China Petroleum Processing and Petrochemical Technology

Catalyst Research Volume 23, Issue 2, October 2023 Pp. 2643-2662

ANTIBACTERIAL ASSESSMENT OF SILVER NANOPARTICLES SYNTHESIZED VIA MICROWAVE-ASSISTED METHOD USING SYZYGIUM CUMINI LEAF EXTRACT

Gulshan Sana
MS Scholar, Department of Chemistry, University of Sialkot, Sialkot, Punjab, Pakistan
gulljutt21@gmail.com

Aqsa Habib
Lecturer, Department of Chemistry, University of Sialkot, Sialkot, Punjab, Pakistan
aqsa.habib@uskt.edu.pk

Faisal Amin
MS Scholar, Department of Chemistry, University of Sialkot, Sialkot, Punjab, Pakistan
faisalmalikamin@gmail.com

Mehwish Tariq
MS Scholar, Department of Chemistry, University of Sialkot, Sialkot, Punjab, Pakistan
Mehwishtariq2042@gmail.com

Mohsin Shehzad
MS scholar, Department of Mathematics, University of Sialkot, Sialkot, Punjab, Pakistan
mohsinshahzad9868@gmail.com
*Corresponding Author: Gulshan Sana (gulljutt21@gmail.com)

Abstract

In the past decades, silver nanoparticles (Ag NPs) synthesis has relied on energy-intensive and toxic methods, hindering their practical use. A greener alternative involves biosynthesis, which is cost-effective, energy-efficient, and eco-friendly. This study focuses on the synthesis of Ag NPs using Syzygium cumini plant extract. Characterization employed UV-visible spectroscopy, Fourier transform infrared spectroscopy, Scanning Electron Microscopy, and X-ray Diffraction. UV-Visible spectra exhibited an SPR band at 427 nm, confirming Ag NPs synthesis. FTIR spectra identified functional groups responsible for capping and stabilization. XRD analysis revealed crystalline nature with distinct planes (111), (220), (200), and (311). TEM imaging indicated spherical Ag NPs of 20 nm size. Antibacterial assessment demonstrated inhibition of Gram-positive and Gram-negative strains—Escherichia coli and Staphylococcus aureus—with a 14 nm inhibition zone. Comparison with literature highlighted the superiority of microwave-assisted green synthesis for biologically active nanoparticles. The findings underscore the potential of these Ag NPs for clinical trials and future drug design. The study recommends further exploration of microwave-assisted green synthesis for its efficacy and safety.

Keywords: Green synthesis, silver nanoparticles, antibacterial activity, Syzygium cumini.

Introduction

The term "nano" originates from Greek, meaning small. Nanotechnology, a promising 21st-century technology, was introduced by physicist Richard Feynman in 1959. It manipulates matter at 1-100 nm scale, exhibiting unique properties. Nanoscience has developed methods for materials like metal nanoparticles (NPs), gold, silver, etc. NPs possess size-driven properties, finding applications in catalysis, medicine, electronics, cosmetics, and more. Silver, a noble metal, is vital in nanotech and has uses in medicine, electronics, alloys, and more.

Ag NPs have many applications in the field of science such as antibacterial, antimicrobial, antifungals, anti-inflammatory and antioxidant due to exceptional characteristics such as facile synthesis, high stability, high surface to volume ratio, non-toxicity, tunable morphology, high reactivity, size dispersion, electronic and optical properties (Ahmed et al, 2016; Sajid & Płotka-Wasylka, 2020).

Two approaches have been established for the synthesis of NPs under nanotechnology (Top-down and Bottom-up). In top-down approach, the bulk material is cracked down into small sized material using different forces such as mechanical and electrical energy. The top-down approach involves break down of complex molecules into smaller fragments which can no longer be fragmented using some forces (Aadim Ph D & Jasim Ph D, 2022; Menazea, 2020).

The second approach is bottom-up approach which has attracted more attention and it is used to build-up nanostructure from molecular components by nucleation and growth processes (Scolaro et al., 2020; Yusuf, 2020).

Metal NPs can be synthesized through different physical, chemical, and biological methods. The physical methods involve laser pyrolysis, grinding and irradiation (Khatoon, Mazumder, & Sardar, 2017). The chemical methods involve some chemical compounds such as reducing and capping agent, chemical vapor deposition, chemical coprecipitation, microemulsion. Biological methods involve green synthesis through bacteria, fungi, and plants (Ahmad et al., 2017).

Physical methods offer advantages like solvent purity and NP uniformity but require costly equipment, substantial energy, and lack capping agents to prevent clumping. Chemical synthesis employs organic and inorganic solvents (e.g., sodium borohydride, Trisodium citrate), involving electron transfer to reduce Ag+ to Ag0 and form Ag NPs. This method necessitates external energy sources (electrochemical, photochemical, Sonochemical, microwave-assisted) for nucleation and growth in NP synthesis (Xu et al., 2020).

Chemical methods have some limitations such as unfriendly for environment, toxicity, uncontrolled size of of NPs, use of expensive chemicals and requirement of high energy. Finally, the era of green synthesis comes to encounter all above limitations and gaining more attention of researchers for development of material science and nanotechnology. Green synthesis is easy, efficient, eco-friendly, consumes less energy and produces safer products and by-products using natural resources (El Shafey, 2020).

In green synthesis, plants' extracts are preferred over microorganisms due to cost-effectiveness and abundant biomolecules. Plants' various parts contain biomolecules like proteins, flavonoids, terpenoids, etc., ideal for converting metal salts to nanoparticles. Ag NPs can be produced from plant extracts without toxic agents, using biomolecules as reducing and capping agents. Factors like temperature, pH, and extract concentration influence nanoparticle size (Chakravarty et al., 2022).

A microwave assisted technique is more important than other techniques for production of desired size of silver nanoparticles. The synthesized nanoparticles thorough this technique have superior characteristics than others such as smaller particle size, with short reaction time, less energy consumption, better yields of NPs, prevent from agglomeration and chemical wastes. Syzygium cumini is a tree which belong to Myrtaceae Family, this plant initially originated from India and now widely distributed in Asian and African countries where it easily adapted to all type of tropic and subtropic climate. It's also known as Java Plum and is 10-30 m in height.

Syzygium cumini is called Worldwide medicinal plant which used in medicine due to its vaunted properties against metabolic disorders. Syzygium cumini (Jamun plant) extract, having a lot of applications in the medicinal field due to presence of highly nutritive minerals like sodium, calcium, phosphorous, iron, potassium and other water-soluble vitamins and sugars such as glucose mannose and sucrose (Asghar et al., 2020).

This study involves green synthesis of Ag NPs using Syzygium cumini leaf extract. In current study leaf extract of plant Syzygium cumini was used to synthesize Ag NPs by microwave assisted method which reduced the reaction time duration with less consumption of energy. The first indication of synthesized Ag NPs was taken upon changing the shade of solution from light yellow to dark brown. Further indication of synthesized Ag NPs was done through UV-Vis spectroscopy, Fourier transmission infrared spectroscopy (FTIR), X-Ray Diffraction technique (XRD). Finally antibacterial activity of Ag NPs was evaluated against Gram positive and Gram-negative bacterial strain which showed activity against both pathogens i.e. Eschericia coli and Staphylococcus aureus (Prasad et al.,2012).

