

**GREEN SYNTHESIS OF SILVER NANOPARTICLES
USING *CAMELLIA SINENSIS* (TEA) LEAVES
EXTRACT: CHARACTERIZATION AND STUDY OF ITS
ANTIMICROBIAL ACTIVITIES**

DISSERTATION SUBMITTED FOR THE PARTIAL FULFILLMENT OF THE
REQUIREMENT OF THE MASTER OF SCIENCE DEGREE IN CHEMISTRY

SUBMITTED BY

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SUBMITTED TO

DEPARTMENT OF CHEMISTRY

AMRIT CAMPUS

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TRIBHUVAN UNIVERSITY

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July 4, 2023

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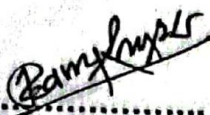
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ACKNOWLEDGEMENTS

First of all, I would like to express my sincere gratitude to my supervisor, Assistant Professor Dr. Deval Prasad Bhattarai, for all his help, support and guidance throughout the preparation of my dissertation work.

I am thankful to the Head of Department of Chemistry Associate Professor Kanchan Sharma and the program coordinator Associate Professor Dr. Bhushan Shakya for their kind support. I am very much thankful to Assistant Professor Hari Bhakta Oli for his kind support and help. I owe a great deal of thanks to all the respected teachers as well as staffs of library for their immense support, continuous guidance and valuable suggestions. I am also indebted to the Amrit campus family where I believe to develop my academic and research career.

Last, but not least, I want to thank all my dear friends and family for their continuous support, help and words of encouragement throughout the report preparation.



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ABSTRACT

Chemical synthesis of nanoparticles has been found to be somewhat expensive, malignant to the environment, and less efficient with respect to materials and energy use. Therefore, the synthesis of ecologically friendly nanoparticles that do not produce harmful waste throughout the manufacturing process has piqued people's curiosity. This can be accomplished by using biological synthesis procedures that are regarded safe and environmentally friendly as an alternative to traditional physical and chemical approaches. The aim of this research is to determine the true potential of green nanotechnology in the synthesis of silver nanostructures and the possibility of modifying present techniques to be more environmentally friendly, energy-efficient, less hazardous, simpler, and cheaper.

In this study, silver nanoparticles (AgNPs) have been synthesized using Tea (*Camellia sinensis*) leaves extract as a reducing agent and stabilizer onto the silver nitrate precursor. Then, the antimicrobial activity of as-synthesized AgNPs has been evaluated against Gram-negative bacterium (*Escherichia coli*), Gram-positive bacterium (*Bacillus subtilis*) and pathogenic fungus (*Candida albicans*). Also, the as-synthesized AgNPs were characterized by Ultra-Violet Visible spectrophotometry (UV-VIS), X-ray Diffraction (XRD), Fourier Transform Infrared (FTIR) spectroscopy, Field Emission Scanning Electron Microscopy (FESEM) and Energy-Dispersive X-ray Analysis (EDX). UV-VIS spectroscopy determined the Surface Plasmon Resonance (SPR) peak at 400-500 nm. Tauc's plot analysis disclosed its band gap energy as 3.605 eV which manifested the potential of AgNPs to be semiconductors. The particle size of silver nanoparticles was determined from XRD and the Scherrer equation and average size of 66.87 ± 16.74 nm from SEM. EDX analysis confirmed the AgNPs' purity with a strong silver signal at 3 keV. The anti-microbial activity of *Escherichia coli* gave the maximum zone of inhibition (12 mm) higher than that of *Bacillus subtilis* (11 mm) as compared to the control kanamycin (20mm) for both and *Candida albicans* (10 mm) as compared to the same control (18mm) confirming their antimicrobial property. Based on supporting data, it has been found that *Camellia sinensis* leaves extract is good enough in the green synthesis of silver nanoparticles that can be used for antimicrobial applications.

Keywords: *Camellia Sinensis*, leaves extract, silver nanoparticles, green synthesis, Tauc's plot, antimicrobial activity.

LIST OF ACRONYMS AND ABBREVIATIONS

AgNPs	: Silver Nanoparticles
FTIR	: Fourier Transform Infrared
MNPs	: Metallic Nanoparticles
NPs	: Nanoparticles
SEM	: Scanning Electron Microscope
SPR	: Surface Plasmon Resonance
keV	: kilo electron Volt
TEM	: Transmission Electron Microscope
UV-VIS	: Ultra-Violet Visible Spectroscopy
XRD	: X-ray Diffraction
EDX	: Energy Dispersive X-Ray

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CHAPTER 1

INTRODUCTION

1.1 General Introduction

Nanoscience focuses on the study of nanomaterials (1-100 nm) whereas nanotechnology is interdisciplinary field that enables the production of nanomaterials of tunable properties (Hernández-Morales *et al.*, 2019; Neupane *et al.*, 2016; Poole *et al.*, 2003).

Recently, overwhelming work on design, fabrication and application of nanomaterials are going on, because of their dimension dependent unique properties like mechanical, electrical, catalytic, magnetic, diffusive, optical, microbial etc. (Allafchian *et al.*, 2018; Behravan *et al.*, 2019; Vélez *et al.*, 2018). On the basis of dimensions, they are classified into four types as in Figure 1.1.

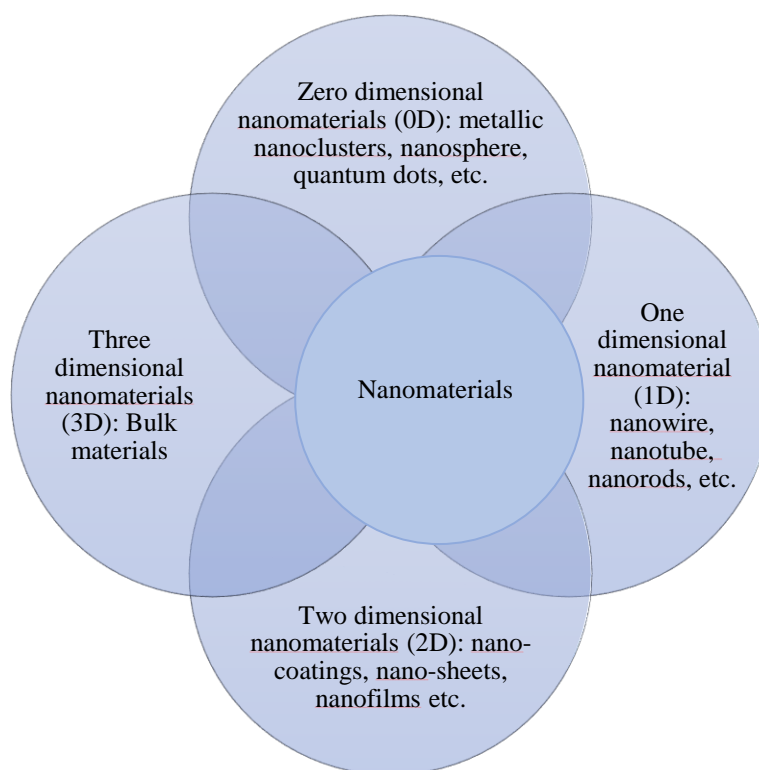


Figure 1.1: Classification of Nanomaterials based on Dimension

Nanoparticles are zero dimensional nanomaterials (Neupane *et al.*, 2016) having different properties associated to their shape, size, surface charge, and surface area (Salem *et al.*, 2021). They are metallic, semiconducting and non-metallic on the

basis of electrical properties. Research on the production and commercialization of metallic nanoparticles (MNPs) is tremendously going on because of their possible applications in a variety of sectors, including health care, synthetic biology, and cellular transportation (Shaik *et al.*, 2018).

Metallic nanoparticles (MNPs) are considered flexible nanostructures due to their ability to control composition, shape, size, structure, encapsulation, assembly, and optical properties during synthesis (Jamkhande *et al.*, 2019). They can be prepared by physical as well as chemical methods among which electrochemical changes, chemical reduction, and photochemical reduction are generally employed for the preparation and stabilization of metallic nanoparticles. In the present days, green synthesis method is flourishing as it involves the use of plant parts such as root, stem, leaf etc. as well as bacteria and fungi (Shnoudeh *et al.*, 2019).

Among several nanoparticles, the synthesis of nanoparticles from diverse noble metals such as palladium, tin, copper (Thiruvengadam *et al.*, 2019), silver (Arumai *et al.*, 2018), and gold (Abdel-Raouf *et al.*, 2017) is now receiving tremendous interest because of its unique characteristics and applications in many sectors. Amid them, silver nanoparticles (AgNPs) are one of the most important and intriguing. In addition, silver nanoparticles are essential in the pharmaceutical industry because they serve as antibacterial agents with minimal toxic effects (Zhang *et al.*, 2016). Silver has wide antibacterial action against Gram positive and Gram-negative bacteria, fungi, protozoa, and certain viruses. The antibacterial activities of silver and silver-based compounds, such as AgNPs, have been exploited by antibiotic-resistant bacteria (Beyene *et al.*, 2017).

1.2 Synthesis of Silver Nanoparticles (AgNPs)

The synthesis approaches of silver nanoparticles include top-down approach and bottom-up approach. Top-down approach (Lee *et al.*, 2019) is a physical method of nanoparticle synthesis where bulk material is converted into small nano-sized particles. The approach involves the methods such as mechanical milling, thermal and laser ablation. Bottom-up approach involves chemical and biological method which is based on formation of nanoparticles from smaller molecules like joining of atoms, molecules or small particles (Jamkhande *et al.*, 2019).

Chemical Approach includes chemical precipitation, vapor deposition, Atomic/Molecular condensation, sol-gel process, spray pyrolysis, laser pyrolysis and

aerosol pyrolysis. Silver nanoparticles can be prepared by chemical reduction method. Silver nitrate can be taken as the metal precursor and hydrazine hydrate as a reducing agent (Zia *et al.*, 2018). Biological Approach involves nanoparticles synthesis using bacteria, fungi, plant and plant extracts. Plant extracts have often been employed as reducing agents instead of chemical reducing agents in this technique. Therefore, there is no need of introduction of any hazardous substances as the process is renewable and affordable (Yadi *et al.*, 2018).

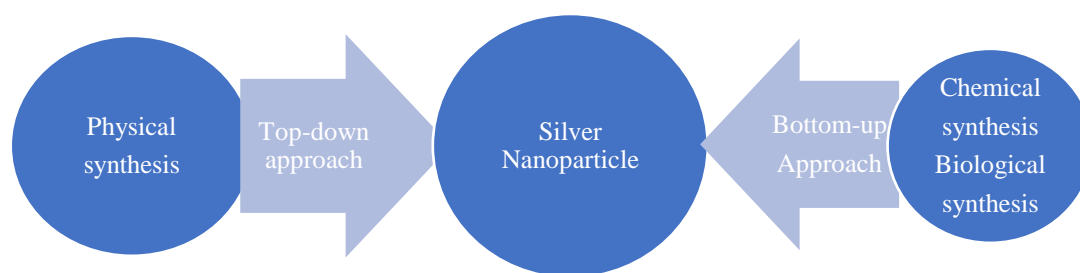


Figure 1.2: Methods of Preparing Silver Nanoparticles.

Nanoparticle can be synthesized by chemical and physical approaches which are designated as not cost-effective. Moreover, the chemicals utilized in chemical procedures are extremely reactive and pose a risk to the environment. Because these materials are encompassed in the nanoparticle manufacturing process, their medicinal and clinical applications are limited (Hamedi *et al.*, 2019). Therefore, the demand for environmentally friendly nanoparticle production processes has resulted in a growing interest in biological approaches that avoid the use of harmful chemicals and the formation of toxic byproducts. In this respect, the demand for "green nanotechnology" is growing (Arumai *et al.*, 2018).

Green synthesis is the creation of nanoparticles using environmentally friendly components such as bacteria, fungi, and plants; and are free of the drawbacks that come with traditional synthetic techniques. Synthesis from biologically generated extracts has various advantages, including quick synthesis, high yields, and, most significantly, the absence of costly downstream processing. As a result, nanoparticle synthesis from plant extracts may provide a route to large-scale commercial nanoparticle production (Moodley *et al.*, 2018). It has been reported that biomass filtrate derived from a variety

of biological systems, including yeast, bacteria, actinomycetes, fungus, algae, and plant extract can be used to synthesize green NPs (Salem *et al.*, 2021).

