PHYTOCHEMICAL SCREENING, GREEN SYNTHESIS OF SILVER NANOPARTICLES USING AQUEOUS LEAF EXTRACT OF MANGO LEAF (*Mangifera indica*) AND INVESTIGATION OF THEIR ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES

BY

ANIAKOR, SHARON CHIMAOBI
U22/NAS/BCH/313

DEPARTMENT OF BIOCHEMISTRY,
FACULTY OF NATURAL SCIENCES AND ENVIRONMENTAL STUDIES,
GODFREY OKOYE UNIVERSITY, ENUGU

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TITLE PAGE

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A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF NATURAL SCIENCES AND ENVIRONMENTAL STUDIES, GODFREY OKOYE UNIVERSITY, ENUGU

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE (B.Sc.) IN BIOCHEMISTRY

SUPERVISOR: DR. E. L. AYUK

JULY, 2026

APPROVAL

This research titled "Phytochemical Screening, Green Synthesis of Silver Nanoparticles using Aqueous Leaf Extract of Mango Leaf (*Mangifera indica*) and Investigation of their Antibacterial And Antioxidant Properties.." has been assessed and approved by the Committees of the Department of Chemical Sciences and Faculty of Natural Sciences and Environmental Studies, Godfrey Okoye University Enugu Nigeria.

DR. E. L. AYUK — **DATE**
SUPERVISOR

DR. JOY ANOSIKE — **DATE**
HEAD OF DEPARTMENT

EXTERNAL EXAMINER — **DATE**

CERTIFICATION

This is to certify that this project, titled Phytochemical Screening, Green Synthesis of Silver Nanoparticles using Aqueous Leaf Extract of Mango Leaf (*Mangifera indica*) and Investigation of their Antibacterial And Antioxidant Properties., was carried out by ANIAKOR, SHARON CHIMAOBI with Registration Number GOU/U22/BCH/313, in the Department of Biochemistry, Faculty of Natural Sciences and Environmental Studies, Godfrey Okoye University, Enugu, under the supervision of Dr. E.L AYUK. This work has not been previously submitted for any degree in this or any other institution.

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DR E.L AYUK **DATE**

SUPERVISOR

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HEAD OF DEPARTMENT

DATE

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DEAN FACULTY OF NSES

DATE

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EXTERNAL EXAMINER **DATE**

DEDICATION

This work is dedicated to my family who has been of great support to this project work, with the help of the Almighty and intercession of our Blessed Mother Mary.

ACKNOWLEDGEMENT

I give all glory, honour, and gratitude to Almighty God for His grace, wisdom, strength, and guidance throughout the course of this research.

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Finally, I am deeply grateful to my parents, family, friends, and colleagues for their prayers, encouragement, understanding, and moral support throughout my academic journey. May God richly bless everyone who contributed in one way or another to the successful completion of this project.

ABSTRACT

This research aimed to determine the phytochemical constituents, antioxidant and antibacterial activities, and to demonstrate the green synthesis of silver nanoparticles (AgNPs) using aqueous Mangifera indica leaf extract. Phytochemical screening revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, and phytosterols. Successful synthesis of AgNPs was confirmed by the colour change from pale yellow to dark brown, a UV–Vis absorption peak at 438 nm, and FTIR peaks at 3421.35 cm^{-1} (O–H stretching), 1602.41 cm^{-1} (C=C stretching), and 1384.53 cm^{-1} (O–H bending), indicating the involvement of phytochemicals in the reduction and stabilization of the nanoparticles. SEM analysis revealed predominantly spherical, well-dispersed nanoparticles with an average particle size of approximately 45–60 nm. EDX analysis confirmed the successful synthesis of AgNPs, with silver (68.4%) as the major element, alongside oxygen (18.7%), carbon (10.9%), and chlorine (2.0%), demonstrating the elemental composition of the nanoparticles. DPPH and FRAP assays showed moderate antioxidant activity, with AgNPs exhibiting slightly higher activity than the crude leaf extract. No antibacterial activity (zone of inhibition = 0 mm) against Aspergillus niger, Escherichia coli, and Staphylococcus aureus was observed at a concentration of 9 mg/mL. Mangifera indica leaf extract is an effective and eco-friendly reducing and capping agent for the green synthesis of AgNPs with considerable antioxidant potential.

