT-IR spectrum of synthesized AgNPs located at about 665.16cm-1, 1634.21cm-1, 2073.60cm-1, 3435.39cm-1. The absorption peak at 665.16cm-1 showed the stretch of silver nanoparticles. The peak found at 1634.21cm-1 shows the bond stretch of N-H. The peak at 2073.60cm-1 shows C-H bond and the peak corresponding to 3435.39cm-1 shows O-H stretching, H- bonded alcohol and phenols. Therefore, proteins and metabolites with functional groups, such as terpenoids, were found to surround the produced nanoparticles. (Alsalhi et al., 2016).

Figure 2: FTIR peak for fabricated Nanoparticles and extract



From the analysis of FT-IR studies, we confirmed that the carbonyl groups from the amino acid residues and proteins have the stronger ability to bind metal indicating that the proteins could possibly form a layer covering the metal nanoparticles (i.e.) capping of silver nanoparticles to prevent agglomeration and thereby stabilize the medium. These results suggest that the biological molecules perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium.

DLS Analysis

The DLS approach is a diagnostic tool that is frequently used in science and business to analyse the particle size distribution of silver nanoparticles in solution or colloidal suspensions. Figure 3 displays the DLS size distribution of created silver nanoparticles. Silver nanoparticles have an estimated average particle size distribution of 90 nm. The DLS analyzer's wide spectrum confirms that, in comparison to the SPR peak (437 nm) found in UV spectra, the particle size has decreased. Thus, DLS is described as a method for determining the sample's average hydrodynamic diameter by varying the scattering light intensity fluctuations. DLS has been used to identify metal ions and cancer biomarkers due to its quick and sensitive solution phase detection measuring process.

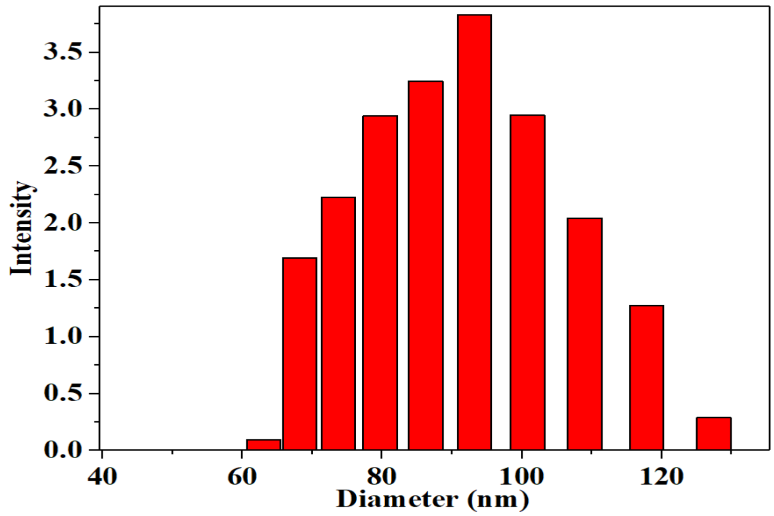


Figure 3: DLS analysis of fabricated nanoparticles

SEM Analysis

SEM is a method that creates an output image using electrons as opposed to light. The size, shape, morphology, and dispersion of produced silver nanoparticles are all described using the SEM examination. The silver nanoparticles that were produced, as shown in figure 4, are pure and polydisperse, according to SEM micrographs. According to SEM pictures, silver nanoparticles are typically spherical in shape, smooth on the surface, evenly scattered, and arranged closely together. The produced nanoparticles were larger than expected; they should have been between 1 and 100 nm, but instead, their size ranged from 100 to 140 nm. The proteins that were bound to the surface of the nanoparticles caused the size to be larger than what was desired.