Biosynthesis of metal, metal oxides and metal composite of nanoparticles for numerous applications from different plant parts such as leaves, roots, flower, seeds etc. are growing (Beyene *et al.*, 2017). Biological materials such as bacteria, fungus, yeast, algae, and plants have been shown to have a strong bio-reduction capacity to produce metallic nanoparticles of diverse sizes and shapes. The reduction of metals into metallic nanoparticles by plants and plants extract is due to the presence of biomolecules such as terpenoids, flavones, ketones, aldehydes, proteins, amino acids, vitamins, alkaloids, tannins, phenolics, saponins, and polysaccharides (Vijayaraghavan *et al.*, 2017).

1.3 Applications of AgNPs

Silver nanoparticles with high surface-to-volume ratio possess unique properties which help in molecular diagnostics, therapies, as well as in several medical equipment (Abou El-Nour *et al.*, 2010). Not limited to this, it attracted a great interest because of their applications in electronics, biosensors, catalysis, good conductivity, chemical stability, photonic, optoelectronic, and antimicrobial properties (Baruah *et al.*, 2019; Manosalva *et al.*, 2019).

AgNPs are used more commonly with respect to other NPs. Since ancient times metallic silver as well as other silver compounds are well recognized for their antimicrobial properties and had been used in burn, wound, and antibacterial infections. Their use decreased in recent years due to the development of sophisticated antimicrobial medicines. However, they are reviving as an alternative to overcome the antibiotic resistance developed by the bacteria (Sajid *et al.*, 2020). The antibacterial application of AgNPs in the dentistry is remarkable including orthodontic, endodontic, restorative, prosthetic, implant and periodontal treatment (Yin *et al.*, 2020). It can be used to induce the apoptosis-programmed cell death more strongly than necrosis in cancer cell line (Dehghanizade *et al.*, 2018) that has also been reported against gastric cancer cell lines (Mousavi *et al.*, 2018), human breast cancer cell line (MCF-7) and human colon carcinoma cell line (HCT-116) (Alkhulaifi *et al.*, 2020). AgNPs show antimicrobial, antifungal (Abbasi *et al.*, 2021), antioxidant (Bedlovičová *et al.*, 2020), anti-diabetic (Jini *et al.*, 2020), anti-inflammatory effects which can be used to produce new and cost-effective drugs (Kumar *et al.*, 2019; Naveed *et al.*, 2022).

The AgNPs synthesized from green approach can exhibit good activity against the isolated dermatophytes which can be used to create drug resistant towards broad-spectrum antibiotics (Najimu *et al.*, 2014). AgNPs has a lot of potential in the medical field for treating infectious diseases (bacterial, fungal, viral, and parasitic), gastrointestinal diseases (including acne), skin diseases (including acne), disinfectant (Deshmukh *et al.*, 2019) and some types of wounds and burns (Paladini *et al.*, 2019), as well as their ability to act in oral rehydration solutions and disinfectants (Salleh *et al.*, 2020).The drug- and targeted-delivery systems are prolonged with AgNPs (Tan *et al.*, 2020).

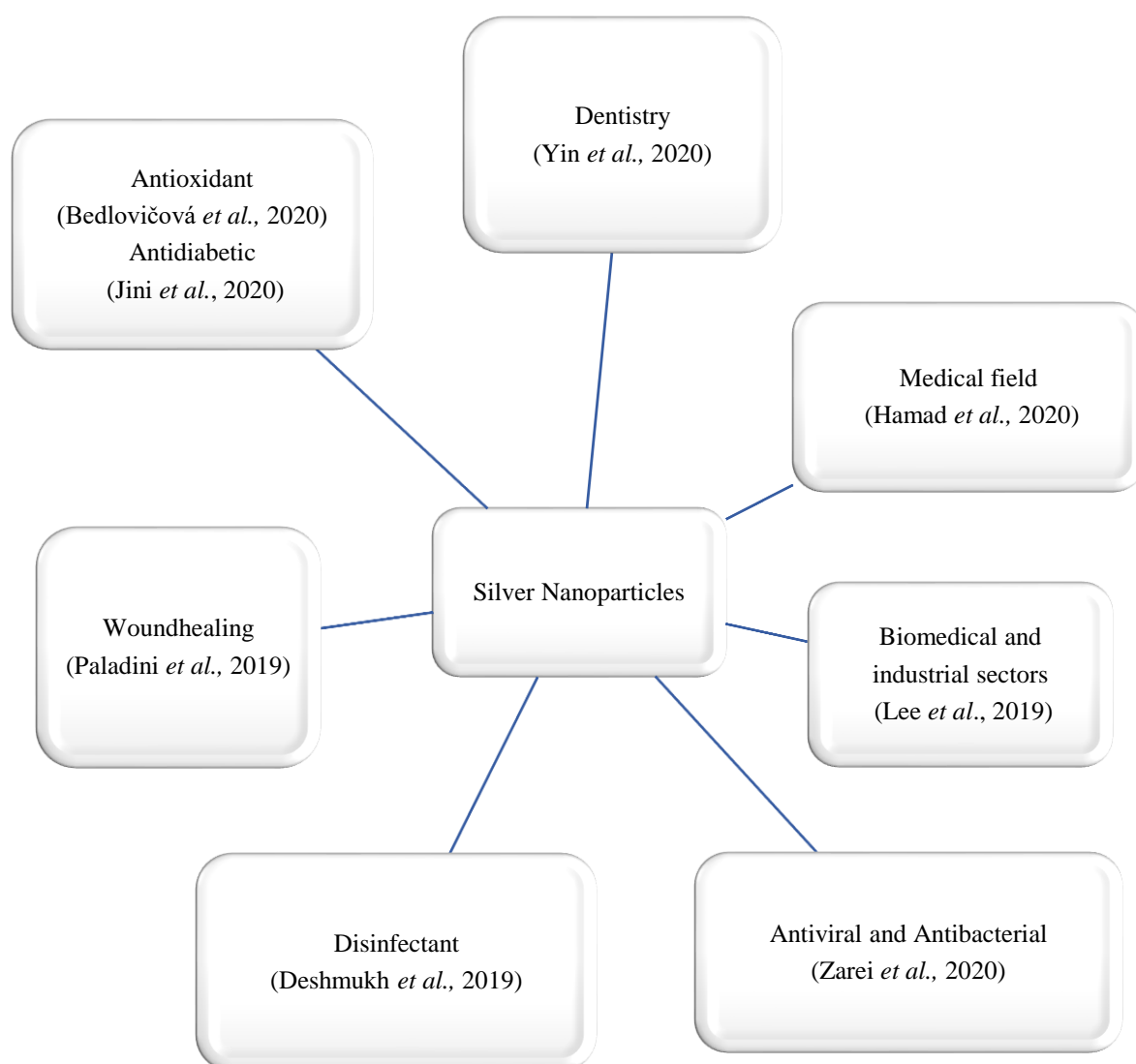


Figure 1.3: Applications of Silver Nanoparticles

1.4 Antimicrobial Activity of AgNPs

Antibiotic resistance is one of the world's greatest health risks, and micro-organisms resistance grows faster than the current rate of discovering new and effective medicines. To counter these antibiotic-resistant microorganisms, metal-based antimicrobial compounds such as AgNPs with readily adjusted physicochemical characteristics can be created. The ability of silver nanoparticles (AgNPs) to damage the extracellular membrane and intracellular components of bacteria allows them to have a broad antibacterial impact (Zheng *et al.*, 2018). Silver are these days used as antibacterial agents in the medical field in the form of nanoparticles and ions, where silver ions proved to be effective against active bacteria, viruses, and fungi (Hamad *et al.*, 2020). According to a study (Parthiban *et al.*, 2019), the mechanism of antimicrobial activity AgNPs may be due to the free radicals generation, where they interact with bacteria and release the silver ions inside of the cell which results the contents of the cells to be leaked out and ultimately leads to the protein denaturation. Many study also suggests the silver ions released from silver nanoparticles kill microbes facilitating antibacterial effects (Yin *et al.*, 2020). Therefore, AgNPs can be considered as a better alternative to treat the infectious disease caused by pathogens.

The present research is focused on green synthesis of silver nanoparticles and its characterization using *Camellia sinensis* (Tea) leaves extract and investigation of its antibacterial property.

1.5 *Camellia Sinensis* (Tea)

Camellia sinensis, a plant native to Southeast Asia, is a vital plant for humans all over the world. Humans have been drinking tea brewed from the leaves for thousands of years as a stimulant, and it is now the second most frequently consumed beverage after water. It is a widely cultivated evergreen crop of the *Camellia* genus of the Theaceae family (Xia *et al.*, 2019).

Tea contains polyphenolic compounds that can help with cardiovascular disease, cancer, digestive issues, and metabolic problems including obesity and diabetes. *Camellia sinensis* leaves produced polyphenolic chemicals that include epigallocatechin, caffeine, tannins, catechin, and epicatechin, which protect plants from dangerous infections in addition to free-radical scavenging and antioxidant activities. The extract of *C. sinensis*, as well as the primary polyphenolic constituent catechin, has

medicinal use in the prevention and treatment of a variety of disorders (Heck *et al.*, 2009)

Furthermore, it has long been linked to health advantages against a variety of disorders, including diabetes, inflammation, clastogenesis, vomiting, diarrhea, heart problems, and several forms of cancer. Tea has lately been demonstrated to prevent liver harm caused by alcohol and a variety of other variables in a significant number of scientific experiments using experimental animals (Shivashankara *et al.*, 2019).

Scientific Classification

Kingdom : Plantae
Clade : Tracheophytes
Clade : Angiosperms
Clade : Eudicots
Clade : Asterids
Order : Ericales
Family : Theaceae
Genus : *Camellia*
Species : *sinensis*



Figure 1.4: *Camellia sinensis* leaves

1.6 Rationale of Study

There are several well-known methods for synthesizing nanoparticles via physical, chemical reduction methods which are quite expensive, hazardous, and laborious to conduct. To overcome the problems of conventional method, green method of synthesizing nanoparticles can be used. Green synthesis of silver nanoparticles has been done extensively using different plant extracts due to their wide applications in several fields as they possess unique physicochemical, optical, electrical, thermal activities and magnificent disinfectant properties. Moreover, Silver nanoparticles have excellent anticancer as well as antimicrobial efficacy resulting wide applications as antimicrobial, wound healing, antidiarrheal, and antifungal agents. An alternative drug invention has been the subject of importance to overcome the antibiotic resistant bacteria. One of the alternatives against various microbial pathogens can be the use of silver nanoparticles (AgNPs).

The silver nanoparticles have been grown predominantly in pharmaceuticals and other drug industries, because of a great impact on antibacterial properties due to a different size variation. Hence, the main rationale of this research is to study green synthesis of silver nanoparticles using locally available plant extracts so as to replace expensive chemicals by plant extract using *Camellia sinensis* (Tea) leaves and study of their antimicrobial activity.

1.7 Objectives

1.7.1 General Objective

- Green synthesis and characterization of silver nanoparticles (AgNPs) using *Camellia sinensis* (Tea) leaf extract and the study of antimicrobial activity of AgNPs.

1.7.2 Specific Objectives

- Extraction of leaf extract from *Camellia sinensis* in aqueous media.
- Synthesis of AgNPs using *Camellia sinensis* (Tea) leaf extract as reducing and capping agent.
- Characterization of AgNPs by using Ultra-Violet Visible Spectroscopy (UV-VIS Spectroscopy), Energy-dispersive X-ray (EDX) Spectroscopy, Fourier Transform Infrared (FTIR), X-ray Diffraction (XRD), Scanning Electron Microscope (SEM) analysis technique.
- Study of antibacterial activity of synthesized AgNPs against Gram-positive bacteria (*Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli*) and Gram-positive fungus (*Candida albicans*).

CHAPTER 2

LITERATURE REVIEW

2.1 Green Synthesis of Silver Nanoparticles

Green synthesis utilizes its natural agents and nontoxic chemicals which results high yield of AgNPs (Salleh *et al.*, 2020). Many research have been done on the green synthesis of AgNPs using plants and plants parts. Some of the research studies which is focused on the synthesis of silver nanoparticles by using green method are described as follows:

Silver Nanoparticles has been synthesized utilizing a green method that included *Combretum erythrophyllum* leaf extract as reducing and capping agents. The formation of Ag-NPs with a cubic crystal structure was indicated by UV–VIS and XRD investigations, while FTIR verified the capping of Ag-NPs by the functional groups of aqueous extract. According to TEM images, the Ag-NPs were spherical in form with an average particle size of 13.62 nm (Jemilugba *et al.*, 2019).