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CHAPTERONE

INTRODUCTION

1.1 Background of the Study

In recent years, a substantial breakthrough in the biomedical sciences was achieved thanks to growing interest in both the research of plant-based bioactive chemicals and the technology of nanoparticles. This was triggered primarily by the necessity to come up with new medicinal remedies that are more secure, effective, and environmentally friendly (Ajiboye *et al*., 2021). The link between the two fields is tightened when plant-based mediated (green) synthesis of metal nanostructures, which exhibited increased biological activity, is evolved (Kahsay *et al*., 2020).

Medicinal Chemistry and the Significance of Medicinal Plants

Phytochemistry

It is defined as the study of plant-derived chemical compounds that exhibit a form of biological or other scientific property. Phytochemicals of plants consist of compounds, namely alkaloids, flavonoids, phenols, tannins, terpenoids, and saponins that have demonstrated antimicrobial, antioxidant, anti-cancer, and anti-inflammatory properties (Islam *et al*., 2022). The medicinal property of those compounds has been established, and the evidence of beneficial roles to help against degenerative disorders linked to oxidative stress and microbial attack increases daily. The bioactive chemicals, also known as secondary compounds, have got great medicinal significance. For example, phenolic compounds and flavonoids are identified to be able to act as free radical scavengers.

Plant(s): *Mangifera indica*

Description Morphology of *Mangifera indica*

A tree that commonly goes by the name mango (*Mangifera indica*), the tree grows in the family of Anacardiaceae. The tree grows up to a height of 10-40 meters. This evergreen perennial grows at a large rate. A thick canopy of a dense and broad and round shape tops the tree. Underneath the surface, a deep taproot system grows downwards. It has a thick and sturdy trunk that grows to be 2 meters high approximately and has rough grayish-brown bark; it gives off a gummy and resinous substance when it's wounded. The bark on younger parts of the tree is smooth and green but turns brownish and harder when it ages. Leaves are simple alternate leaves and have a leathery texture and can be either lanceolate or oblong-lanceolate and about 15 to 35 cm long. Younger leaves are colored red or purplish in shade or copper. At maturity, they become hard and a lustrous dark green color. The tree has leaves with multiple secondary chemicals found within, like flavonoids, phenolic acids, tannins, saponins, alkaloids, and other types of phytocompounds. These chemicals are all capable of performing a wide range of actions and have proved themselves biologically effective (Anand *et al*., 2017). There are smaller, yellowish to pink-colored fragrant flowers, which are grown in groups on very large terminal panicles, which could reach many thousands of flowers. Most of these flowers only do male functions, but some can be both functionally male and female and form fruits. When ripe, these fruits can be fleshy drupes of a large variety of shapes, weights, and sizes depending on how they were raised, but typically these may range from orange to red and green to yellow-colored drupes. Each large drupe bears within a flat seed that is usually protected by a tough woody layer, which can have one or even many tiny embryos inside depending on the cultivar (Kittipong, K.). The tree (*Mangifera indica*), also called the mango tree, is one of the leading fruit trees in the globe and has numerous potentially beneficial medical aspects, as its parts have already been employed for healing by many societies since a long time ago. In recent days, it's been acknowledged as a medicinal and useful plant in modern systems of medicine along with traditional uses. This fact is attributed to the numerous bioactive constituents, including phenolics, flavonoids, carotenoids, and mangiferins, contained in various parts of mango, like the seeds, peel, leaves, bark, seed, and fruit; all of these possess medicinal properties (Donga *et al*., 2021). Mango was utilized in ancient days to stop and treat a variety of diseases, including but not limited to those in the Indian subcontinent and some African native countries in Ayurveda and traditional medicine (Akinmoladun *et al*., 2019). With their remarkable array of bioactive compounds, these mango-based components have garnered modern attention for their antioxidant, antidiabetic, anti-inflammatory, and antibacterial activities (Kumar *et al*., 2021). As an example, the leaves of mango trees that are rich in mangiferin have indicated they play an important function in managing blood sugar by increasing insulin sensitivity and decreasing sugar levels, thereby aiding in treating the conditions related to type 2 diabetes (Aderibigbe *et al*., 2020). Traditional use has traditionally been associated with the treatment of different digestive problems like constipation, diarrhea, and dysentery because of the tannic compounds found in its bark and peels that exhibit an astringent effect (Donga *et al*., 2021). Antioxidant components that are present in the mango tree neutralize the unstable, harmful compounds known as free radicals that damage cells and contribute to cardiovascular disease and other health problems (Adam *et al*., 2021). Its use in the traditional method for the treatment of inflammation is common. Inflammation may also be associated with joint disorders such as arthritis and body pain. Some research suggests that the mango extract has antibacterial and antifungal actions that can make it helpful in treating oral infections and skin disorders (Akinmoladun *et al*., 2019; Bhakya *et al*., 2018). Origin of *Mangifera indica*: A tree of origin from Indo-Burma in Southeast Asia that belongs to the family Anacardiaceae of the order Dicotyledon is primarily sourced from parts of Asia and Africa but is found extensively in other parts of the world as well since 4000 yrs. Of Domestication (Yao, 1998). The mango tree needs to be kept at an ambient temperature range between 24 and 30 degrees Celsius and also have good rainfall varying from 750 to 2500 mm annually; and to ensure growth, it requires fertile soil ranging from sandy loam or loam having a light acid to neutral pH (Yao, 1998). Mango fruit is widely commercialized and distributed in countries like India, China, Indonesia, Mexico, Nigeria, Pakistan, Thailand, etc. In Nigeria, mango trees are abundantly found everywhere, either in communities, farms, or roadsides; at various institutes; or even on the borders of forest areas. This makes the *Mangifera indica* tree one of the most highly cultivated trees in the world. It can be grown in communities as well as northern and southern parts of the country, in almost every homestead (Akinmoladun *et al*., 2019).