Problem Statement

Ag NPs Ag NPs) synthesized through reported physical and chemical methods have requirement of high energy, expensive chemicals as a reducing agent which show toxic impact on the environment. So, there is a need to introduce a more facile method to synthesize NPs with reduced side effects.

Physical and Chemical methods of synthesis of NPs are not reliable because they require high energy and have toxic effect on an environment. So, there is need to use facile, cost effective and environmentally friendly approach for the synthesis of Ag NPs with controlled shape and size (Hebeish et al., 2011).

Research Objectives

The objectives of present study are as follows:

- To prepare a plant extract of Syzygium cumini.
- To utilize a microwave-assisted approach for the synthesis of silver nanoparticles (Ag NPs).

- To perform characterization of the synthesized Ag NPs using various techniques including UV-Visible spectroscopy, FTIR (Fourier Transform Infrared) spectroscopy, Transmission Electron Microscopy (TEM), and X-ray Diffraction (XRD).
- To assess the antibacterial effectiveness of the synthesized Ag NPs.

Significance of the Study

Microwave assisted green synthesis of Ag NPs is expected to be a better choice than other techniques due to short reaction time, Less energy consumption, and better yields of NPs (Awwad & Salem, 2012).

Literature Review

Silver nanoparticles (Ag NPs) are highly sought after in nanotechnology due to their unique properties such as chemical stability, conductivity, catalytic, antimicrobial, and anti-inflammatory activity. Green synthesis using a top-down approach is preferred due to its wider applications compared to physical-chemical techniques (Ahmad et al., 2019). Chemical reduction with organic compounds like ascorbate, sodium borohydride, and Tollen's reagents is commonly used to synthesize Ag NPs by reducing silver ions to metallic silver in solution (Gudikandula & Charya Maringanti, 2016).

While various chemical methods exist, they often involve toxic substances and are not environmentally friendly. This has led to the development of green synthesis methods that are both eco-friendly and economically viable, using plant materials to produce non-toxic nanoparticles with applications in biomedicine and the environment (Abdelghany et al., 2018).

Biological approaches to nanoparticle synthesis involve a wide range of organisms, from simple bacterial cells to multicellular fungi and plants. Different organisms are used for different types of nanoparticles, such as bacteria for gold, iron, cadmium, and zinc NPs, yeast for silver, cadmium, and lead NPs, fungi for gold, cadmium, and silver NPs, algae for gold and silver NPs, and plants for gold, silver, zinc, palladium, platinum, and magnetite NPs (Kalpana & Devi Rajeswari, 2018). Biological methods focus on various aspects like organism properties, reaction conditions, and biocatalyst selection to synthesize well-characterized and stable NPs. By controlling factors such as substrate concentration, reaction conditions, buffer strength, and exposure time, the size and shape of NPs can be tailored to meet desired specifications. For example, bacterial supernatant from Bacillus Licheniformis yields stable 40 nm silver NPs, while B. Subtilis supernatant irradiated with microwaves produces monodisperse Ag NPs (5-50 nm) (Kalishwaralal et al., 2009). Phytonanotechnology involves synthesizing NPs using plant extracts, an eco-friendly and cost-effective approach with high potential for medical applications. Moringa leaf extract mixed with $AgNO_3$ at elevated temperatures yields antimicrobial Ag NPs. Neem leaf extract and other plant parts have also been used for Ag NP synthesis, with experimental parameters affecting NP characteristics (Moodley et al., 2018). Mulberry leaf extract and latex of Jatropha curcas have been employed to synthesize Ag NPs of various sizes, characterized using techniques like UV-Vis, XRD, and SEM (Bar et al., 2009).

Green synthesis of metallic nanoparticles, such as Ag NPs using Jatropha latex, offers an eco-friendly alternative to chemical methods. Characterization techniques like TEM, XRD, and UV spectroscopy reveal size (10-20nm) and stabilization mechanisms, with cyclic octapeptide for smaller particles and latex enzymes for larger ones (Kumar et al., 2017). Ag NPs' antimicrobial potential is explored in nanobiotechnology research (Demissie & Lele, 2013). Eucalyptus chapmaniana leaf extract successfully synthesizes stable Ag NPs, indicated by color change, and characterized using SEM, TEM, XRD, and UV spectroscopy (Majeed et al., 2018). The synthesized Ag NPs demonstrate antimicrobial activity against Pseudomonas aeruginosa through agar well diffusion tests.

K. Anandalakshmi et al. used Pedalium murex leaf extract to synthesize silver nanoparticles (Ag NPs) through a green approach. The NPs were characterized using UV-Vis spectroscopy, FTIR, XRD, and TEM. They displayed antibacterial effects against various bacteria (Anandalakshmi et al., 2016). Banana peel extract was employed as a capping agent for Ag NP synthesis, affected by factors like silver nitrate concentration, pH, and incubation time. Characterization techniques included UV-Vis, XRD, and TEM, showcasing antibacterial activity against different pathogens, with larger inhibition zones for Gram-negative bacteria (Pratikno et al., 2021).

Qian Sun et al. utilized tea leaf extract to synthesize Ag NPs via a green approach, studying variables such as dosage, reaction time, and temperature. Tea extract served as both a reducing and capping agent. Characterization methods encompassed TEM, XRD, FTIR, and TGA, revealing size (20-90 nm), crystalline structure, and antibacterial properties against E. coli (Prema et al., 2022).

Shakeel Ahmed and Saifullah employed Azadirachta indica leaf extract for a straightforward green synthesis of Ag NPs, demonstrating reducing and capping roles. Characterization utilized TEM, UV-Vis spectroscopy, and FTIR, with NPs showing antibacterial activity against diverse microorganisms (Ramar et al., 2019).

Salvia spinosa plant extract provided an eco-friendly and rapid method for synthesizing Ag NPs in just 15 minutes, with the extract serving as the reducing agent. The approach exhibited strong antibacterial activity and was characterized through UV-Vis spectroscopy, SEM, and FTIR. UV-Vis indicated absorption at 450 nm, and XRD revealed crystalline planes, primarily in round-shaped NPs. This method's non-toxic nature is advantageous for biomedical applications (Akintelu et al., 2020).

Mostafa M.H. Khalil and colleagues employed a green synthetic approach using olive leaf extract to synthesize small, spherical Ag NPs of around 20 nm. Various factors such as temperature, pH, and contact time were studied, with plant extract concentration influencing NP size. The biomolecules in the plant extract were found to stabilize and reduce the size of the synthesized Ag NPs (Rodino et al., 2019).

The nature of Ag NPs was determined using variable characterization techniques such as UV-Visible spectroscopy, X-ray Diffraction and Scanning electron microscopy. SPR of synthesized Ag NPs were showed at 440-458 nm and XRD clear its crystalline nature, FTIR technique was used to categorize the biomolecules that are responsible for capping and stabilization of synthesized Ag NPs such NPs further used for antimicrobial activity against different strain of bacteria using different concentration of synthesized Ag NPs (Atalar et al., 2021).