Silver nanoparticles (AgNPs) were reported to be synthesized from *Cynara scolymus* (Artichoke) leaf extracts. AgNPs had an absorption spectrum that ranged from 330 to 640 nm, with a strong peak at 434 nm. AgNPs were detected in broad bands in the FTIR spectrum. The decrease of Ag^+ to Ag can also be ascribed to stretching vibrations at 538 cm^{-1} . The SEM pictures seemed to be agglomerated and dispersed. Silver nanoparticles were found in 59.59 percent of the EDX spectrum (Erdogan *et al.*, 2019). The biological synthesis of silver nanoparticles has been reported using *Enicostemma axillare* leaf extract which described that AgNPs were mostly spherical. UV-VIS spectrophotometer analysis was used to monitor the formation and stability of reduced silver nanoparticles in colloidal solution. DLS research verified the zeta potential. The size of the particles was measured between 15 and 20 nm, and the mean particle diameter of silver nanoparticles was determined using TEM and SEM. The spherical form of the particles was identified using TEM. Peaks in the XRD pattern corresponding to [111], [200], [220], and [311] planes indicated the crystalline nature of the nanoparticles and their face-centered cubic structure (Raj *et al.*, 2018).

An aqueous leaf extract of *Givotia moluccana* was used as reducing and stabilizing agent for silver nanoparticles formation. SPR of AgNPs at 435 nm was due to reduction of pure Ag^+ ions that were monitored by UV-VIS spectra. FTIR revealed plant metabolites like polyphenols, flavonoids, quinones and organic acids were responsible

for the bio-reduction of silver ions into AgNPs. XRD pattern showed the face centered cubic structure which confirmed the crystalline nature of synthesized AgNPs. SEM and TEM were used to confirm the size and shape of the synthesized AgNPs with a narrow-size distribution at about 30 nm (Sana *et al.*, 2018).

Likewise, Novel silver nanoparticles synthesis from *Gleichenia Pectinata* was studied. Noteworthy absorption was observed at 460 nm due to the surface plasmon resonance (SPR) which was best at 105 minutes contact time. SEM and TEM displayed a spherical shape of AgNPs with an average size of 7.51 nm. The XRD result showed a crystalline and polydispersed AgNPs (Femi-Adepoju *et al.*, 2019).

In another study, AgNPs were reported to be synthesized from the aqueous leaf extract of *Holoptelea integrifolia*. The presence of -OH and -NH functional groups of secondary molecules of extract capped on the AgNPs surface was verified by FTIR measurements. FESEM analysis indicated the development of spherical aggregates with a diameter of 32–38 nm. EDX tests verified the presence of elemental silver. XRD study determined the face centered cubic (FCC) crystal structure of biosynthesized AgNPs (Kumar *et al.*, 2019).

Similarly, UV–vis spectra of the leaf extract of *Thymbra spicata L. var. spicata* and AgNO₃ mixture of different concentration revealed clear peaks at 452 and 444 nm for AgNPs formed (Erci *et al.*, 2018). The surface plasmon resonance (SPR) bands were consistent with the literature reports for AgNPs.

The variation in the absorption spectra of AgNPs synthesized using Tea extract at different temperature from 5–35 °C was observed. It was found that with an increase in the reaction temperature, the absorption peak blue shifted towards lower wavelength, from 455 to 436 nm. There was no noticeable peak in the absorption spectra at 5 °C, indicating that AgNPs were not formed.

In another research report, similar result was obtained when Tea leaf extract was added to AgNO₃ solution and exposed to sunlight, the color of the solution changed from colorless to light brown and then to a dark brownish red color. This surface plasmon excitations caused color variance in the samples. UV–vis spectrum of AgNPs synthesized by using sunlight irradiation gave sharp and strong plasmon resonance at 430 nm on varying the different light sources (UV, microwave, sunlight) and methods (refluxing, room temperature) (Singh *et al.*, 2018).

According to Abbasi *et al.*, the interaction of AgNO₃ with *Citrus limon* leaf aqueous extract resulted in Ag nanoparticles. Nanoparticles were characterized using TEM, FE-

SEM, UV–VIS, and FT-IR techniques. FT-IR spectra showed the presence of large number of the antioxidant compounds that provided appropriate conditions for the reduction of Ag (Abbasi *et al.*, 2021).

The potential of *Nigella arevensis* aqueous leaf extract for the biogenesis of silver nanoparticles (AgNPs) was investigated. Colour changes and UV–visible spectroscopy verified the production of AgNPs, with absorbance maxima peaking at 416 nm. The AgNPs in the TEM images were anisotropic and generally spherical, with diameters ranging from 5 to 100 nm. The flavonoids, alkaloids, and phenolic groups found in leaf extract were responsible in the reduction, according to FTIR analyses (Chahardoli *et al.*, 2018).

Similarly, an aqueous solution of Saudi *Origanum vulgare L.* plant extract was stated to be used as a bioreducing agent to synthesize AgNPs. Various microscopic and spectroscopic methods were used to characterize the AgNPs as they were produced. The findings suggested that crystalline face-centered cubic (FCC) AgNPs were formed. Furthermore, an FT-IR investigation revealed that the *O. vulgare L.* extract not only acted as a bio-reductant but also as a capping agent, stabilizing the surface of the AgNPs (Shaik *et al.*, 2018).

Table 2.1: Green Synthesis of Ag Nanoparticles by Different Researchers using Plant Extracts.

Plant	Plant's part	Applications of AgNPs	References
<i>Camellia sinensis</i> (Tea)	leaf	Antimicrobial activity, antidiabetic activity	Malapermal <i>et al.</i> , 2017
<i>Camellia sinensis</i>	leaf	Antibacterial property	Jain <i>et al.</i> , 2017
<i>Camellia sinensis</i>	leaf	Catalysis	Singh <i>et al.</i> , 2018
<i>Citrus limon</i>	peel	Antibacterial and cytotoxic properties	Alkhulaifi <i>et al.</i> , 2020
<i>Annona reticulata</i>	leaf	Mosquito larvicidal and antimicrobial activity	Parthiban <i>et al.</i> , 2019
<i>Alpinia nigra</i>	leaf	Antimicrobial and photocatalytic activities	Baruah <i>et al.</i> , 2019
<i>Anthemis atropatana</i>	Aerial parts	Antibacterial and anticancer effects	Dehghanizade <i>et al.</i> , 2018

<i>Artemisia turcomanica</i>	leaf	Anti-cancer effect and apoptosis induction on gastric cancer cell line (AGS)	Mousavi <i>et al.</i> , 2018
<i>Artocarpus heterophyllus Lam.</i>	seed	Antibacterial activity	Jagtap <i>et al.</i> , 2013
<i>Berberis vulgaris</i>	leaf, root	Antibacterial activity	Behravan <i>et al.</i> , 2019
<i>Combretum erythrophyllum</i>	leaf	Antibacterial activity	Jemilugba <i>et al.</i> , 2019
<i>Elephantopus scaber</i>	leaf	Environmental and biological effects	Francis <i>et al.</i> , 2018
<i>Holoptelea integrifolia</i>	leaf	Antioxidant, anti-inflammatory, antidiabetic and antibacterial activities	Kumar <i>et al.</i> , 2019
<i>Origanum vulgare</i>	leaf	Microbicidal activities	Shaik <i>et al.</i> , 2018
<i>Prosopis juliflora</i>	bark	Antimicrobial, anticancer and catalytic agents.	Arya <i>et al.</i> , 2018
<i>Curcuma longa</i>	rhizome	Antibacterial activities	Alsammarraie <i>et al.</i> , 2018
<i>Achillea millefolium</i>	leaf	Antibacterial and antioxidant properties	Yousaf <i>et al.</i> , 2020
<i>Salvia hispanica</i>	seeds	Antibacterial activity	Hernández-Morales <i>et al.</i> , 2019

2.2 Antibacterial Activity of Green Synthesized AgNPs

Antibacterial activity of silver nanoparticle synthesized from *Givotia moluccana* was tested against Gram positive *S. aureus*, *S. epidermidis*, and Gram-negative *E. coli* and *P. vulgaris* as reported by a study. In comparison to streptomycin, AgNPs were more efficient against *S. epidermidis* (Jemilugba *et al.*, 2019). Accordingly, Synthesized AgNPs tested against three gram negative bacteria *E.coli*, *P. vulgaris*, *K.pneumonia* and gram positive bacteria *Staphylococcus aureus* (*S. aureus*) also revealed effective suppressive activity (Sana *et al.*, 2018).

AgNPs synthesized from *Gleichenia Pectinata* (Willd.) C. Presl. showed effective antimicrobial activity against antibiotic resistant pathogens *Pseudomonas aeruginosa*,

Escherichia coli, *Klebsiella pneumoniae* and *Candida albicans* and were comparable with standard antibiotics. The study of AgNPs hence showed the potential as an antimicrobial and therapeutic agents against multidrug resistant micro-organisms (Femi-Adepoju *et al.*, 2019).

According to research, the antibacterial effect of nanoparticles was confirmed when analyzed against two bacterial strains (*Escherichia coli* and *Salmonella typhi-murium*) by using the disc diffusion method where Zone of inhibition was concentration dependent. The obtained result could be used to synthesize new and cost effective drugs by using green approach (Kumar *et al.*, 2019).

In accordance with a study, the antibacterial properties of AgNPs were tested under well diffusion and MIC methods against different bacterial strains such that the gram-negative bacteria strains of *Staphylococcus marcescens*, *E. coli* and *Pseudomonas aeruginosa* showed a maximum zone of inhibition at 20 mm, 17 mm and 16 mm respectively whereas *Subtilis epidermidis* strain showed complete inhibition zone among the Gram negative and Gram-positive bacteria (Chahardoli *et al.*, 2018).

Additionally, the antimicrobial activity of the NPs was tested against various bacterial microorganisms including *Shigella sonnei*, *Micrococcus luteus*, *Escherichia coli*, *Aspergillus flavus*, *Alternaria alternate*, *Paecilomycesvariotii*, *Phialophora alba*, and so on. Among the several bacterial strains examined, the AgNPs had the most action against *P. aeruginosa* (Gram-negative strain) and *S. aureus* (Gram-positive strain)(Shaik *et al.*, 2018).

2.3 *Camellia sinensis* (Tea) Mediated Green Synthesis of AgNPs

Green chemistry has been used to synthesize silver nanoparticles (AgNPs) using leaf extracts of *Camellia sinensis* (Tea) as a precursor. The study investigated the role of biomolecules present in Tea in the formation of AgNPs from cationic silver under different physicochemical conditions such as pH, temperature, reaction time, and reactants concentration. Depending on the size, shape, and morphology of the resultant particles, the produced AgNPs had a high absorption maximum between 400–450 nm. The average particle size of AgNPs synthesized using the green method ranged from 10 to 20 nm. The fabricated AgNPs were shown to have improved antibacterial properties against Gram-negative bacteria (*E-Coli*), implying biomedical potential (Jain *et al.*, 2017).

Aqueous leaf extract from *Camellia sinensis* was used to synthesize stable AgNPs with a size range of 3–25 nm. UV–VIS spectroscopy, TEM, SEM–EDX, dynamic light scattering (DLS), and zeta potential were used to determine the size, shape, and elemental analyses. The AgNPs derived from *Camellia sinensis* exhibited an inhibitory effect at $89.31 \pm 5.32\%$ against *Bacillus stearothermophilus* indicating an enhanced biocatalytic potential. As a result, the bioderived AgNPs displayed antimicrobial activity against bacterial species *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Salmonella* species (Malapermal *et al.*, 2017).

The research work reported the synthesis of biogenic silver nanoparticles (AgNPs) from Tea leaf extract which is a clean and sustainable approach. The study involved the application of solar radiations at mild temperature condition for AgNPs formation. The synthesis of stable and monodisperse AgNPs with a strong absorption band at 430 nm and an average diameter of 5–10 nm has been shown by spectroscopic and electron microscopic characterization investigations. In an alkaline media, the catalytic potential of AgNPs was studied for the reduction of 4-nitrophenol to 4-aminophenol by UV–vis spectroscopy with high reduction rate (Singh *et al.*, 2018).

The methanolic leaf extract of *Camellia sinensis* was used to make stable silver nanoparticles in a green way. The antibacterial activity was tested using the disc diffusion method against microorganisms that cause urinary tract infections, such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. A lower concentration of Tea methanolic extract was found to be beneficial against urinary tract infections (Priyadarshini *et al.*, 2019).