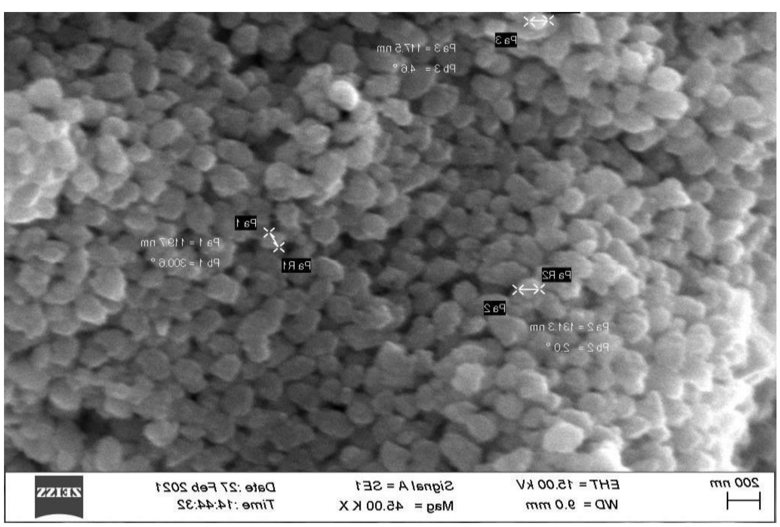


Figure 4: SEM analysis of fabricated Nanoparticles

Anti-microbial activity

The images show the anti-bacterial effects of various concentration of star anise seed extract (25, 50, 75, 100 µl). The silver nanoparticles against *S. aureus* (Gram positive bacteria) and *E. coli* (Gram negative bacteria) was studied by agar well diffusion method. Silver nanoparticles showed significant anti-bacterial activity against these pathogens. The inhibition zone formed were silver nanoparticle dose dependent and maximum zone of inhibition was obtained at 100µl. The high anti-bacterial potential of silver nanoparticles may be due to large surface area, shape and ultrafine structure. The synthesized silver nanoparticles helps to deactivate cellular enzymes and damage the bacterial cell membrane because of positively charged silver ions. This leads to enzymatic dysfunction and damage to proteins and DNA because of reactive oxygen species by silver nanoparticles ending in cell death. These spherical shaped silver nanoparticles are responsible for the inhibition of gram negative bacterium *E. coli*. Anti-bacterial activity of synthesized silver nanoparticles is observed against both *E. coli* and *S. aureus* at a higher concentration of 75µl and 100µl and a clear zone is also formed and shown in figure 5. Here antibiotic Streptomycin is used as control. These bacterial group incubations around the wall are due to the release of diffusible inhibitory compounds from silver nanoparticles, these biosynthesized silver nanoparticles are used in cancer therapy, wound healing and anti-microbial activity and also in medical field. The zone of inhibition possess that silver nanoparticle has effective antibacterial property (Prabhu et al., 2022).

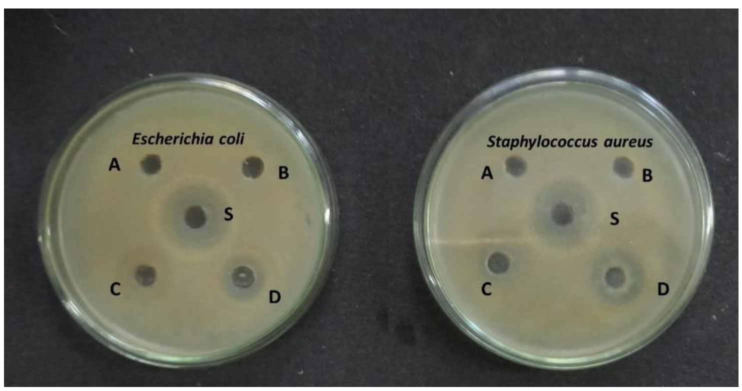


Figure 5: Anti-Bacterial activity of fabricated silver nanoparticle against *E. coli* and *S. aureus* (A - 25 µl of AgNPs, B - 50 µl of AgNPs, C - 75 µl of AgNPs, D - 100µl of AgNPs, S – Streptomycin)

Conclusion

The green synthesis of silver nanoparticles using Illicium verum seed extract is an eco-friendly, simple, and efficient route for the synthesis of nanoparticles. They are inexpensive and can produce silver nanoparticles at room temperature. The silver nanoparticles were characterized by UV-visible spectrophotometer, FT-IR, DLS, and SEM analysis. The surface Plasmon resonance of green synthetic silver nanoparticles was confirmed by UV-visible spectral studies. The UV absorption peak at 437 nm clearly indicates the synthesis of silver nanoparticles. The SEM images confirmed that the synthesized nanoparticles were spherical in shape and that their sizes ranged from 100 to 140 nm. It was helpful to analyse their morphology and distribution. FT-IR measurements confirmed that the biomolecules were responsible for reducing and capping silver nanoparticles. The calculated average particle size distribution of silver nanoparticles was found to be 90 nm from the DLS data. The AgNPs biosynthesized from Illicium verum seed extract exhibit excellent anti-microbial activity against *E. coli* and *S. aureus*. Hence, green synthesis of silver nanoparticles is an effective and eco-friendly process for nanodrugs for pharmacological application as well as large-scale commercial production.

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