Plant extract of tulsi, banana and neem has been used to synthesize Ag NPs in ecofriendly way for therapeutic applications.it is observed that Ag NPs synthesized by banana plant extract display high antibacterial activity against Gram positive and Gram- negative bacteria. Besides this it is also reported that seed treated with Ag NPs showed better result in germination antioxidative stress of enzyme. Synthesized Ag NPs were characterized through different characterization techniques such as transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) (Verma & Mehata, 2016).

Hemali Padalia and his coworkers synthesized Ag NPs through marigold flower extract. This flower extract acts as a stabilizing agent. Silver ions are reduced to Ag NPs when interacting with the flower extract. In this study synergetic effect of antibiotics and Ag NPs has been reported. It is observed that 15 antibiotics with Ag NPs combination have better results. It is reported that Ag NPs have better results as compared to antibiotics. Plant mediated Ag NPs can be used as alternative of multi drug resisting bacteria (Padalia et al., 2015).

Mostafa and colleagues synthesized Ag NPs using olive leaf extract as a stabilizing agent, controlling shape and reaction rate through extract concentration, contact time, pH, and temperature. Basic conditions favored Ag NP formation, characterized by UV-visible spectroscopy, XRD, and SEM. The NPs were spherical, stable (20-25 nm), and exhibited antibacterial potential (Nasir et al., 2016).

Naheed Ahmad and Seema Sharma used Ananas comosus extract for green synthesis of Ag NPs, characterized via UV-Vis spectroscopy, XRD, and HRTEM. UV-Vis displayed absorption in 200-800 nm, FTIR identified phenol as a reducing agent, and TEM revealed round particles (12 nm). XRD indicated face-centered cubic Ag NPs (Baran et al., 2021).

The synthesis of synthesized Ag NPs physically conformed by color modification of the reaction solution from yellowish to dark brown and further different characterization techniques were used to conform such as UV-Visible Spectroscopy (UV-Vis), Fourier Transforms Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM). The maximum absorbance peak through UV-Visible was observed at 420 and 440 nm for Clitoria ternatea and Solanum nigrum respectively. FTIR was used to identify the biomolecules present in leaf extract of Clitoria ternatea and Solanum nigrum behaves as capping and stabilizing agent showed absorption band of O-H stretch at 3317.34 cm-1 for Clitoria ternatea at 2933.88 cm-1 for Solanum nigrum (Francis et al., 2018).

XRD investigation used to identify the size of particles which were mostly crystalline in nature, TEM further clear exact size of synthesized Ag NPs was 20nm for Clitoria ternatea and 28 nm for Solanum nigrum. The synthesized Ag NPs were used at diverse concentration for antibacterial activity against various pathogens. The Ag NPs of Clitoria ternatea showed higher activity than Solanum nigrum against pathogens, antimicrobial activity is confirmed through disc diffusion method (Kumar et al., 2010).

Microwave-assisted synthesis offers advantages over conventional methods for producing silver nanoparticles (Ag NPs), with improved size distribution, crystallization, and energy efficiency. Using cellulose or sodium as reducing agents, microwave synthesis yields uniform and stable Ag

NPs with sizes controlled by reducing agent concentration (Sökmen et al., 2017). Amino acids in microwave-assisted synthesis control Ag NP size and morphology, with proteins playing a role in reduction and stabilization (Jayaprakash et al., 2017).

Syzygium cumini leaves and bark act as both reducing and capping agents for Ag NP synthesis, confirmed through UV-Vis, FTIR, SEM, and XRD. Particles range in size (100-160 nm), exhibit antimicrobial activity, and can be synthesized using different parts of the plant (Kumar et al., 2010). Bark extract, utilized for Ag NP reduction and size control, undergoes characterization using UV-Visible, FTIR, DLS, and TEM, resulting in spherical NPs of around 14 nm (Kumar et al., 2010).

Material and Methods

Different approaches have been described in literature for the formation of Ag NPs with some shortcomings. The present research work aims at reducing these shortcomings using microwave assisted method for the synthesis of Ag NPs using plant extract Syzygium cumini for antibacterial activity. The synthesized Ag NPs were used for further antimicrobial evaluation. All the experimental work was carried out in chemistry lab of University of Sialkot. The list of chemicals and apparatus which is used in present study has been given below.

Table 1: List of chemicals

Sr. No	Chemical Name	Molecular Formula	Mol. mass (g/mol)	Purity (%)
1.	Silver nitrate	AgNO3	169.87	99.8
2.	Ethanol	C2H5OH	46	99.5
3.	Acetone	C3H6O	58.08	97.2

Table 2: Instruments and glassware

Sr. No	Instruments and glassware	Functions
1	Measuring cylinder	To measure volume
2	Pipette	Transfer measured amount of liquid
3	Beaker	Mixing and stirring
4	Erlenmeyer flask	Mixing, shaking, filtration
5	Volumetric flask	Precise dilution of solution
6	Pestle mortar	For sample griding
7	Burette	For quantitative analysis of sample
8	Glass stirrer	Stirring and mixing
9	Dropper	Transfer sample drop wise
10	Sample vials	To hold sample tubes
11	Petri dishes	Used to hold growth culture
12	Magnetic stirrer	For sample stirring

13	Electrical balance	To weigh the sample
14	Microwave oven	To speed up the synthesis of Ag NPs
15	Vacuum Oven	For sample drying
16	Centrifuge	For centrifugation of NPs suspension
17	UV-Visible	To identify the absorbance peak of Ag NPs
18	FTIR	To identify the active functional group
19	XRD	To confirm the crystalline nature of Ag NPs
20	SEM	To confirm size and shape of AgNPs

Sample Collection

Syzygium cumini leaves were collected from the nearby areas of Sialkot. These leaves were washed continuously using tap water first and after rinsing with distilled water to eliminate dirt and scraps. The leaves were shade dried for 5 days.

Figure 1: (a). Syzygium cumini fresh leaf

Figure 1: (b). Syzygium cumini dry leafs

Sample Preparation

The dried leaves were grounded well into a fine powder with an electric blender and stored in an airtight container for further experimental use. Different solutions of concentration such as 2mM, 4mM, 6mM, 8mM, 10mM of silver nitrate precursor solutions were prepared.

Figure 2: Syzygium cumini leaf powder

Extraction Preparation

10 g of Syzygium cumini plant leaf powder was mixed with 100 ml distilled water and was stirred for 3h at room temperature. The suspension was filtered through Whatman filter paper. The filtrate was stored at 4 0C for further analysis (Jagetia, 2017).