Research on isolation of AgNPs using leaf filtrate of *Camellia sinensis* was reported. The change in color of leaf extract was imminent when the silver nitrate was added to the basil leaf filtrates, indicating the formation of AgNPs. The UV-visible spectroscopy and SEM analysis confirmed the formation of AgNPs. It exhibited antibacterial activity against *Klebsiella*, *B. aureus*, *S. aureus* and *E. coli* and the activity varied significantly with the different concentrations of silver nitrate used. It has been observed that most of the plant extracts yielded silver nano particles of size less than 100 nm (Swamy *et al.*, 2020).

CHAPTER 3

MATERIALS AND METHODS

3.1 Sample Collection and Experimental Site

Tea (*Camellia sinensis*) leaves were collected from Ilam in the month March, 2022 which is situated in the Mahabharata hilly region of Province No. 1 in eastern Nepal which also serves as the headquarters for the Ilam District. The scientific name of the plant was identified with the help of Department of Botany, Amrit Campus, Tribhuvan University, Kathmandu, Nepal.

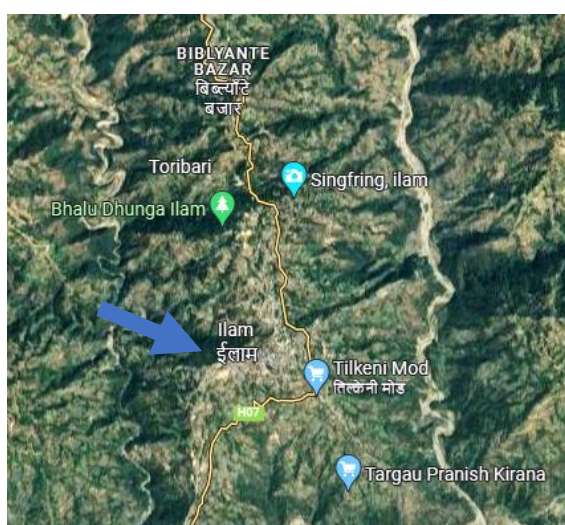


Figure 3.1: Geographical Location of the Sample Collection Area.

The research work for the synthesis of silver nanoparticles was carried out in Chemistry Research Laboratory of Amrit Campus. FTIR spectroscopic characterization and UV-Visible Spectroscopy Characterization were also carried out in research laboratory of Amrit campus. XRD was done in Jawaharlal Nehru Center for Advanced Scientific Research (JNCASR), Bengaluru, research laboratory of India. SEM and EDX were done in Jeonbuk National University, research laboratory of Korea. Antibacterial and antifungal tests were carried out in Himalayan Research Institute of Nepal.

3.2 Apparatus and Instruments

UV-Visible single beam spectrophotometer (UV-2510), XRD, EDX, FTIR spectrometer, Centrifuge tubes, weighing machine, volumetric flasks, Pipettes,

Magnetic stirrer, Conical flasks were major instruments/ equipment used for this research work.

3.3 Chemicals and Reagents

Silver Nitrate (AgNO_3 , 99.9% Merck) as chemical precursor, Distilled water, and Tea leaves as reducing and stabilizing agent.

3.4 Experimental Methods

3.4.1. Preparation of *Camellia sinensis* Solution

Tea (*Camellia sinensis*) leaves were first washed with tap water and then with distilled water to remove the dust and unwanted particles which may be present on them. The leaves were then dried at room temperature under shade to eliminate any remaining water on their surface. About 10 g of finely incised dried Tea leaves were heated for about 15 minutes in 100 mL distilled water at 60 °C. Then, Whatman filter No.1 paper (cellulose filters, 90 mm diameter, pore size; 11 μm) was used to filter the supernatant solution. The light-brown transparent solution was obtained which was kept at 4–8 °C for further use.

3.4.2. Preparation of Silver Nitrate Solution

The solution of 0.1 M AgNO_3 solution was prepared by dissolving 1.69 g of AgNO_3 crystals in 100 mL of distilled water. In this way, stock solution of AgNO_3 was prepared.

3.4.3. Green Synthesis of Silver Nanoparticles

First of all, 10 mL of tea extract and 100 mL of 0.1 M AgNO_3 was added to 250 mL of conical flask. The mixture was constantly stirred on a magnetic stirrer for about an hour at room temperature. During the time of stirring, the reddish-brown colored suspension was obtained. The color change indicated the formation of silver nanoparticles. The suspension obtained was centrifuged at 2500 rpm for 10 minutes and the supernatant liquid was decanted off and residue was washed with distilled water followed by ethanol. Centrifugation and decantation followed by washing process was repeated to remove the possible impurities. The obtained precipitate was dried to obtain the AgNPs in solid form. A general procedure for the synthesis of AgNPs using the aqueous extract from *Camellia sinensis* is given below.

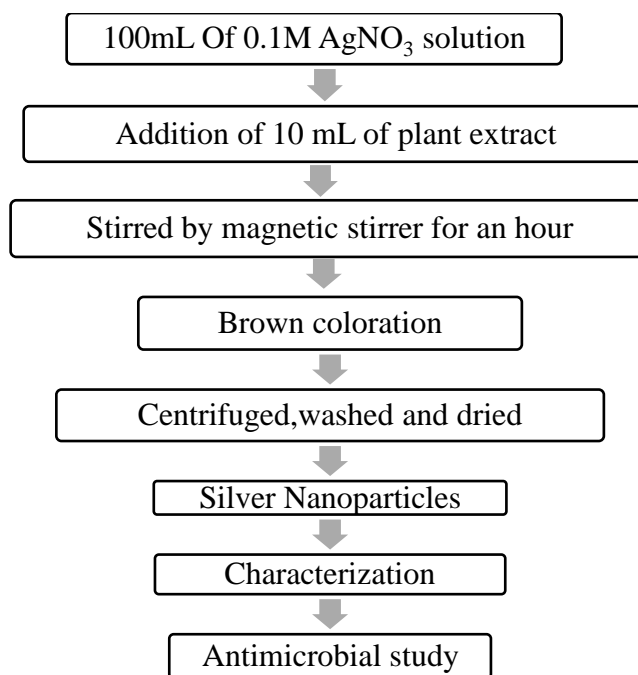


Figure 3.2: Flow Chart Diagram of Green Synthesis of Silver Nanoparticles.

3.4.4. Antibacterial Test of the Synthesized AgNPs

Clinical isolates of *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* were tested. A loop of single colony of each test strain was inoculated in Mueller Hinton Agar medium and incubated in a temperature-controlled shaker (120 rpm) at 37°C overnight. Antibiotics used for the analysis was kanamycin as standard for this test. The petri-dish and agar were autoclaved before use.

Disc diffusion method was used for Zone of inhibition analysis in which pure bacterial cultures were uniformly spread on nutrient agar plates using an L-rod. Three major pathogens (*E.coli.*, *B.subtilis*, *C.albicans*) were taken for analysis. Three plates for each organism containing solidified agar media were prepared and swabbed with respective microbial inoculum. Two disks in each plate were fixed at equal distance. The first disk was loaded with 50 µg of silver nanoparticle and the second disk was impregnated with 50 µg of reference antibiotic kanamycin which served as a positive control. All these plates were kept at 37°C for incubation overnight. After 24 hrs of incubation, the zone of inhibition was measured and compared.

3.5 Physicochemical Characterization

Various characterization techniques were used to know the characteristics of the nanomaterials.

3.5.1. Visual Observation (Color Change Test)

The primary confirmation of the synthesized silver nanoparticles was done by visual basis. The formation of nanoparticles was detected by the color change. Reduction of silver ion into silver particles during exposure to the plant extract is followed by color change.

3.5.2. UV-Visible Spectroscopic Studies

UV-Vis spectroscopic study is a broadly used analytical technique capable of measuring the concentration of an analyte species in a transparent liquid. The absorbance can be used to measure the concentration of a solution by using Beer-Lamberts Law.

$$A = \log_{10} \frac{I_0}{I}$$

Where, I is the measured intensity of the transmitted light, I_0 is the reference intensity of light without analyte (Shard *et al.*, 2020).

The reduction of metal ions was estimated by measuring the absorption level using UV-visible spectroscopy from the wavelength of 200-800 nm by using distilled water as blank. The measurements were taken by taking small aliquot of the distilled water in a cuvette as a blank followed by small aliquot of sample in cuvette which was then kept in UV-visible spectrometer. Finally, the amount of light absorbed by the sample was measured.

The optical property of the silver nanoparticles was also determined by calculating the optical band gap E_g by using Tauc's plot by plotting $(\alpha h\nu)^2$ in $(\text{cm}^{-1}\text{eV})^2$ and $h\nu$ in eV followed by extrapolation on the x-axis (Yusoff *et al.*, 2015).

Tauc's relation (Patidar *et al.*, 2008) is given by the equation,

$$(\alpha h\nu)^2 = K(h\nu - E_g)$$

where,

$$\alpha = 2.303 \log \frac{A}{t}$$

α is the absorbance coefficient, A is the absorbance,

t is the thickness of cuvette usually taken 1cm.

h is the planck's constant,

v is the frequency of incident UV-radiation, K is the constant,

E=hv is the energy of incident wave

In order to convert the wavelength into energy,

$$Eg = \frac{1240}{\lambda}$$

Where, λ is the wavelength of light in nanometer

and Eg is the band gap.

3.5.3. X-Ray Diffraction (XRD) Spectroscopic Studies

It is a crystallographic technique used to identify and quantify numerous crystalline phases present in solid materials and powders.

According to Bragg's law, the waves are constructively added in specific directions.

$$2 d \sin \theta = n\lambda$$

where,

d= spacing between diffracting angles,

θ = incident angle,

n = integer,

and λ = wavelength of the beam.

Practically, the Scherrer equation derived from Bragg's law is used to determine the size of crystallites;

$$t = \frac{k\lambda}{\beta \cos \theta}$$

Where, t is the thickness or grain size of the crystal,

k is a constant which depends on the crystallite shape,

and β is the full width at half maximum of the broadened peak.

If a Gaussian function is used to describe the broadened peak, then the constant k is equal to 0.89 otherwise it is given as 0.94 depending on the particle shape (Cullity *et al.*, 1978).

X-ray diffraction was carried out using X-ray diffractometer instrument to investigate the formation, crystalline behavior and the quality of synthesized silver nanoparticles

powder. The scanning was done in the region of 2θ from 10° to 80° . Thus, the nature of silver nanoparticles was studied. Origin Pro software was used to analyze XRD data and calculate the crystallite size.

3.5.4. Energy Dispersive X-Ray (EDX) Spectroscopic Studies

EDX provides an overall mapping of the sample by investigating near-surface elements and evaluates the elemental proportion at different positions. An electron beam with an energy of 10–20keV hits the conducting sample's surface, causing X-rays to emit from the material.

The EDX can be used to determine the composition or quantity of nanoparticles close and at the surface, as long as they include some heavy metal ions. EDX can easily identify nanoparticles such as silver, gold, and palladium on the surface (Titus *et al.*, 2019).

EDX was carried out to observe the pattern of synthesized silver nanoparticles for the determination of elemental composition of synthesized nanoparticles.

3.5.5. Fourier Transform Infrared (FTIR) Spectroscopic Studies

FTIR spectroscopy analysis is a method based on the principle of infrared spectroscopy. and is used to determine the nature of associated functional groups and structural features of biological extracts with nanoparticles. It can identify and characterize unknown materials, detecting contaminants in a material, finding additives, and identifying decomposition and oxidation.

IR of about $10,000\text{--}100\text{cm}^{-1}$ is passed through the sample with part of the radiation absorbed which is converted to vibrational or rotational energy by the sample. The resultant signal obtained is a spectrum generally from 4000 to 400 cm^{-1} that represents molecular fingerprint of the samples.

FTIR is a powerful tool for chemical identification as each molecule has its own fingerprints (Titus *et al.*, 2019).

FTIR spectroscopy was carried out in the wavelength range of $4000\text{--}500\text{ cm}^{-1}$ at the resolution of 4 cm^{-1} in Attenuated Total Reflectance (ATR) mode. FTIR spectra obtained was used to identify bonds and detect functional groups.

3.5.6 Scanning Electron Microscopy Techniques

Scanning electron microscopy (SEM) is a useful technique for micro- and nanostructures analysis. It provides information regarding composition and topography

of surfaces which include adjustable magnifications from reading glass magnification and imposition of structure visualization (Kumar *et al.*, 2019).