Economic values of *Mangifera indica*

The *Mangifera indica* tree has multiple uses, including nutritional, industrial, and medicinal properties. In most regions, ripe mangoes are edible and used to make wines, juices, jams, dried fruits, canned fruits, jellies, and other products. Mango fruit is high in carbohydrates, fiber, and potassium, as well as vitamins A and C, both of which are important antioxidants. All parts of the *Mangifera indica* tree seed, stem bark, leaf, peel, fruit, and flower—have medicinal value and contain numerous bioactive compounds that could be used as precursors in drug development for a variety of diseases, (Rakesh kumar, 2026)

The leaves of the *Mangifera indica* tree have significant antimicrobial, anti-inflammatory, anti-cancer, and anti-diabetic properties. The presence of various beneficial phytocompounds in it also helps to prevent cellular damage. *Mangifera indica* stem bark and root contain antiseptic, anti-inflammatory, and astringent properties. It has been used in many indigenous medicines as an antibacterial and anti-inflammatory agent. Tannins are also abundant in the stem bark of *Mangifera indica*, which can be used in leather processing. Mango wood is primarily used for furniture and timber production, but it can also be used to build structures and pallets. Packaging, wood pulp used to make newsprint. Mango seed kernel, an animal feed ingredient, also produces fats useful in the pharmaceutical and food industries. The bark extract is a yellow-brown viscous liquid that is used to treat skin infections, (Islam, 2022).

The flowers of this tree are used in folk remedies to treat asthma, nausea, and catarrh, while the leaves have anti-diarrheal properties.

Seed has abortifacient properties, and seed powder, along with other constituents, is used to treat diabetes. Phytochemistry is the study of phytochemicals, chemicals, or naturally occurring compounds derived from plants.(Aisida, 2020) These phytochemicals, or chemicals, are plant-derived compounds, with the majority classified as secondary compounds (Devi *et al*., 2016).

The most common natural compounds include alkaloids, saponins, steroids, and phenolic compounds (polyphenols, flavoglycosides, glycosides, and tannins).

These natural products are not necessary for plant growth or reproduction, but they do aid in disease and pest control and attract insects. Plant-derived products are being studied and researched to determine their bioactivities, which include anti-inflammatory, anti-tumorigenic, anti-cancer, and antioxidant properties. Many phytochemicals are becoming increasingly important globally due to their diverse applications, (Kahsay, 2020).

They are safe in their nature, and are a better alternative as supplements and medicine to their synthetically prepared analogues (Vijayan *et al*., 2018). For instance, phenolic components derived from plants act as strong antioxidants by scavenging reactive free radicals, hence they serve an efficient antioxidant that can be utilized for treating cancer, diabetes, cardiovascular diseases and inflammatory disorders. Taking into account of the advantages, the development of cheaper and effective agents using plants as natural resource is being practiced for discovering alternative medicines. (Rani, 2023)

The extract from the leaves of the plant, *Mangifera indica*.

Indica is known to contain more polyphenolic compounds, including gallic acid, catechin, mangiferin, and quercetin (Chung *et al*. 2016). Mangiferin, a compound found in *M. indica* leaves, is well known for its powerful antibacterial and antioxidant properties. Aside from biomedical applications, they are used as oxidizing and reducing agents in nanotechnology, where they aid in the formation of metal ions into Nanoparticles while inhibiting aggregation of formed Nanoparticles, (Ssekatawa, 2021)

The green synthesis method produces Nanoparticles using plant extract as a reducing and capping agent, avoiding the use of hazardous chemicals, low yield, and difficult reaction conditions (Manikandan *et al*., 2022).