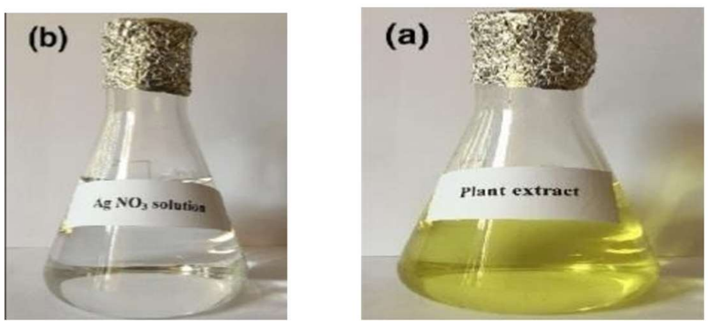

Figure 3: (a). plant extract Figure 3: (b). precursor solutions

Microwave Assisted Synthesis

The 10 ml of aqueous extract was added drop-wise in different concentration of salt solution (AgNO3). i-e 2 mM (0.034g), 4 mM (0.068g), 6 mM (0.102g), 8 mM (0.136g), 10 mM (0.17) separately.

Figure 4: Leaf Extract with Pressure Solution

Catalyst Research Volume 23, Issue 2, October 2023 Pp. 2643-2662

The reaction mixture was irradiated employing a microwave oven (Dawlance operating at 700 W and 2450 MHz) until the color altered from light yellow to dark brown.

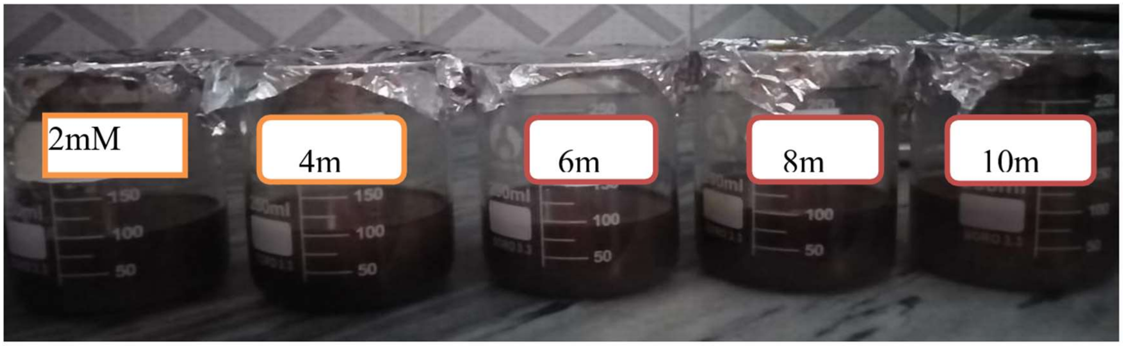

Figure 5: Solution after microwave treatment

The prepared solution was subjected to centrifugation at 10000 rpm for 10 mins. The obtained pellet was washed thrice with ethanol. After ethanol washing the prepared pellet is dried using vacuum drying oven for 6h to change the suspension to dried stable powder of nanoparticles which stored at 4 0C for further characterization. Ag NPsshow sensitivity under light so during whole methodology falcons covered with aluminum foil. (Seku et al., 2018).

Analysis of Sample

The synthesized Ag NPs were characterized using different characterization techniques such as UV-Visible spectroscopy, Fourier Transmission Infrared spectroscopy (FTIR), X-Ray Diffraction (XRD) and Transmission electron microscope (TEM) to get information about size, shape, and formation of silver nanoparticles.

UV-Visible Spectroscopy

UV-Visible spectra were recorded at University of Agriculture Faisalabad (Cecil 7400S Uv spectrophotometer) in the range of 300-700 nm to determine lambda max for Ag NPs (Zhang et al., 2021).

Fourier Transform Infrared Spectroscopy

FTIR spectra were recorded at University of Agriculture Faisalabad using FTIR Cary 630 spectrophotometer to identify the components that are responsible for the formation of Ag NPs. Different peaks of energetic compounds corresponding to functional groups were identified. The metabolites which are accountable for the reduction of silver ion were indicated by FTIR spectrum (Kahrilas et al., 2014).

X-ray Diffraction

x- ray diffraction analysis was carried out in university of Peshawar To reveal crystalline nature and size of synthesized Ag NPs The crystalline size was calculated using Scherrer equation:

$D=K\lambda/(\beta \cos \theta)$

Weíe D= Cíystalline Size (nm)

K= 0.94 Scheííeí constant

λ = 0.15406 nm (wavelength of X-íay souíce) β = Full width half maximum (FWHM) íadians θ = Peak position (íadians)

Transmission Electron Microscope

The morphology and size of synthesized Ag NPs were measured by image recorded from Transmission Electron Microscope (TEM) in university of Hong Kong (Varghese Alex et al., 2020).

Antibacterial Activity

Antibacterial efficiency against both Gram positive (Staphylococcus aureus) and Gram negative (Escherichia coli) bacteria was evaluated by agar well diffusion method. Antibiotic ciprofloxacin was used as positive control. 50 ml of agar was autoclaved at 121 0C for each bacterial strain and 20 ml was placed into labeled petri- plates using measuring cylinders. After the medium had solidified, plates were shifted in an incubator for an hour. The samples were injected by micropipette of size 1mg/ml. The plates were allowed to stand for half an hour before being incubated at 37 0C for 24 hours to ensure optimal diffusion. Further plates were examined and zones of inhibitory were measured in millimeters using a scale (Rao et al., 2013).

Results and Discussion

In present research work Ag NPs have been synthesized using microwave-assisted biological approach. Syzygium cumini leaf extract was used as a reducing and oxidizing agent. The synthesized Ag NPs have been characterized using UV-Vis, FTIR, XRD and TEM. Antibacterial activity of produced Ag NPs has also been evaluated.

Synthesis of Ag NPs

Syzygium cumini fresh leaf were collected from the local area of Sialkot. leaves were washed with distilled water and then shaded dry. The dried leaves were grounded into fine powder. Aqueous natural extract was prepared using 10g powder of leaf in 100 mL of distilled water and stirred well on magnetic stirrer. After stirring, filtration was done using Whatman filter paper and filtrate was stored at 4 0C. Different concentration (2mM, 4mM, 6mM, 8mM, 10mM) precursor solution of $AgNO_3$ were prepared. 10 mL plant extract was added in 100mL of precursor solution and further treated in microwave. The physical identification about synthesis of Ag NPs was confirmed by the color alteration from light yellow to dark brownish after 10min of microwave treatment. The obtained suspension was centrifuged at 10000 rpm for 10 min and washed thrice with ethanol. After centrifugation, the suspension was dried in vacuum oven and then Ag NPs were converted into fine powder.