SEM was used to determine the shape and size of AgNPs.

Table 3. 1: Summary of Characterization Techniques

Methods	Information
UV-VIS	Absorbance, optical properties and concentration of a solution. Confirmation of the formation of NPs and detection of absorbance peak.
FTIR	Detection of bonds and Identification of functional groups
EDX	Evaluation of Atomic structure of NPs. Chemical composition and elemental identification of a sample.
XRD	Composition of molecule, electronic structure of molecule and crystal phase of NPs.
SEM	Size and morphological appearance of NPs.

CHAPTER 4

RESULTS AND DISCUSSIONS

The aqueous extract from *Camellia sinensis* was successfully extracted by cold percolation method. The extract was used for the preparation of silver nanoparticle. The results achieved from the green synthesis of silver nanoparticles using tea (*Camellia sinensis*) extract are as follow.

4.1 Visual Observation

The bio-reduction of the silver nitrate solution was observed using tea (*Camellia sinensis*) leaves extract by mixing them. The appearance of reddish-brown color was observed, which indicated the formation of silver nanoparticles in the reaction mixtures. The initial color of mixture of tea extract and silver nitrate solution transformed into final dark brown color indicating the formation of silver nanoparticles. The color change was observed with the increase in time with continuous stirring in magnetic stirrer. The color change further indicated that the plant started to reduce the silver nitrate solution. Hence, tea extract was effective to reduce the silver nitrate solution and silver nanoparticles was obtained.

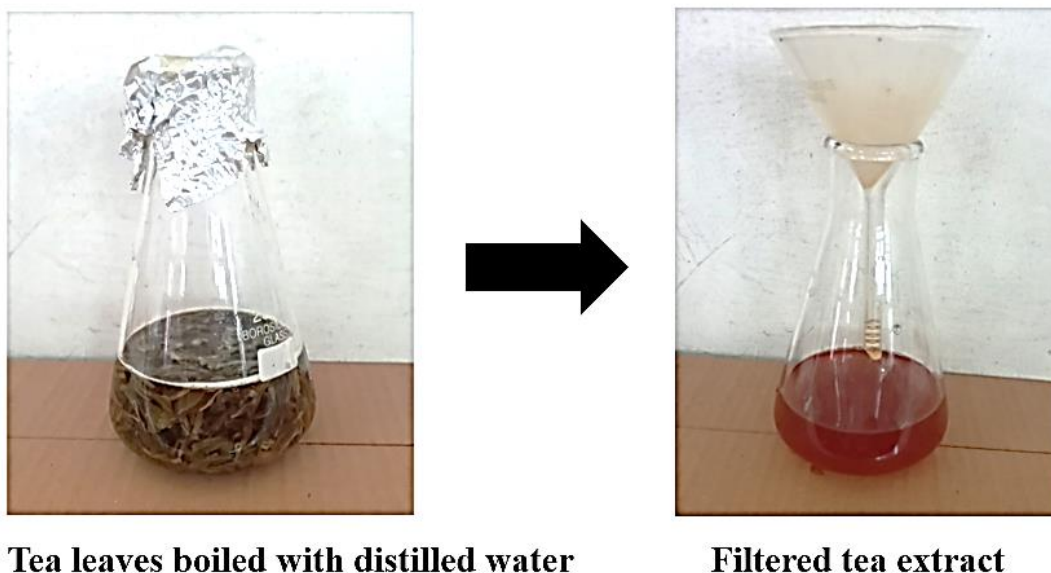


Figure 4.1: Preparation of Tea (*Camellia sinensis*) Extract

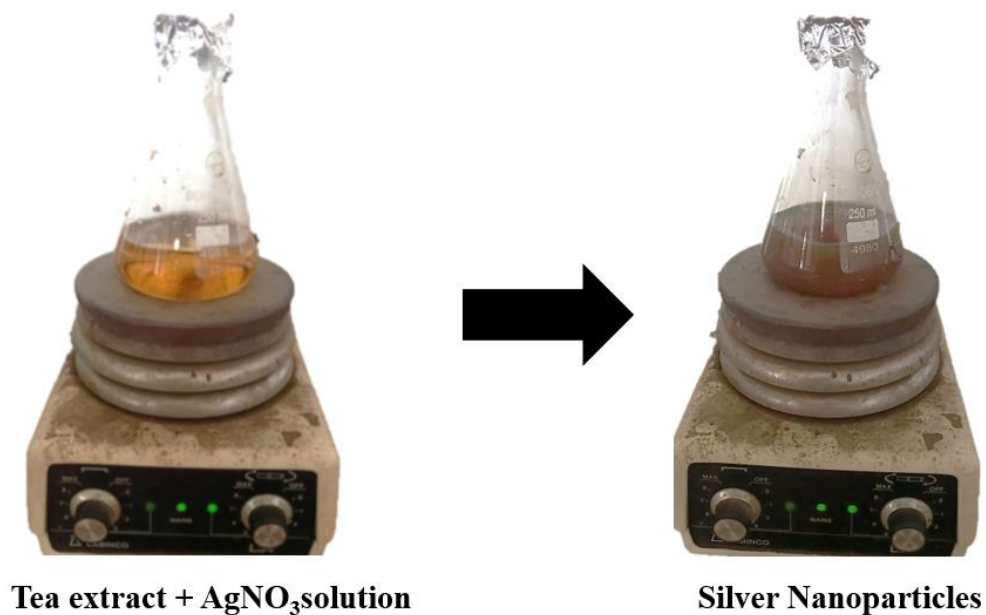
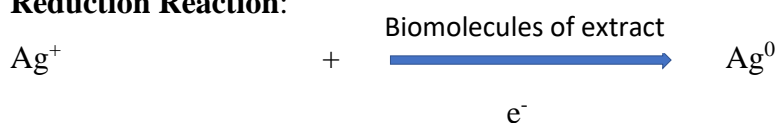


Figure 4.2: Color Change Observation during Synthesis of Silver Nanoparticles.

Reduction Reaction:



The result gives the primary confirmation of synthesized AgNPs which was further characterized by UV-visible spectroscopy.

4.2 UV-Visible Spectroscopic Studies

UV-visible spectroscopy is most widely used technique to investigate the optical properties of the particles. The mixture of tea (*Camellia sinensis*) leaves extract and silver nitrate (AgNO₃) solution are shown in figure which results in the formation of colloidal brown colored silver nanoparticles with the increase in time.

A visible color change from pale yellow to dark yellow and finally to the colloidal brown color indicated the formation of silver nanoparticles by green synthesis, which was confirmed by UV-Visible spectrometer as reported in the article (Jain *et al.*, 2017). The absorption peak centered at 425 nm, which is associated with absorbance of Ag nanoparticles. AgNPs exhibited brown color due to the Surface Plasmon Resonance (SPR) phenomenon.

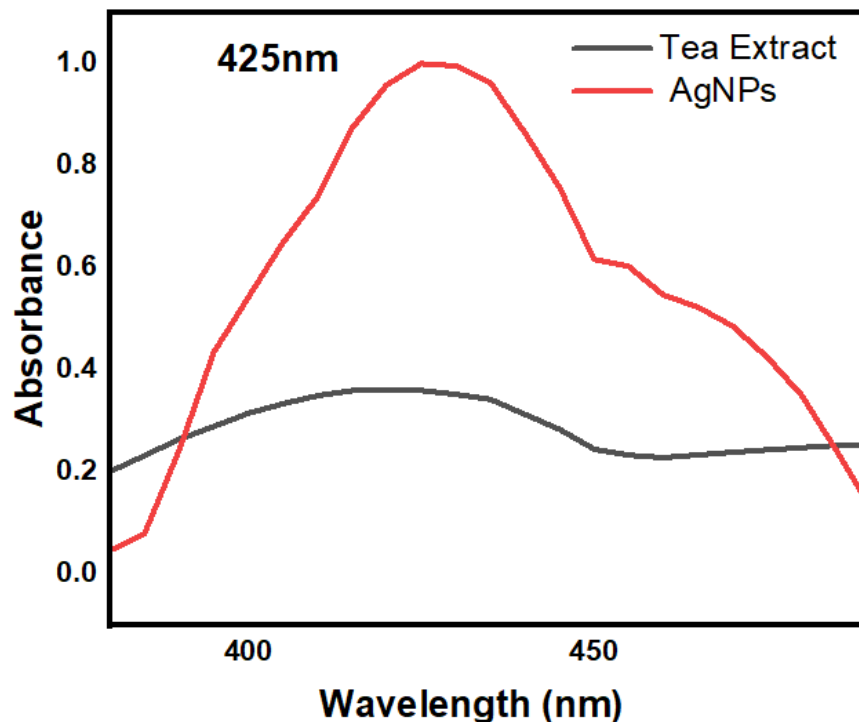


Figure 4.3: Absorption Spectra of Tea Leaf Extract and AgNPs Synthesized using *Camellia sinensis* Leaf Extract at Room Temperature.

The formation of silver nanoparticles was examined by measuring the absorption spectra at regular time intervals in the range of 200–600 nm for the reaction of silver salt by Tea extract. An increase in absorbance was observed with the passage of time indicating an enhancement in the formation of AgNPs (Jain *et al.*, 2017).

The results obtained in this study was found coherent with a study where the consequent colour changes were observed from a light yellow to a brown colour and as we know that AgNPs are known to exhibit a UV–Visible absorption maximum in the range of 400–500 nm because of surface plasmon resonance, the synthesized AgNPs exhibited a well-defined absorption peak at 425 nm which was nearly equal to the absorption peak observed at 438 nm from *O. basilicum*, 439 nm for *Camellia sinensis* reported in the literatures (Malapermal *et al.*, 2017).

Accordingly, the result was in accordance with the absorption spectrum 416 nm of the synthesized AgNPs using *Annona reticulata* (Parthiban *et al.*, 2019); 430 nm using *Origanum vulgare* (Shaik *et al.*, 2018); 435 nm using *Givotiamoluccana* leaf extract (Sana *et al.*, 2018).

A study (Fang *et al.*, 2021; Xia *et al.*, 2019) also reported the presence of phytochemical constituents such as the phenolics, flavonoids, terpenoids, polysaccharides, flavonoids and other. Such phytochemicals can reduce the metal ions into metal nanoparticles.

As the green synthesized nanoparticles were formed by the interaction with the biomolecules of *Camellia sinensis* leaf studied, it can be confirmed that there is the reduction of Ag^+ ions to Ag^0 by the secondary plant metabolites that are in turn oxidized to other species. Results obtained has been found to be consistent with the observations of many researchers reported globally.

4.2.1 Band gap energy by Tauc's Plot

The bandgap energy of synthesized silver nanoparticles was calculated from Tauc's plot by extrapolating the linear portion of the UV-Visible curve. Several model have been used to describe the quantum confinement of nanoparticles. The most widely used is Tauc model.

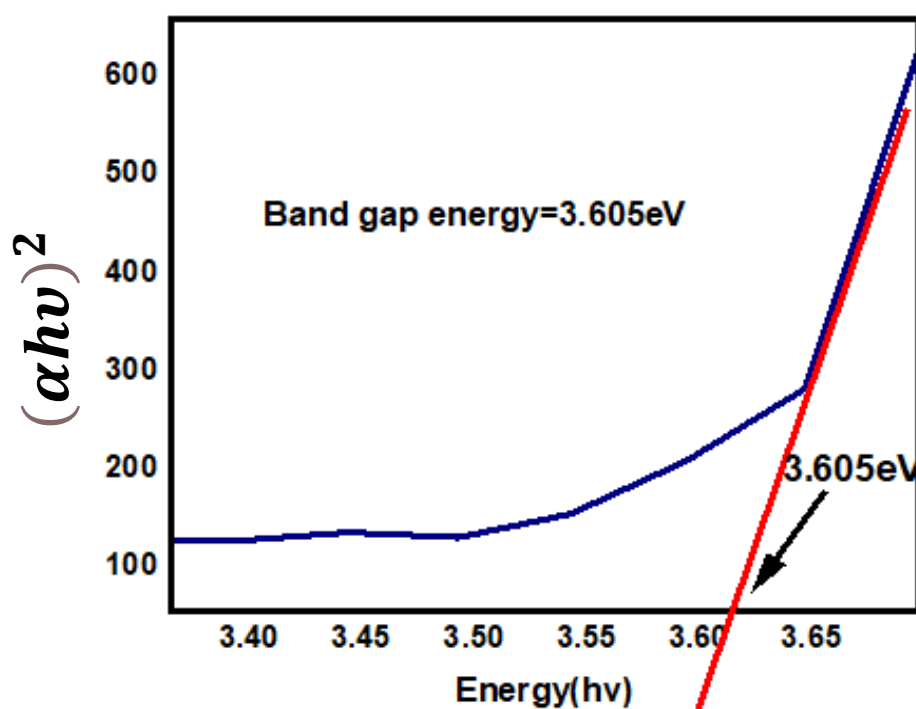


Figure 4.4: Tauc's Plot of Synthesized AgNPs

Quantum confinement refers to the prominent effect in the electronic and optical properties when the sample is sufficiently nano sized.