Metal nanoparticles (NPs) ranging in size from 1 nm to 100 nm have properties that differ significantly from those of bulk material due to their higher surface-to-volume ratio and chemical reactivity (Abubakar *et al*., 2018). However, these altered properties of Nanoparticles have sparked significant research interest in fields such as medical sciences, energy, electronics, and environmental sciences (Rao *et al*., 2018). Various traditional Nanoparticles preparation techniques, such as physical methods (microemulsion, sol-gel, PVD, and CVD) and chemical reduction (citrate and boron hydride), have a negative environmental impact due to the use of toxic reagents, expensive precursors, and high energy requirements.(Desai, 2022)

Green synthesis approaches provide cost-effective, efficient and environmentally friendly route by using different natural sources like plant, microbial biomass for Nanoparticles synthesis. (The bottom-up approaches utilize basic building units to produce nanomaterial's), (Elemike, 2020).

Plant mediated NP synthesis requires the extraction of plant-derived material such as roots, leaves, stems and flowers, which are then utilized to induce the reduction, capping and stabilization of metal ions in the aqueous medium (Ajitha *et al*., 2015).

The secondary compounds (phytochemicals) naturally present in the plant extract are effective in reducing the metal ion to its zero oxidation state leading to the formation of metal NP (NPs) in aqueous solution. (Behravan, 2019)

For instance, Ag NPs synthesized from the *M. indica* extract, have reduced Ag ions to zero valent silver, a reaction carried out by plant derived compounds (Alqahtani *et al*., 2022). Several reports show the efficacy of *M. indica* extract to synthesize Ag NPs that effectively fight against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. (Al-Zahrani, 2021). Moreover, these biologically synthesized Nanoparticles from plants exhibit a pronounced antioxidant activity as synergistic effect of the metal Nanoparticles with natural Phytochemical antioxidants found in plant extracts improve antioxidant properties by increasing the free radical scavenging capacity of metal NPs (Kotakadi *et al*., 2020).

In general, green NPs with antimicrobial and antioxidant activity have a high potential for biomedical applications (Balashanmugam *et al*., 2021).

Integrating Phytochemistry and Nanotechnology

Nanotechnology is the process of designing and fabricating nanometer-scale devices and products (1-100 nm), whereas phytochemistry is the study of chemical constituents derived from plants. Among numerous approaches to NP synthesis, the green synthesis method using plant extracts stands out due to its distinct advantage over chemically reduced NPs; the latter have less bioactivity than plant-synthesized ones due to plant-based phytochemicals attached to the surface (Nakkala *et al*., 2018). These factors enhance the antimicrobial and antioxidant properties of the resulting NPs, potentially making them new therapeutic agents (Gowramma *et al*., 2017).

The growing threat of drug-resistant microbes, rising rates of noncommunicable diseases, growing environmental concerns, and the rapid development of green technologies are all driving the development of novel and environmentally friendly solutions. (Tyavambiza, 2021)The green synthesis of Ag NPs using *M. indica* leaves is therefore both practical and scientifically interesting, (Khatoon, 2023)

The proposed methodology will result in the synthesis of potentially highly active plant-based therapeutics and novel nanomaterials.

1.2 Statement of the problem

Antimicrobial Resistance has become a significant problem in global health, resulting in difficult and persistent infections and reduced effectiveness of commonly used antibiotics. Pathogenic microorganisms like *Escherichia coli* and *Staphylococcus aureus* have become resistant to different classes of drugs, which has reduced the therapeutic options and increased the morbidity and mortality rates in the world (Chinnasamy *et al*., 2021)

Because microbial resistance to synthetic antibiotics is increasing and spreading, there is an urgent need to find environmentally friendly and cost-effective antibacterial drugs with comparable or improved efficacy.Oxidative stress, caused by excessive ROS (reactive oxygen species) production, plays a vital role in the pathogenesis of chronic and disabling diseases including cancer, diabetes, cardiovascular and neurodegenerative diseases. Tissue damage and disease occurs when the body becomes unable to handle ROS, and ROS levels become too high.