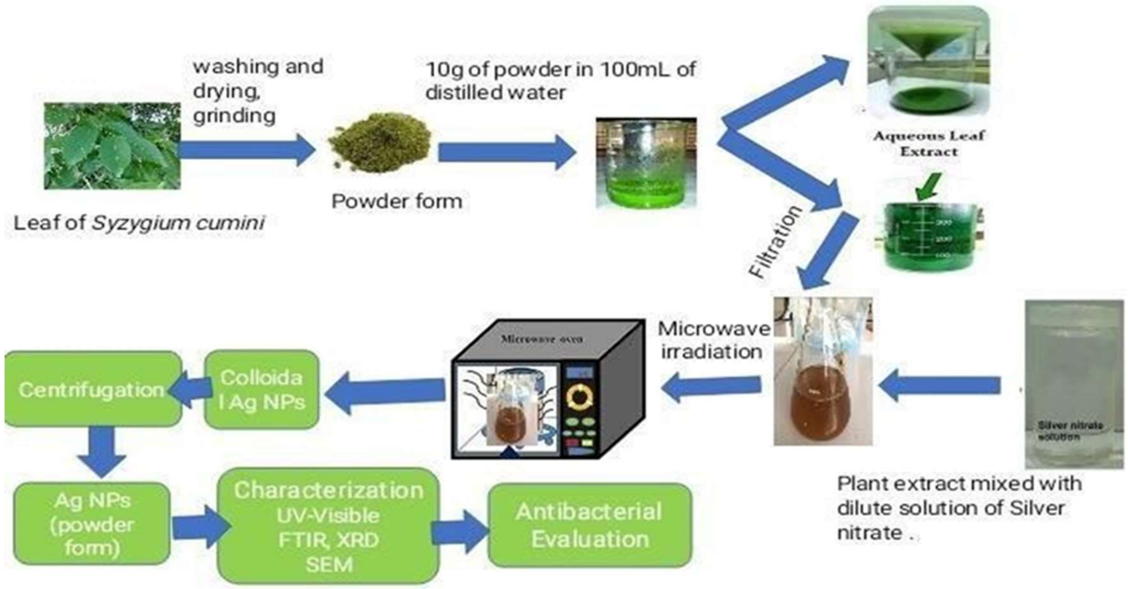

Figure 6: Flow sheet diagram for Ag NPs formation

Characterization of Ag NPs

The synthesized Ag NPs have been characterized using UV-Vis, FTIR, XRD and TEM.

UV-Visible Spectroscopy

The synthesized Ag NPs showed the phenomena of surface plasmon resonance through UV-Vis Spectroscopy in wavelength range of 300-700 nm. The sample of different molarity 4 mM, 6 mM, 8 mM, 10 mM were further diluted in small aliquots and monitored by adjusting the wavelength range through double beam spectrophotometer (CESIL 7400). The synthesis of Ag NPs was indicated by UV-Vis spectrophotometer. UV-Visible spectrum of synthesized Ag NPs showed sharp peak at 427cm-1 and broadening of peak also indicated that synthesized Ag NPs were polydisperse.

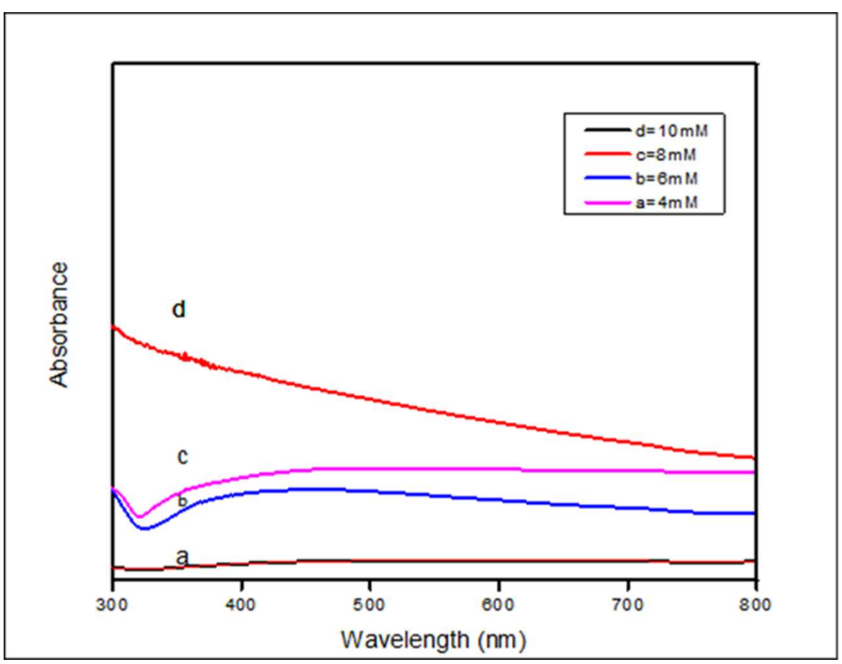

Figure 7: UV-Vis Spectra of Ag NPs (a=4 mM, b=6 mM, c=8 mM and d=10 mM)

In figure 7 the peak at 415 nm is attributed to the presence of Ag NPs showing SPR which is due to the collective oscillations of electrons on the surface of Ag NPs using 4mM AgNO3 concentration. At 6 mM, 8 mM, and 10 mM bathchromic shift at 427 nm 434 nm and 447 nm respectively could be due to increase in size of Ag NPs. In earlier work the synthesis of Ag NPs using Syzygium cumini leaf extract has been done which showed SPR band at 446nm (Ojo et al., 2018; Varghese Alex et al., 2020). So, it can be concluded that both fruit and leaf can be used for the synthesis of Ag NPs.

FTIR Analysis

FTIR analysis was used to identify the synthesis of Ag NPs and its association with other biomolecules. Different peaks of active compounds corresponding to functional groups were identified. The phytochemicals present in leaf extracts of Syzygium cumini which are responsible for the reduction of silver ion in the synthesis of AgNPs were identified by FTIR spectrum.

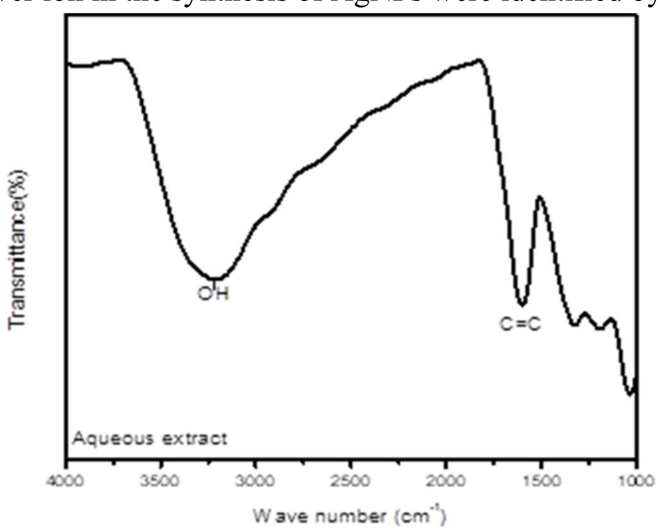

Figure 8: FTIR Spectra of aqueous leaf extract

Figure 8 shows FTIR spectra for aqueous Syzygium cumini leaf extract similar to reported green tea plant extract (Rónavári et al., 2017; Varghese Alex et al., 2020) where signal at 3300cm-1 represents the hydroxyl (OH) group, which usually starts from 3200cm-1 but due to the presence of C-H group, it appeared that the peaks are merged, while peak at 2431cm-1 and 2395cm-1 indicated the presence of aldehyde group. The absorption band at 1636.9cm-1 corresponds to the stretching vibrations of alkene group with aromatic ring.