Larger the band gap energy, smaller the size of nanoparticle and vice-versa. The electron-hole pairs or charge carriers present in a material are excitons. The electron

and hole are held together by attractive forces and the distance of separation is called exciton-bohr radius. This results exciton to be confined into a critical quantum dimension and exciton cannot extend to its natural limit which results in quantum confinement (Anantharaman *et al.*, 2020).

The Tauc optical band gap associated with the nanoparticles can be determined through the extrapolation of the linear portion of plot of $(\alpha h\nu)^2$ over a limited range of photon energies $h\nu$.

The optical band gap was determined to be 3.605 eV suggesting the movement of size towards quantum confinement for the synthesized nanoparticles. These results indicated the large value of band gap energy which can be further used in advance optoelectronic devices as a semi-conductive material (Mistry *et al.*, 2021). The value of band gap is much similar to earlier reported literature and this value could be due to quantum confinement effect.

4.3 X-Ray Diffraction (XRD) Spectroscopic Studies

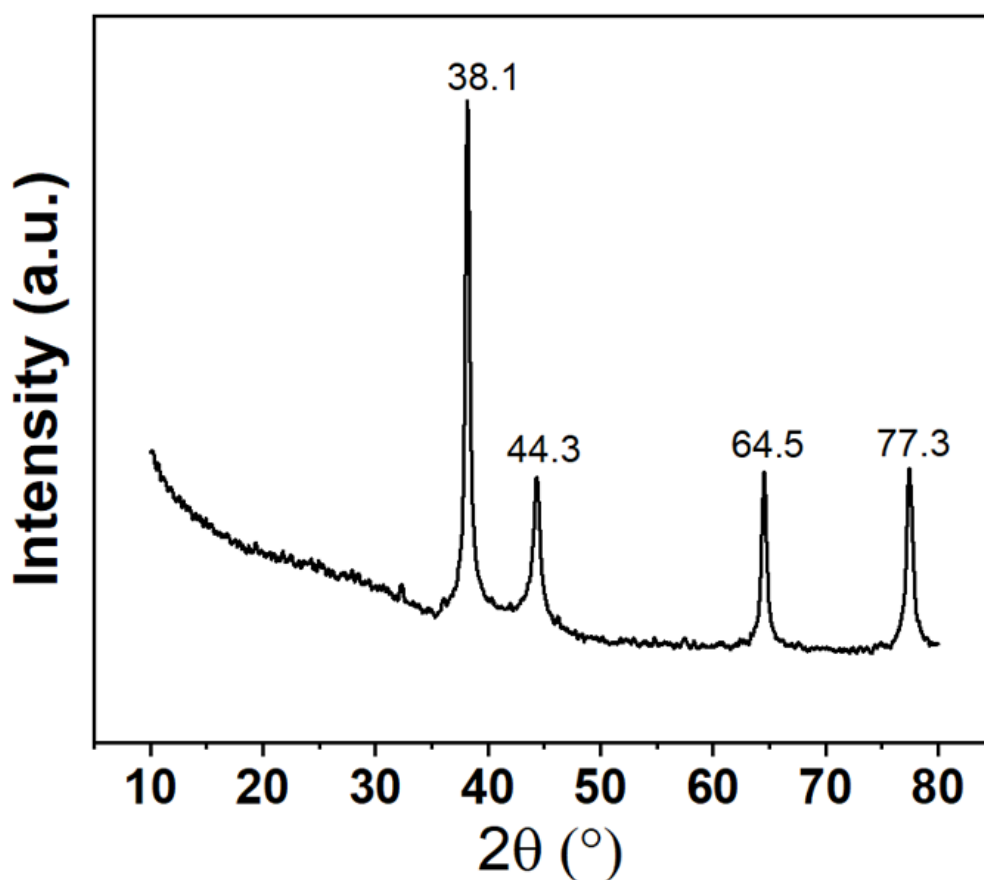


Figure 4.5: XRD Patterns of AgNPs obtained using Tea Extract as Reducing Agent

X-ray diffraction pattern was studied to determine the crystalline nature and surface morphology of the synthesized AgNPs.

A number of Bragg reflections corresponding to $2\theta = 38.1^\circ$, 44.3° , 64.5° and 77.3° that were ascribed to (111), (200), (220) and (311) respectively, reflection planes of the silver cubic face-centered (FCC) crystalline form (JCPDS No 87-0720). The results proved that, the bio-synthesized Ag-NPs due to Ag^+ ions reduction are crystalline in nature and the sharp peak at $2\theta = 38.1^\circ$ indicating the position (111) of Ag corresponded to FCC (Aref *et al.*, 2020). The analysis also confirmed that the bio-synthesized Ag-NPs were composed of crystalline silver with high purity.

The average particle size was calculated by Debye-Scherrer equation where full width at half maximum (FWHM) data were used. The particle size estimated was approximately 20.62 nm from the most intense peak in the case of AgNPs synthesized using Tea extract as shown in Table 4.1. The crystallite size was in accordance with other characteristic study where it was 10-20 nm (Singh *et al.*, 2018).

Similar diffraction peaks of AgNPs using *Annona reticulata* extract were reported at 2θ angles of approximately, 37.56° , 43.25° and 64.10° , were obtained which correspond to the crystallographic planes of faced-centered cubic (FCC) structures of silver nanoparticles [111],[200], and [220], respectively (Parthiban *et al.*, 2019). It matched the database of the Joint Committee on powder diffraction standards (JCPDS) file no: 04–0783. Similarly, Shaik *et al.* observed additional peaks in the diffractogram of AgNPs prepared from *O.vulgare* leaf extract (Shaik *et al.*, 2018) which also were found in line with several other studies of AgNPs using various extracts (Behravan *et al.*, 2019; Sana *et al.*, 2018).

Table 4.1: d-spacing Value of Synthesized Ag Nanoparticles

Plane	2θ (in degree)	θ (in degree)	$d_{hkl} = n\lambda / 2 \sin \theta$ in Å n=1 and λ for Cu source=1.54Å
111	38.1	19.05	2.36
200	44.3	22.15	2.04
220	64.5	32.25	1.44
311	77.3	38.65	1.23

Table 4.2: Characterization Parameters for AgNPs as per XRD Data

Plane 2θ (in degree)	β (FWHM, in degree)	β =FWHM (rad)	$D=0.9\lambda/\beta\cos\theta$ (in nm)
(111) 38.13	0.4897	0.00855	20.62
(200) 44.32	0.7632	0.01332	14.54
(220) 64.55	0.5876	0.01026	24.21
(311) 77.38	0.6456	0.01127	56.31

4.4 FTIR studies

FTIR analysis confirmed that the biomolecules present in plant extract were responsible for the reduction of silver ions to AgNPs.

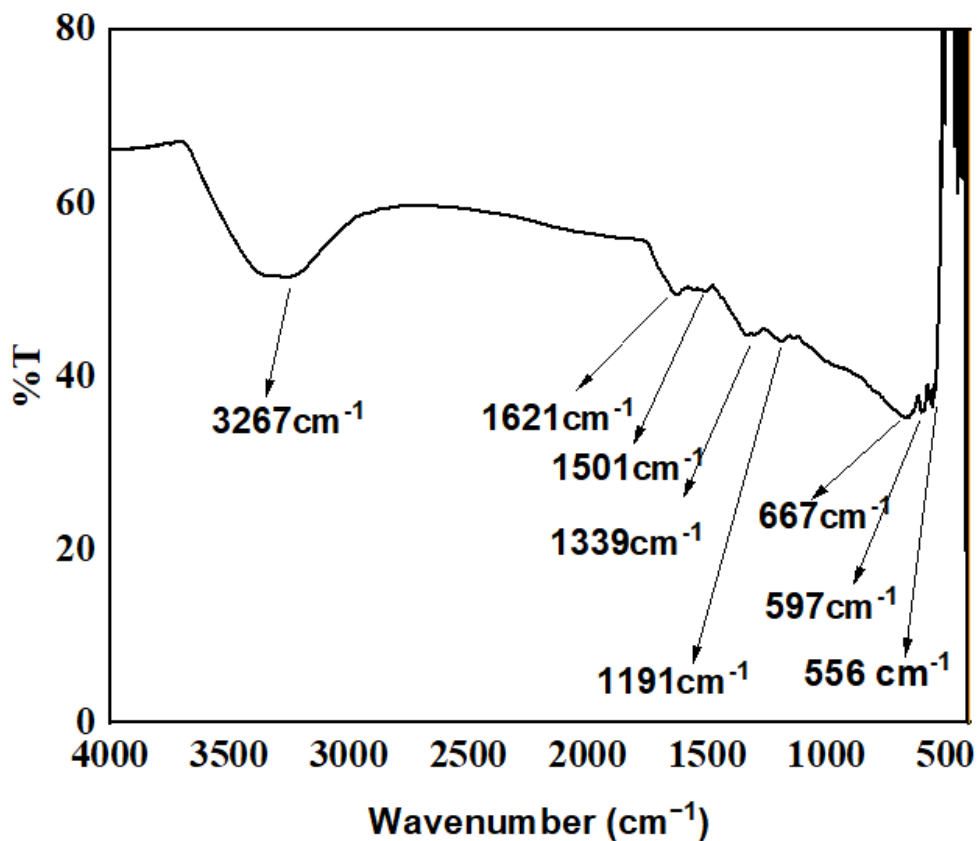


Figure 4.6: FTIR Spectra AgNPs Synthesized using Tea Extract as a Reducing Agent.

The biomolecules present in the synthesis of AgNPs was analyzed by FTIR spectrometer. The peaks were discovered at 597, 667, 1191, 1339, 1501, 1621, 3267 cm⁻¹ indicating the presence of capping and stabilizing biomolecules with the nanoparticles.

The peaks and the corresponding functional groups are shown in the table which are consistent with the other literatures.

Table 4.3: FTIR Data of Synthesized AgNPs using Tea Extract.

Wavenumber (cm ⁻¹)	Functional groups	Information
3267	Broad -OH stretching and N-H amide stretch	H-bonded alcohols, phenols
1621	C=O stretching	carboxylic acid, Amide (Benakashani <i>et al.</i> , 2016)
1501	C=C stretching	benzene (Chandra <i>et al.</i> , 2020)
1339	C-C and C-N stretch	Amines
1191	O-H stretch	polyphenols and phenols (Gomathi <i>et al.</i> , 2017)
667,597,556	-CH out of plane bending vibrations	substituted ethylene systems (Devaraj <i>et al.</i> , 2013)

The peak at 3267 cm⁻¹ is attributed to -OH vibrational stretching allocated to -OH group of polyphenols such as catechins, tannins and stretching of the -NH band of amino groups (Otunola *et al.*, 2018; Ren *et al.*, 2019). The carboxylic acid frequency range is 1700-1300 cm⁻¹. The band at 1621cm⁻¹ may arise from C=O vibration of bonded conjugated ketones, quinones, carboxylic acids, alkenes and esters (Rolim *et al.*, 2019). The band at 1339 cm⁻¹ and 1191 cm⁻¹ is associated to C-N stretching vibration of aromatic amines and -OH stretch indicating the presence of water-soluble caffeine respectively (Otunola *et al.*, 2018). These functional groups have role in stability/capping of AgNP as reported in many studies.

FTIR study showed that the hydroxyl (-OH) and amine (N-H) groups in the extracts are mainly involved in reduction of Ag⁺ ions to Ag⁰ nanoparticles (Yadav *et al.*, 2018). The similar features were detected in the previous reports on AgNPs synthesis observed by FTIR indicating the contribution of capping agents as functional groups involved in stabilization of AgNPs (Behravan *et al.*, 2019; Dehghanizade *et al.*, 2018; Malapermal *et al.*, 2017b; Parthiban *et al.*, 2019).