There are a number of synthetic antioxidants that have been reported, but one big drawback is that these may be toxic to cells and the long-term safety of these antioxidants is a matter of much debate. Therefore, natural antioxidants are gaining popularity. Silver nanoparticles (AgNPs), or nanoscale particles, have recently received a lot of attention in biomedical science due to their unique physical and chemical properties, as well as their excellent antibacterial and antioxidant activity (Bbindhu *et al*., 2016).

The most widely used methods of nanoparticle synthesis, however, use expensive, energy-intensive and hazardous chemical agents, producing high levels of byproduct toxicity which may restrict their use in medicine (Noruzi *et al*., 2015). Even though green synthesis using various plant extracts are considered to be safe, the characterization of phytochemicals, the green synthesis of silver nanoparticles and the antibacterial activity testing using a single plant system have not received much attention (Logeswari *et al*., 2020).

A few studies have reported antibacterial activity of plant based nanoparticles and the reported antibacterially active phytochemicals or the size/shape of the AgNPs are not correlated to the microbial activity (Philip *et al*., 2021). The leaves of *M. indica* are well known for their various medicinal activities; however, there is still a lack of studies to report on the synthesis of silver nanoparticles using the extract, along with integrated phytochemical profiling and assessment of the antibacterial and antioxidant activity of the resulting product in a standardized experimental setup (Alqahtani *et al*., 2022).

The present work was focused on synthesis of a new eco-friendly plant-mediated route to silver nanoparticles (AgNPs) using *Mangifera indica* leaf extract and characterization of phytochemical composition as well as their antimicrobial and antioxidant activities as potential alternatives to the synthetic antibacterial and antioxidant agents (Iravani *et al*., 2017).arable or superior efficacy to those currently available (Velmurugan *et al*., 2018).

1.3 Aim of the study

The objective of this work was to carry out the phytochemical screening, green synthesis of silver Nanoparticles using *Mangifera indica* leaf extract and evaluate their antimicrobial and antioxidant activities

1.4 Specific objectives of the study

The following specific objectives were given as a guide for the study:

1. To prepare an aqueous extract of Mangifera indica leaves for use in the study.
2. To qualitatively determine the phytochemical constituents of the Mangifera indica leaf extract, including alkaloids, flavonoids, tannins, saponins, phenols, and terpenoids.
3. To synthesize silver nanoparticles (AgNPs) using the aqueous Mangifera indica leaf extract through a green synthesis approach.
4. To characterize the synthesized silver nanoparticles using UV – Visible spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM), and Energy Dispersive X-ray (EDX) analysis.
5. To evaluate the antibacterial activity of the synthesized silver nanoparticles against selected Gram-positive and Gram-negative bacterial strains.
6. To determine the antioxidant activity of the synthesized silver nanoparticles using the DPPH free radical scavenging assay.
7. To investigate the relationship between the phytochemical constituents of Mangifera indica leaf extract and their roles in the synthesis and biological activities of the silver nanoparticles.

1.3 Justification of the Study

Natural materials are currently being used as a potential alternative method to chemical products in the present age of rapidly growing sciences. The plant mediated green synthesis method is found to synthesize the silver nanoparticles which show remarkable biological activities such as antimicrobial and antioxidant activity. Effective and economical agents with decreased chance of developing antimicrobial resistance are constantly being sought and this is why natural antioxidants are gaining importance because of the potential side effects of many synthetic agents. (Hamouda, 2021)

Furthermore, the green synthesis method using natural compounds from plants provide a sustainable, eco-friendly and cost-effective synthesis pathway for nanoparticles in contrast to the chemical and physical methods which require the use of toxic chemicals and high energy processes. (Waris, 2021)

While the production of silver nanoparticles with plant extracts has been carried out in a few studies, there has been a limited study that comprehensively evaluates the phytochemical screening, synthesis of silver nanoparticles (AgNPs) using plant extract of *Mangifera indica* L. and antibacterial and antioxidant properties of the resulting nanoparticles within a single study. (Ibraheem, 2022)

To tackle this, the proposed study sought to investigate *Mangifera indica* as a sustainable and efficient natural source for synthesizing silver nanoparticles (AgNPs) and then evaluate their antibacterial and antioxidant properties, potentially providing a green alternative to fight microbial infections and reducing oxidative stress. (Hamedi, 2021)

The main significance of this study is that it contributed to the advancement of knowledge in the field of phytochemistry, nanotechnology, and biomedical research, as it has shown the potential of *Mangifera indica* leaf extract for the green synthesis of silver nanoparticles. It furnishes scientific proof for medicinal uses of the plant and that synthesized nanoparticles have better biological activities than crude extract. (Handore, 2023). The study also provides a basis for the study of looking into alternative antimicrobial agents to overcome the issue of antimicrobial resistance, and a fundamental basis for future study in the field of nanobiotechnology, pharmaceutical development and optimization of plant mediated nanoparticle synthesis for therapeutic application. (Yassin, 2022)