Catalyst Research Volume 23, Issue 2, October 2023 Pp. 2643-2662

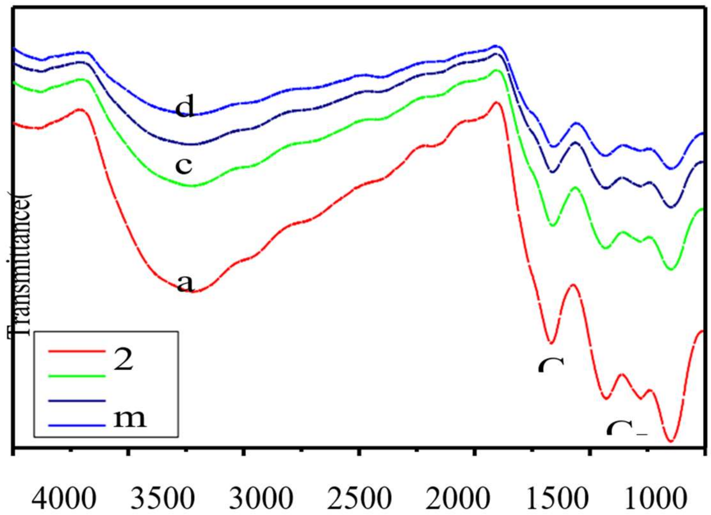

Figure 9: wave number (cm-1)s

Figure 9. FTIR Spectra of synthesized Ag NPs containing different concentration of precursor solution of silver nitrate (a=2 mM , b=4 mM , c= 6 mM ,d= 8 mM)

X-ray Diffraction

XRD analysis was performed to check the crystalline nature of synthesized silver nanoparticles, which displayed four strong reflections at 38.160, 47.080, 64.360,76.720.

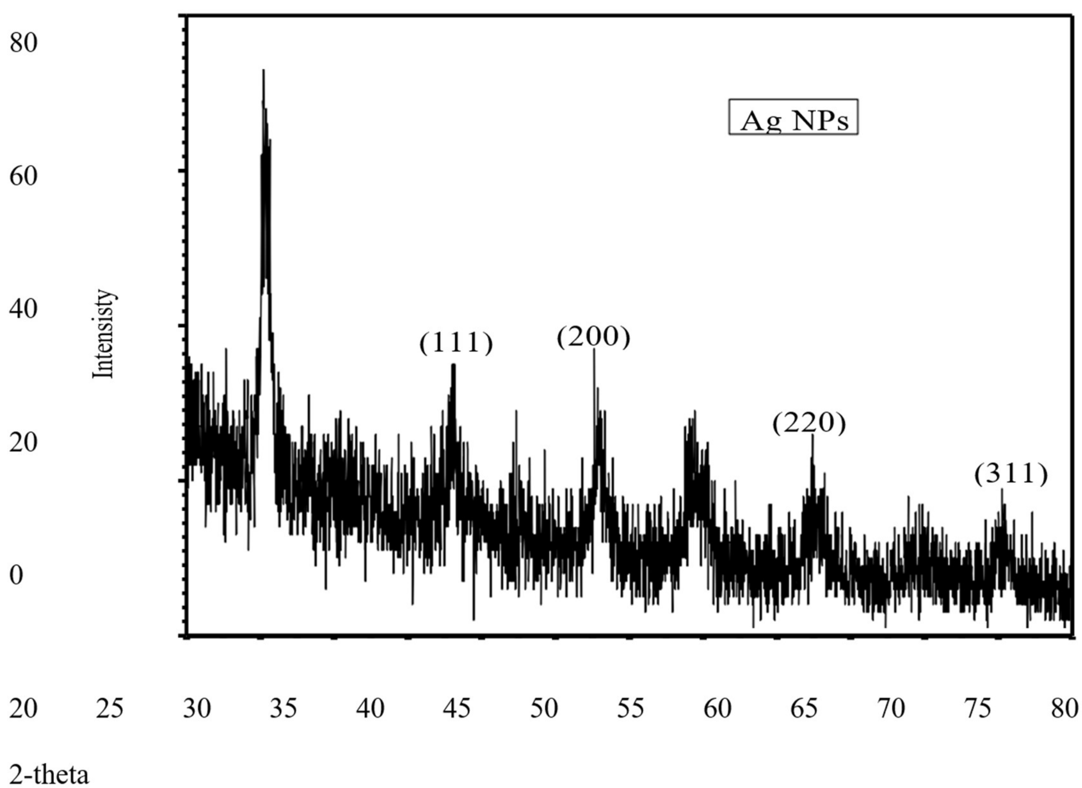

Figure 10: X-ray Diffraction analysis of Ag NPs

(111), (200), (220) and (311) planes respectively. The signals appeared are in close agreement with those reported in literature and are clear indication of face -centered cubic crystalline structure of Ag NPs. The results are comparable with available literature (Danish et al., 2021).

Crystal size of Ag NPs and comparison of the FWHM of (111), (200), (220) and (311) diffraction peaks for the sample.

SamplePeak
Position
Peak Orientation FWHM Crystallite size(nm)

	(2 Theta)	(Miller indices)
AgNPs	38.16^0	111
	47.08^0	200
	64.36^0	220
Mean value=	76.72^0.	311

Transmission Electron Microscope

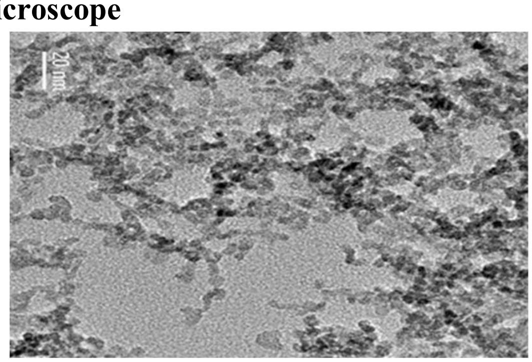

Figure 11: TEM image of synthesized Ag NPs (a) 20 nm

Transmission Electron micrograph showed the morphology of synthesized Ag NPs at university of Hong Kong. Figure 11 indicates that most of the synthesized Ag NPs are spherical in shape with average size of 20 nm. These results showed strong agreement with literature (Awwad & Salem, 2012).

Antibacterial Evaluation

Antibacterial activity of synthesized Ag NPs was evaluated against Gram positive and Gram-negative bacteria Staphylococcus aureus and Escherichia coli.

Table 3: Zone of Inhibition against Gram positive and Gram-negative bacteria at different AgNO3 concentration for Ag NPs

Sr. No	Sample Name	Precursor (AgNO3) concentration	(Zone of inhibition Staphylococcus aureus) (mm)	(Zone of inhibition Eschericia coli) (mm)
1	GST 1	2 mM	12.0	3.3

Catalyst Research Volume 23, Issue 2, October 2023 Pp. 2643-2662

2	GST 2	4 mM	12.5	4
3	GST 3	6 mM	13.7	6.2
4	GST 4	8 mM	14.0	12.4
5	GST 5 Ciprofloxacin positive control	10 mM	15 43	12.0 42

GST 5 was found to be more effective against Gram positive bacteria with 15 mm inhibition zone while GST 4 was found more effective against Gram negative bacteria. The results are comparable to antibacterial action of synthesized Ag NPs to literature (Ahmad et al., 2017).