4.5 Surface Morphology and Elemental study

The surface morphology of as-synthesized silver nanoparticles was studied in terms of field emission scanning electron microscopy (FESEM) equipped with energy dispersive X-rays (EDX) and elemental mapping. The surface morphology of silver nanoparticle is shown in different magnification as given in Figure 4.7. The image shows that the silver nanoparticles are homogeneously formed with narrow size distribution. This is evidenced from the measure of size of silver nanoparticles using Image software. The result shows the particle size of 66.86 ± 16.73 nm. The silver nanoparticles are formed in nanorange. The particle size of as-synthesized silver nanoparticles was also determined from Debye Scherer formula in the XRD measurement (20.62 nm).

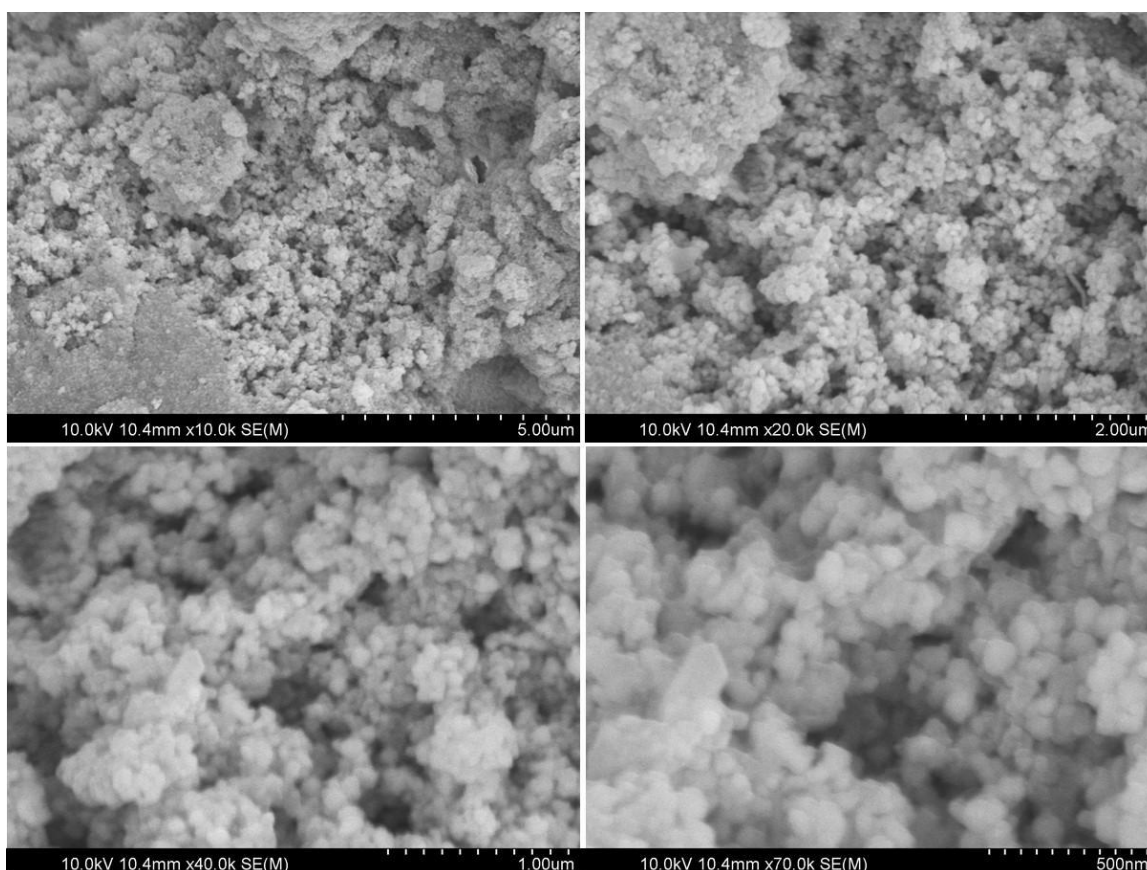


Figure 4.7: Field Emission Scanning Electron Microscopy (FESEM) of Silver Nanoparticles at Different Magnification. (a) ($\times 10,000$ magnification) in $5\mu\text{m}$. (b) ($\times 20,000$ magnification) in $2\mu\text{m}$. (c) ($\times 40,000$ magnification) in $1\mu\text{m}$. (d) ($\times 70,000$ magnification) in 500 nm.

The study was found to be consistent with literatures (Ahamed et al., 2011; Esmaili et al., 2020). The Figure 4.8 shows the 3D image of the AgNPs in 500 nm ($\times 70,000$ magnification) and the frequency of SEM size distribution of AgNPs were determined in this scale with the particle size ranging from 28-74.42 nm. SEM average diameter was calculated by measuring the particles in random fields of SEM view. The average particle size of AgNPs was 66.87 ± 16.74 nm as shown in histogram of Figure 4. 9. The biomolecule coating of the biosynthesized AgNPs as shown in the SEM images confirms the role of plant metabolites in the synthesis and stabilization of AgNPs. These results are in agreement with findings (Mortazavi-Derazkola *et al.*, 2020; S.S. *et al.*, 2019).

The FESEM micrographs of AgNPs in three-dimensional view in 500 nm scale are shown in Figure 4. 8.

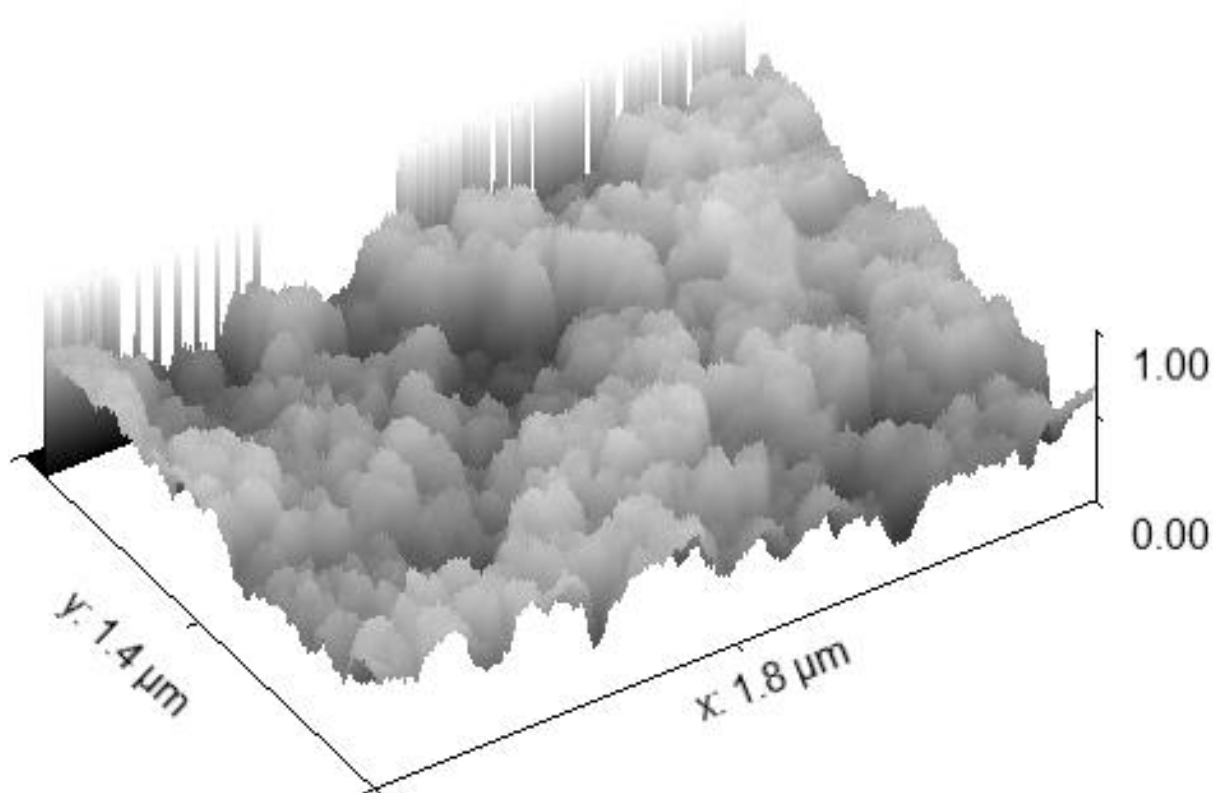


Figure 4.8: 3D Image of AgNPs in 500 nm Scale (Particle Size Determination).

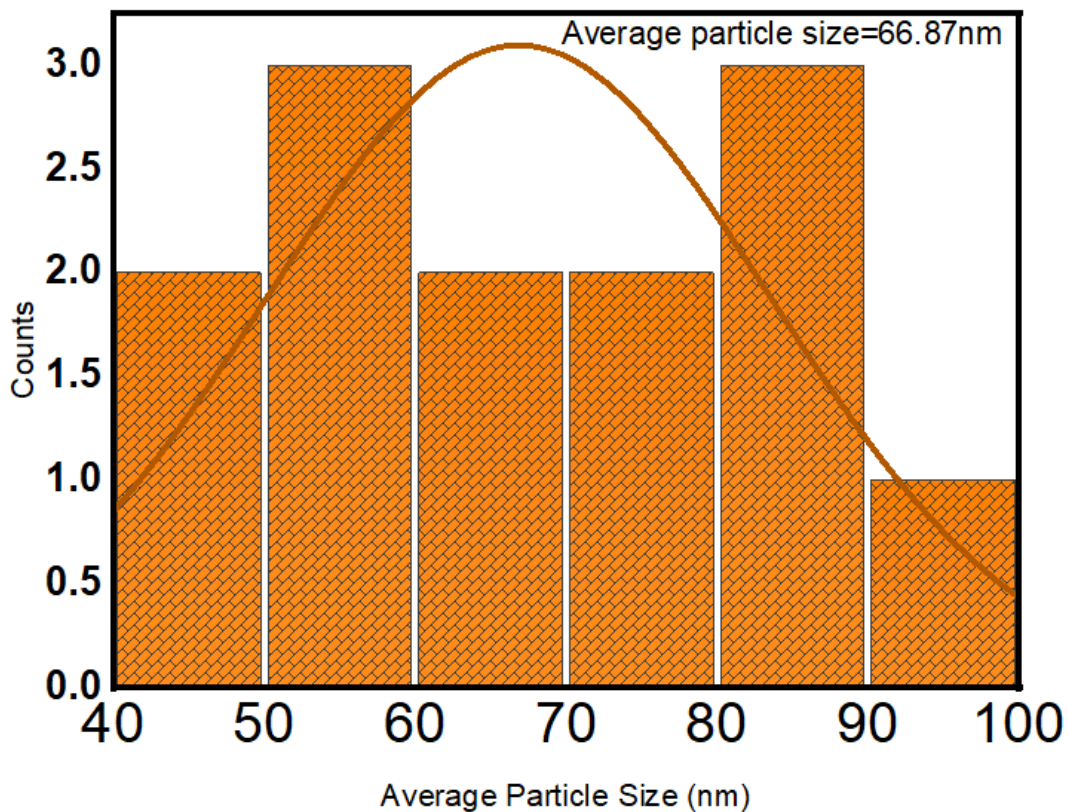


Figure 4.9: A Size Histogram of Ag Nanoparticles Counted from SEM Image Showing the Distribution of AgNPs (Particle Size Analysis from AgNPs Biosynthesized by *C. sinensis* Extract from SEM Image in 500 nm scale).

In addition, to ensure the synthesized product, energy dispersive X-ray with elemental mapping was performed which is shown in the Figure 4.10. Result shows the predominantly a silver nanoparticle with some oxygen and carbon. As the nanoparticle was synthesized using the aqueous extract of *Camellia sinensis*, the aqueous extract served as a capping as well as stabilizing agent. Such adhered organic moieties could be associated with the composition of carbon and oxygen.

In addition, partly, silver may have undergone aerial oxidation in due process of storage which impart the presence of such elemental. However, majority of the content in the nanoparticle is silver. The XRD result also supports the formation of silver nanoparticles but not silver oxide nanoparticles from their study of crystallinity. This fact supports that the presence of carbon and oxygen are due to the presence of organic moieties from aqueous extract of *Camellia sinensis*.