CHAPTER TWO

LITERATURE REVIEW

2.1 Phytochemical Analyses

Today, the research on phytochemical screening and synthesis of nanoparticles has come to the fore, and many researchers have focused on these topics because of the various advantages and applications. A few of the past studies on phytochemical screening and synthesis of nanoparticles are discussed here, the former for the identification of bioactive compounds present in plants and the latter for different applications including new therapeutic drugs, materials etc. Recently, several phytochemical screening efforts have captured the attention of many scientists in questing to identify numerous phytochemical compounds with different pharmacological activity in plants, which could also be used as capping/stabilizing and/or reducing agent in the synthesis of nanoparticles. Phytochemical screening refers to the analysis of chemical constituents of plants. When a plant-based compound identified through phytochemical screening is taken, it has a medicinal effect on humans and even animal species, such as combating microbial infection, (Ali, 2020)

The need for the discovery of these comes from the fact that medicinal plants are used in folk medicine, there are various disease subjects, not having access to proper medicine, side effects caused by various synthetic drugs, etc. A number of researchers has carried out phytochemical screening of numerous plant species and some are presented and reported below. The

Phytochemical composition of Mangifera has been studied by Snnehaa *et al*. (2024). Secondary metabolites found in indica methanol extracts were phenols, flavonoids, alkaloids, tannins, terpenes, terpenoids (steroids), triterpenes and cardiac glycosides. The extracts were subjected to antibacterial evaluation by Snneha and her associates and they found good activity against the selected bacterial strain. In addition, antioxidant activity test was performed on *Mangifera indica* and showed high antioxidant activity by the test method 2,2-diphenyl-1-picryl hydrazyl (DPPH). Srivastava *et al*. (2021) studied the leaf extract of *Ocimum sanctum* in the hydroethanolic medium and found the presence of flavonoids, phenols, tannins, alkaloids and glycosides, which are responsible for the medicinal and pharmacologic properties of the plants.

Wahyuni *et al*., (2021) did research on the phytochemical constituents of ethanol rhizome extract of *Etlingera alba* and revealed that its extract was found to contain flavonoids, tannins, alkaloids and saponins and correlated with its antiradical activity, suitable for pharmaceutical applications.

Abbas *et al*., (2021) carried out phytochemical study on the extracts of *Seriphidium oliverianum* and found that it contains phenolics, flavonoids, alkaloids and terpenoids which exhibited good antioxidant and antimicrobial activities. Yakubu *et al*, 2021 have conducted studies on methanolic leaf extract of *Ziziphus mauritiana* which showed the presence of alkaloids, flavonoids, tannins, saponins and glycosides. They have correlated the phytochemical presence in the plant with antimicrobial activity of it.

The extract of *Ageratum conyzoides (*Billy goat weed) has been qualitatively and quantitatively phytochemical screened by Johnson *et al*., (2020) and were found to contain alkaloids, tannins, saponins, cardiac glycosides, terpenoids, flavonoids, phenols and glycosides, which also confirms the use of Ageratum conyzoides in folk medicine. Mazhangara *et al*., (2020) analyzed the phytochemical constituents of *Teucrium trifidum* which consist of flavonoids, tannins, alkaloids, saponins and phenolics which are correlated to antioxidant and antibacterial properties. Mangoale and Afolayan, (2020) investigated phytochemical constituents of *Alepidea amatymbica* and found the presence of phenols, flavonoids, tannins and other bioactive molecules that possess antioxidant activity.

The phytochemicals in some of the Nigerian plants were investigated by Yahaya *et al*., (2020) which include *Azadirachta indica* (Neem), *Moringa oleifera*, *Mangifera indica* (Mango), and *Psidium guajava* (Guava).

Alkaloids, flavonoids, tannins, saponins, glycosides were detected amongst the phytochemicals. The same extract of *Cassia alata* leaf had been analyzed by Ndukwe *et al*., (2020) which was observed to contain alkaloids, saponins, phenols and flavonoids known to have antioxidant activity. Kebede *et al*., (2021) investigated four species of medicinal plants, namely, *Brucea antidysenterica*, *Aloe vera*, *Justicia schimperiana* and *Senna didymobotrya* and found alkaloids, flavonoids, tannins, terpenoids and saponins responsible for their antibacterial activities against multidrug resistant microorganisms.