(a) Staphylococcus Bacillus (b) Eschericia coli

Fig 4.7: Antibacterial activity of synthesized Ag NPs against

Conclusion

Ag NPs are of great interest due to their unique characteristics such as facile synthesis, high stability, high surface to volume ratio, non-toxicity, tunable, adequate morphology, high reactivity, size dispersion, electronic and optical properties. The green synthesis route was adopted to produce silver nanoparticles. In current study leaf extract of plant Syzygium cumini was used microwave assisted method which reduced the reaction time duration with less consumption of energy. The first indication of synthesized Ag NPs was taken upon changing the color of solution from light yellow to dark brown. Further indication of synthesized Ag NPs was done through UV-Vis spectroscopy, Fourier Transmission Infrared spectroscopy (FTIR), X-Ray Diffraction technique (XRD). Synthesized Ag NPs show lambda maximum at 380cm-1 due to excitation of electron in surface plasmon resonance phenomena under UV-Vis spectroscopy. FTIR spectra of plant extract

is compared with spectrum of synthesized Ag NPs which was cleared the peaks shifting from 3200 cm-1 to 3365 cm-1 due to phytochemicals that involve in synthesis of silver nanoparticles. XRD shows the crystalline nature of synthesized silver nanoparticles. The synthesized Ag NPs displayed four strong reflections at 38.160,44.830,64.690,77.540 having (111), (220),
(200) and (311) planes respectively. The synthesized Ag NPs showed effective antimicrobial activity against Gram positive and Gram-negative bacterial species, Staphylococcus aureus and Escherichia coli with maximum inhibition zone of 14 nm. It is expected that the synthesized AgNPs could be of great benefit for further clinical trials and drug designing.

References

Aadim Ph D, K. A., & Jasim Ph D, A. S. (2022). Silver nanoparticles synthesized by Nd: YAG laser ablation technique: characterization and antibacterial activity. Karbala International Journal of Modern Science, 8(1), 71-82.

Abdelghany, T., Al-Rajhi, A. M., Al Abboud, M. A., Alawlaqi, M., Magdah, A. G., Helmy, E. A., & Mabrouk, A. S. (2018). Recent advances in green synthesis of silver nanoparticles and their applications: about future directions. A review. BioNanoScience, 8(1), 5-16.

Ahmad, A., Wei, Y., Syed, F., Tahir, K., Rehman, A. U., Khan, A., . . . Yuan, Q. (2017). The effects of bacteria-nanoparticles interface on the antibacterial activity of green synthesized silver nanoparticles. Microbial pathogenesis, 102, 133-142.

Ahmad, S., Munir, S., Zeb, N., Ullah, A., Khan, B., Ali, J., . . . Salman, S. M. (2019). Green nanotechnology: A review on green synthesis of silver nanoparticles— An ecofriendly approach. International journal of nanomedicine, 14, 5087.

Ahmed, S., Saifullah, Ahmad, M., Swami, B. L., & Ikram, S. (2016). Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. Journal of radiation research and applied sciences, 9(1), 1-7.

Akintelu, S. A., Bo, Y., & Folorunso, A. S. (2020). A review on synthesis, optimization, mechanism, characterization, and antibacterial application of silver nanoparticles synthesized from plants. Journal of Chemistry, 2020.

Anandalakshmi, K., Venugobal, J., & Ramasamy, V. (2016). Characterization of silver nanoparticles by green synthesis method using Pedalium murex leaf extract and their antibacterial activity. Applied Nanoscience, 6(3), 399-408.

Asghar, M. A., Zahir, E., Asghar, M. A., Iqbal, J., & Rehman, A. A. (2020). Facile, one-pot biosynthesis and characterization of iron, copper and silver nanoparticles using Syzygium cumini leaf extract: as an effective antimicrobial and aflatoxin B1 adsorption agents. PloS one, 15(7), e0234964.

Atalar, M. N., Baran, A., Baran, M. F., Keskin, C., Aktepe, N., Yavuz, Ö., & İrtegun Kandemir, S. (2021). Economic fast synthesis of olive leaf extract and silver nanoparticles and biomedical applications. Particulate Science and Technology, 1-9.

Awwad, A. M., & Salem, N. M. (2012). Green synthesis of silver nanoparticles byMulberry LeavesExtract. Nanoscience and Nanotechnology, 2(4), 125-128.

Bar, H., Bhui, D. K., Sahoo, G. P., Sarkar, P., De, S. P., & Misra, A. (2009). Green synthesis of silver nanoparticles using latex of Jatropha curcas. Colloids and surfaces A: Physicochemical and engineering aspects, 339(1-3), 134-139.

Baran, A., Keskin, C., Baran, M. F., Huseynova, I., Khalilov, R., Eftekhari, A., Kavak, D. E. (2021). Ecofriendly Synthesis of Silver Nanoparticles Using Ananas comosus Fruit Peels: Anticancer and Antimicrobial Activities. Bioinorganic Chemistry and Applications, 2021.

Chakravarty, A., Ahmad, I., Singh, P., Sheikh, M. U. D., Aalam, G., Sagadevan, S., & Ikram, S. (2022). Green synthesis of silver nanoparticles using fruits extracts of Syzygium cumini and their bioactivity. Chemical Physics Letters, 795, 139493.

Demissie, A., & Lele, S. (2013). Phytosynthesis and characterization of silver nanoparticles using callus of jatropha curcas: A biotechnological approach. International journal of nanoscience, 12(02), 1350012.

El Shafey, A. M. (2020). Green synthesis of metal and metal oxide nanoparticles from plant leaf extracts and their applications: A review. Green Processing and Synthesis, 9(1), 304-339.

Francis, S., Joseph, S., Koshy, E. P., & Mathew, B. (2018). Microwave assisted green synthesis of silver nanoparticles using leaf extract of elephantopus scaber and its environmental and biological applications. Artif Cells Nanomed Biotechnol, 46(4), 795-804.

Gudikandula, K., & Charya Maringanti, S. (2016). Synthesis of silver nanoparticles by chemical and biological methods and their antimicrobial properties. Journal of Experimental Nanoscience, 11(9), 714-721.

Hebeish, A., El-Naggar, M., Fouda, M. M., Ramadan, M., Al-Deyab, S. S., & El- Rafie, M. (2011). Highly effective antibacterial textiles containing green synthesized silver nanoparticles. Carbohydrate Polymers, 86(2), 936-940.

Jagetia, G. C. (2017). Phytochemical Composition and Pleotropic Pharmacological Properties of Jamun, Syzygium Cumini Skeels. Journal of Exploratory Research in Pharmacology, 2(2), 54-66. doi:10.14218/jerp.2016.00038

Jayaprakash, N., Vijaya, J. J., Kaviyarasu, K., Kombaiah, K., Kennedy, L. J., Ramalingam, R. J., . . . Al-Lohedan, H. A. (2017). Green synthesis of Ag nanoparticles using Tamarind fruit extract for the antibacterial studies. Journal of Photochemistry and Photobiology B: Biology, 169, 178-185.