The EDX images of the AgNPs showed the immobilization of silver on *C.sinensis* extract. The sharp peak at 3.0 keV obtained illustrated the existence of metallic silver due to surface Plasmon resonance. The other absorption bands that appeared corresponded to that of C and O elements that are characteristic of plant extract with the elemental silver in high quantities. From the study, the optical absorption peak at 3keV indicated the presence of silver nanoparticles with excellent purity as reported in the literature (Ahamed *et al.*, 2011; Esmaili *et al.*, 2020; Mortazavi-Derazkola *et al.*, 2020; S.S. *et al.*, 2019).

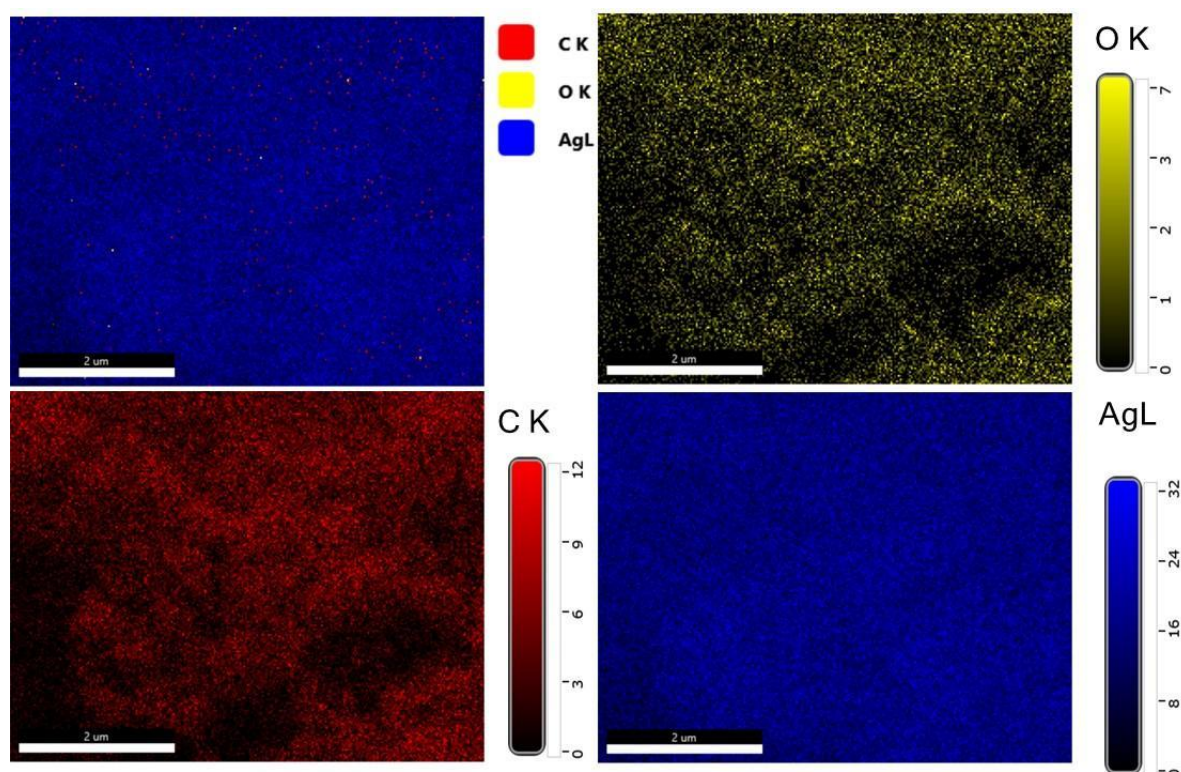


Figure 4.10: Elemental Maps based on Energy-Dispersive X-ray measurements.

Elemental composition analysis from EDX showed strongest signal from silver as shown in Figure 4.11 indicating the presence of large amount of silver nanoparticles and weakest signals from carbon and oxygen was also recorded. The weight % of C K, O K and Ag L was determined 13.6, 5.2 and 81.1, respectively and the atomic % of 51.3, 14.7 and 34.0 for the C K, O K and Ag L as listed in the table.

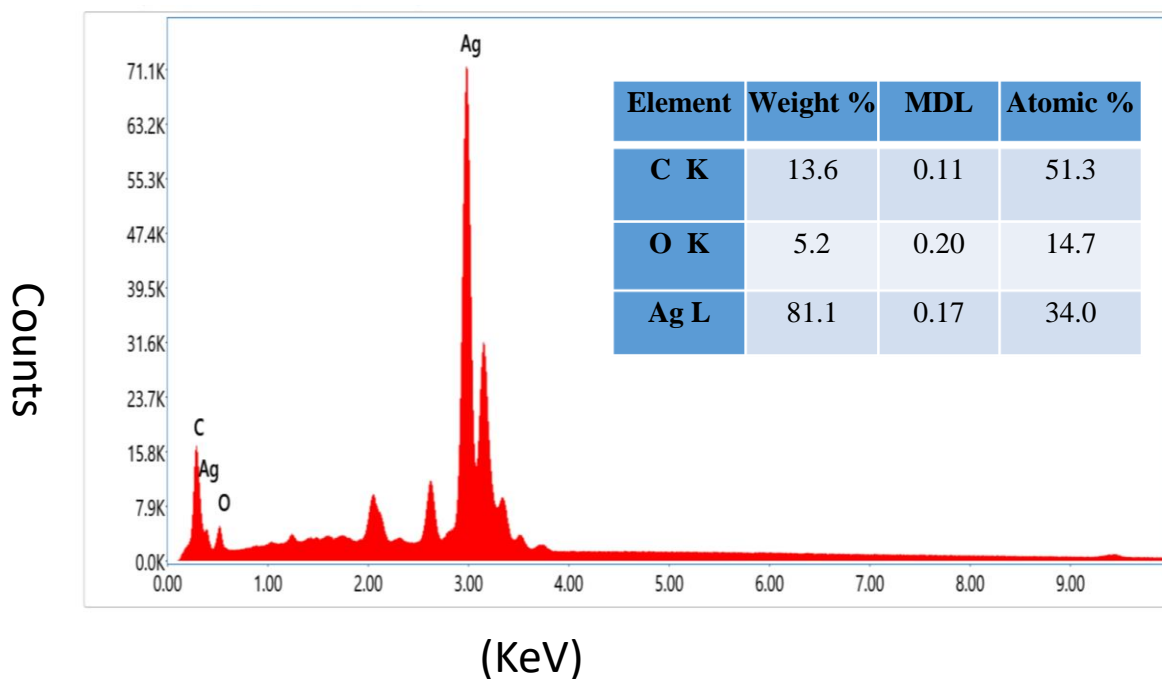


Figure 4.11: EDX Spectrum of Silver Nanoparticles

4.6 Antimicrobial study

Disc diffusion method showed that AgNPs synthesized using tea extract exhibit antimicrobial activity as the zone of inhibition (diameter in mm) observed in case of bacterial strain treated with AgNPs was comparable to the standard antibiotic.

Table 4.4: Zone of Inhibition Observed

Microorganisms Test Organism (ATCC)	Reference Culture	Type	Zone of Inhibition (mm)	Positive Control (ZOI) (mm)
<i>Bacillus subtilis</i>	ATCC 6633 Bacteria	Gram-Positive Bacteria	11	20
<i>Escherichia coli</i>	ATCC 25922 Bacteria	Gram-negative Bacteria	12	20
<i>Candida albicans</i>	ATCC 10231 Fungus	Gram-positive fungus	10	18

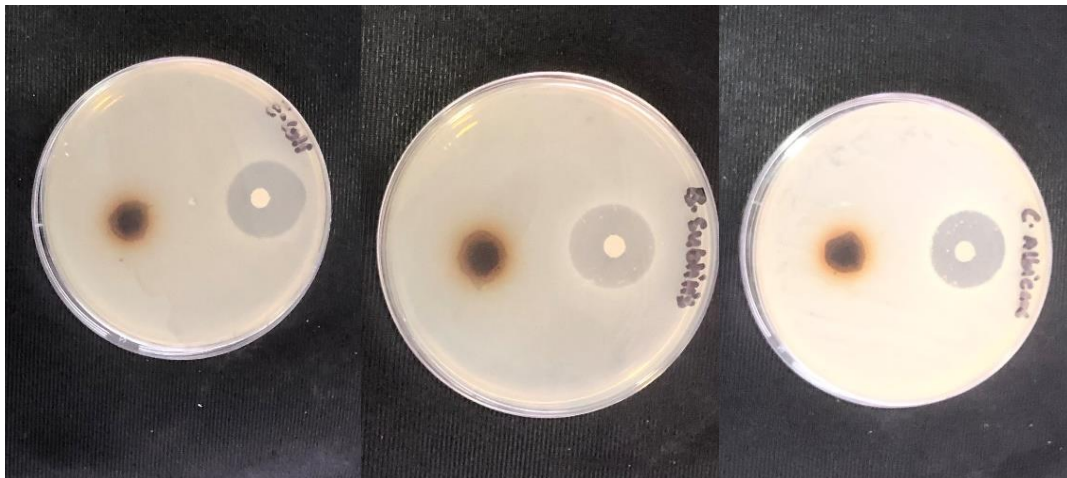


Figure 4.12: Zone of Inhibition Observed when Bacterial Strains are treated with AgNPs

The zone of inhibition calculation for the Gram-negative bacterial samples of *E. coli* (aqueous cultures). Gram-positive bacterial samples of *B. subtilis* and Gram-positive fungus of *Candida albicans* was determined by treating with concentrations 50 $\mu\text{g/mL}$ of AgNPs obtained using Tea extract as reducing agents for 24 hrs.

In this study, the antibacterial effect of silver nanoparticles on Gram-negative bacteria (*E. coli*) was found higher (12 mm) than gram Gram-positive bacteria (*B.subtilis*) (11 mm) and Gram positive fungus (10 mm). It can be concluded that AgNPs obtained using Tea extract has antibacterial potential (Jain *et al.*, 2017).

Similar results were observed where bacterial strains of *E. coli*, *S. aureus* and *P. aeruginosa* also indicated the distinct antimicrobial potential of AgNPs (Sood *et al.*, 2018). The antibacterial effect of silver nanoparticles on Gram-negative bacteria (*E. coli*) was found higher than Gram positive bacteria (*S. aureus*) in a study using *Berberis vulgaris* leaf and root extract (Behravan *et al.*, 2019) whereas leaf extract of neem and Tea mediated AgNPs were more effective against Gram-positive bacteria than against Gram negative ones (Priyadarshini *et al.*, 2019).

Antibacterial potential of AgNPs have also been reported by similar works (Erci *et al.*, 2018) ;(Baruah *et al.*, 2019).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Silver Nanoparticles were successfully synthesized by green method using *Camellia sinensis* (Tea) leaves by focusing the plant extract as a reducing and stabilizing agent and its antibacterial potential were reviewed as well as compared with the similar research articles. It was concluded green synthesis techniques of Ag nanostructures utilizing plant extracts are seen to be a promising option. The study further indicated that Tea leaves can be used as an environment friendly and non-hazardous material for the synthesis of silver nanoparticles, thus aiding in the elimination of using hazardous reducing agents.

The result of the UV spectrum of the AgNPs synthesized using *C. sinensis* leaves extract showed deep absorptions at 400-500 nm which is also the characteristic absorbance peak of AgNPs and hence confirming the formation of AgNPs. The tauc's plot showed the optical band gap of 3.605 eV showing the potential application of AgNPs as semiconductors. The FTIR analysis showed the peaks which were at around 1621 and 3267 cm^{-1} , which corresponds to the functional groups of C=O (around 1640 cm^{-1}) and amine N-H/ O-H vibration stretch (around 3370 cm^{-1}). The XRD patterns indicated the presence of AgNPs of 20.62 nm from intense peak of [111] lattice planes and its crystalline nature whereas average particle size of 66.87 ± 16.74 nm from SEM micrographs. EDX Analysis showed the optical absorption peak at 3 keV. The anti-microbial assay of AgNPs gave an idea about the maximum zone of inhibition (12 mm) for *Escherichia coli* compared with 11mm for *Bacillus subtilis* and 10mm for *Candida albicans* as compared to the control Kanamycin confirming its antibacterial property.

5.2 Recommendations for Further Works

Some of the important future works related to silver nanoparticles are as follows:

- Silver nanoparticles can be characterized by using Dynamic Light Scattering (DLS), Zeta potential, and Atomic Force Microscopy (AFM)) techniques.
- Effects of various parameters such as pH, temperature, concentration, etc. on synthesis of silver nanoparticles can further be explored.
- Various properties like antidiabetic, cytotoxicity, anti-dermatophytic and antioxidant can be further investigated.

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