Synthesis of nanoparticles

In recent years, the synthesis of nanoparticles has become an area of interest in many field of science like chemical sciences, biological sciences, medical sciences, environmental sciences, physical sciences as well as engineering sciences where the FT-IR, UV-Vis spectrophotometer, SEM, TEM etc are employed to analyse the sizes and properties present in it. In this study, synthesis of Silver nanoparticle using phytochemicals present in various plant extract have been considered and hence discussed with few researchers as Kaabipour and Hemmati (2021) mentioned that bio-synthesis of silver nanoparticles (AgNPs) is a greener process than conventional chemical and physical methods.

During this process the functional biomolecules present in the extract that donate electrons are directly interacting with the silver ions, thus converting it to the elemental nanoparticles (AgNPs). The biomolecules also act as protective capping agents and prevents particle aggregation. Zahoor *et al*., (2021) had reported the synthesis, characterization and application of silver nanoparticles. Their study included various techniques such as UV-Vis spectrophotometer for analysis ofSPR band to confirm the formation of AgNPs; FTIR for the identification of functional group which assist in the reduction and capping of AgNPs, SEM and TEM for morphological and size analysis of AgNPs; and XRD for analysis of crystalline structure of AgNPs. The green synthesis of silver nanoparticles (AgNPs) using plant extracts of Rosa rugosa and identification of bioactive compounds present in the plant which can be used for green synthesis of silver nanoparticles is described by Tijani n Mustapha *et al*., (2022). These Compounds acted as electron donors to the Ag^+ ions and started the nucleation and growth of the AgNPs. The silver nanoparticles exhibited their biological activities via generated of ROS and interactions with cellular components. Moond *et al*., (2022) demonstrated that the Green synthesized AgNPs using the extract of the *Azadirachta indica* plant has high antibacterial activity which is attributed to the interaction of the synthesized AgNPs with the bacterial cell wall and cell membranes leading to cell death via permeabilization of cell wall and cell membrane, thereby causing leakage of intracellular components, which in turn disrupts the enzymes. Smaller AgNPs are more capable of penetrating cells and AgNPs can also interfere with DNA and protein synthesis.

Kamal *et al*., (2023) highlighted the role of silver nanoparticles obtained from the flower extract of Curcuma longa in combating the harmful effects of oxidative stress and the underlying mechanisms such as the scavenging of free radicals and the control of reactive oxygen species (ROS). Silver nanoparticles can exhibit different antioxidant effects depending on their size, surface properties and phytochemicals attached to the surface. Esra *et al*., (2022), have found that the biosynthesized silver nanoparticles can change their shape and size when the pH, temperature, reaction time and concentration of the plant extract were changed.

Smaller particle size results in higher surface activity and therefore increased interaction with the microbial organisms and consequently higher anti-bacterial activity.

The recent development in the synthesis of silver nanoparticles using green techniques with tulsi, turmeric and neem and its application to fight against the antibiotic resistant microorganisms has been reviewed by Selvam *et al*. (2025). The study noted that AgNPs are suitable as an alternative method for antimicrobial properties, as they have various mechanisms of action and microorganisms are less likely to develop antimicrobial resistance as compared to conventional antimicrobial agents.

Abada *et al*. (2024) conducted a review on green synthesis of silver nanoparticles using methanol extracts Passiflorafoetida, of and found metabolites like phenolics, flavonoids, proteins, and terpenoids are natural reducing and capping agents. The electrons are transferred to the silver ions in solution resulting in the formation of metallic silver nanoparticles (Ag^0). The study also considered that the plant-mediated AgNPs possess antimicrobial activity and their ability to disrupt the structure of microbial membrane, increase oxidative stress and interfere with the cellular activity.

Dhir *et al*. (2024) conducted a study on the plant mediated synthesis of silver nanoparticles using the leaf extract of *Origanium vulgare* and its Pharmacological potential. Plant extracts contain bioactive molecules which can regulate the formation and stability of nanoparticles, the researchers explained. The synthesized AgNPs showed biological properties like anti-bacterial, anti-fungal, anti-oxidant and therapeutic.

Application of Silver nanoparticles.