Kahrilas, G. A., Wally, L. M., Fredrick, S. J., Hiskey, M., Prieto, A. L., & Owens, J. E. (2014). Microwave-assisted green synthesis of silver nanoparticles using orange peel extract. ACS Sustainable Chemistry & Engineering, 2(3), 367- 376.

Kalishwaralal, K., Deepak, V., Pandian, S. R. K., & Gurunathan, S. (2009). Biological synthesis of gold nanocubes from Bacillus licheniformis. Bioresource technology, 100(21), 5356-5358.

Kalpana, V. N., & Devi Rajeswari, V. (2018). A Review on Green Synthesis, Biomedical Applications, and Toxicity Studies of ZnO NPs. Bioinorg Chem Appl, 2018, 3569758. doi:10.1155/2018/3569758

Kumar, S., Halder, D., & Mitra, A. (2017). Characterization of silver nanoparticles synthesized using latex of Jatropha curcas and Lannea grandis. Journal of Surface Science and Technology, 32(3-4), 115–120.

Kumar, V., Yadav, S. C., & Yadav, S. K. (2010). Syzygium cumini leaf and seed extract mediated biosynthesis of silver nanoparticles and their characterization. Journal of Chemical Technology & Biotechnology, 85(10), 1301-1309.

Majeed, A., Ullah, W., Anwar, A. W., Shuaib, A., Ilyas, U., Khalid, P., . . . Ali, S. (2018). Cost-effective biosynthesis of silver nanoparticles using different organs of plants and their antimicrobial applications: A review. Materials Technology, 33(5), 313-320.

Moodley, J. S., Krishna, S. B. N., Pillay, K., & Govender, P. (2018). Green synthesis of silver nanoparticles from Moringa oleifera leaf extracts and its antimicrobial potential. Advances in Natural Sciences: Nanoscience and Nanotechnology, 9(1), 015011.

Nasir, G. A., Mohammed, A. K., & Samir, H. F. (2016). Biosynthesis and characterization of silver nanoparticles using olive leaves extract and sorbitol. Iraqi journal of biotechnology, 15(1).

Ojo, O. A., Oyinloye, B. E., Ojo, A. B., Ajiboye, B. O., Olayide, I. I., Idowu, O., . . . Adewunmi, F. (2018). Green-route mediated synthesis of silver nanoparticles (AgNPs) from syzygium cumini (L.) Skeels polyphenolic-rich leaf extracts and investigation of their antimicrobial activity. IET Nanobiotechnology, 12(3), 305-310.

Padalia, H., Moteriya, P., & Chanda, S. (2015). Green synthesis of silver nanoparticles from marigold flower and its synergistic antimicrobial potential. Arabian Journal of Chemistry, 8(5), 732-741.

Prasad, R., Swamy, V. S., & Varma, A. (2012). Biogenic synthesis of silver nanoparticles from the leaf extract of Syzygium cumini (L.) and its antibacterial activity. Int J Pharm Bio Sci, 3(4), 745-752.

Pratikno, H., Anggya, P. B., Fadhila, F., Chafidz, A., & Rengga, W. D. P. (2021). Biosynthesis of silver nanoparticles using banana Raja (musa paradisiaca var. raja) peel extract: effect of different concentrations of the AgNO3 solution. Paper presented at the Key Engineering Materials.

Prema, P., Veeramanikandan, V., Rameshkumar, K., Gatasheh, M. K., Hatamleh, A. A., Balasubramani, R., & Balaji, P. (2022). Statistical optimization of silver nanoparticle synthesis by green tea extract and its efficacy on colorimetric detection of mercury from industrial waste water. Environmental Research, 204, 111915.

Ramar, K., Gnanamoorthy, G., Mukundan, D., Vasanthakumari, R., Narayanan, V., & Jafar Ahamed, A. (2019). Environmental and antimicrobial properties of silver nanoparticles synthesized using Azadirachta indica Juss leaves extract. SN Applied Sciences, 1(1), 1-11.

Rao, M. L., Bhumi, G., & Savithramma, N. (2013). Green synthesis of silver nanoparticles by Allamanda cathartica L. leaf extract and evaluation for antimicrobial activity. International Journal of Pharmaceutical Sciences and Nanotechnology, 6(4), 2260-2268.

Rodino, S., Butu, M., & Butu, A. (2019). Application of biogenic silver nanoparticles for berries preservation. Digest Journal of Nanomaterials and Biostructures, 14(3), 601-606.

Rónavári, A., Kovács, D., Igaz, N., Vágvölgyi, C., Boros, I. M., Kónya, Z., . . . Kiricsi,

M. (2017). Biological activity of green-synthesized silver nanoparticles depends on the applied natural extracts: a comprehensive study. International journal of nanomedicine, 12, 871.

Sajid, M., & Płotka-Wasylka, J. (2020). Nanoparticles: Synthesis, characteristics, and applications in analytical and other sciences. Microchemical Journal, 154, 104623.

Scolaro, C., Visco, A., & Torrisi, L. (2020). Laser welding of polymeric nanocomposites filled with silver nanoparticles produced by laser ablation. Journal of Instrumentation, 15(02), C02037.

Seku, K., Gangapuram, B. R., Pejjai, B., Kadimpati, K. K., & Golla, N. (2018). Microwave-assisted synthesis of silver nanoparticles and their application in catalytic, antibacterial and antioxidant activities. Journal of Nanostructure in Chemistry, 8(2), 179-188. doi:10.1007/s40097-018-0264-7

Sökmen, M., Alomar, S. Y., Albay, C., & Serdar, G. (2017). Microwave assisted production of silver nanoparticles using green tea extracts. Journal of Alloys and Compounds, 725, 190-198.

Varghese Alex, K., Tamil Pavai, P., Rugmini, R., Shiva Prasad, M., Kamakshi, K., & Sekhar, K. C. (2020). Green synthesized Ag nanoparticles for bio-sensing and Photocatalytic applications. ACS omega, 5(22), 13123-13129.

Verma, A., & Mehata, M. S. (2016). Controllable synthesis of silver nanoparticles using Neem leaves and their antimicrobial activity. Journal of radiation research and applied sciences, 9(1), 109-115.

Xu, L., Wang, Y.-Y., Huang, J., Chen, C.-Y., Wang, Z.-X., & Xie, H. (2020). Silver nanoparticles: Synthesis, medical applications and biosafety. Theranostics, 10(20), 8996.

Xu, L., Wang, Y. Y., Huang, J., Chen, C. Y., Wang, Z. X., & Xie, H. (2020). Silver nanoparticles: Synthesis, medical applications and biosafety. Theranostics, 10(20), 8996-9031. doi:10.7150/thno.45413

Yusuf, M. (2020). Silver nanoparticles: synthesis and applications. Handbook of Ecomaterials, 2343.

Zhang, H., Chen, S., Jia, X., Huang, Y., Ji, R., & Zhao, L. (2021). Comparation of the phytotoxicity between chemically and green synthesized silver nanoparticles. Science of The Total Environment, 752, 142264.