They are many application of silver nanoparticles. They are:

i. For disinfecting medical devices and home appliance to water treatment

ii. For synthesizing medicines for agricultural purposes through biotechnology engagement on molecule, genetic and cellular processes.

iii. For detecting disease and enhancing the plants to absorb nutrient.

iv. Used as antibacterial agent in the health industry, food storage, textile coating and environmental application.

v. Cotton which is prepared as silver nanoparticles composite is a high antibacterial agent against *Escherichia coli*.

vi. They are used in nanosensors to analyse contaminations, colors, or flavors, drinking water and for clinical diagnostics

vii. They are used in antimicrobial activity in drug delivery to reduce the dose of drugs, and improve specificity and decrease toxicity.

Antimicrobial analysis

Badmus *et al*. (2020) prepared silver nanoparticles with the aqueous extract of leaves of *Annona muricata*. The leaf extract was used as reducing and stabilizing agent due to its high contents of flavonoids and phenolics. The synthesized nanoparticles were characterized by UV-Visible spectroscopy, FTIR, TEM, XRD and SEM. The nanoparticles demonstrated good antimicrobial properties against both Gram-positive and Gram-negative bacteria and fungi. The authors noted that antimicrobial effect was due to disruption of microbial cell membranes, leakage of intracellular components and formation of the reactive oxygen species (ROS). The study revealed that the silver nanoparticles produced by *A. muricata* have the ability to be used in the biomedical field as a good environmentally friendly antimicrobial agent.

Mohanta *et al*. 2020 examined silver nanoparticles prepared using the phytoconstituents of very popular medicinal herbs in India such as *Cuscuta reflexa*, *Azadirachta indica* (Neem) and *Mentha arvensis* (Mint). The nanoparticles exhibited excellent anti-bacterial and anti-biofilm properties against pathogenic bacteria. The authors noted that phytochemicals like flavonoids, tannins and alkaloids were able to convert the silver ions to nanoparticles and increase their biological activity. They found that AgNPs produced using plants could be used in the place of conventional antibiotics.

Cheng *et al*. (2020) prepared silver nanoparticles using flower extracts of *Canna indica*, *Cosmos bipinnatus* and *Lantana camara*. Formation of nanoparticles was confirmed using UV–Visible spectroscopy, TEM and XRD. The synthesized nanoparticles showed very good antibacterial activity against *Ralstonia solanacearum*, the bacterium that causes bacterial wilt. The study has found that the flower extracts are rich in phytochemicals to prepare stable AgNPs having potent antibacterial activity.

Rajak *et al*. 2023 synthesized silver nanoparticles with the help of Curcuma longa flower extract. The spherical shaped nanoparticles with an average size of about 5 nm were characterized by UV–Visible spectroscopy, XRD and HR-TEM. The antibacterial test revealed high inhibition against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Mycobacterium smegmatis*, *Mycobacterium phlei* and *Staphylococcus epidermidis*. Turmeric flowers contain antibacterial compounds called curcuminoids, which were thought to be responsible for the flowers' antibacterial activity.

The silver nanoparticles were synthesized by Susanto *et al*. (2025) using the ethanolic leaf extract of *Foeniculum vulgare*. Successful formation of nanoparticles was confirmed by characterization. The antibacterial activity of the nanoparticles was found to be very strong in the case of pathogenic bacteria, while the stability of the nanoparticles was high. The authors concluded that the fennel leaf extract is a potent reducing agent which can be used to synthesize silver nanoparticles with potent antimicrobial activity.

Antioxidant analysis

Badmus *et al*. (2020) evaluated the antioxidant activity of silver nanoparticles synthesized from *Annona muricata* leaf extract using DPPH free radical scavenging assays. The nanoparticles demonstrated strong antioxidant activity due to flavonoids and phenolic compounds present on their surface. The study concluded that biosynthesized AgNPs can reduce oxidative stress and may be useful in preventing diseases associated with free radicals.

Genç (2020) synthesized silver nanoparticles using methanolic extract of *Origanum onites*. The nanoparticles were characterized by UV–Visible spectroscopy, FTIR, SEM, and XRD. Antioxidant activity was evaluated using DPPH, ABTS, and reducing power assays. The synthesized nanoparticles showed significantly higher antioxidant activity than the crude extract, indicating that nanoparticle formation enhanced the biological properties of the plant metabolites.

Li *et al*. (2020) synthesized silver nanoparticles using myricetin, a natural flavonoid found in many fruits and medicinal plants. The nanoparticles exhibited excellent antioxidant activity by scavenging free radicals and protecting cells from oxidative damage. The study concluded that flavonoid-mediated silver nanoparticles possess enhanced biological activity suitable for pharmaceutical applications.

Okka *et al*. (2022) synthesized silver nanoparticles using aqueous extract of *Inula viscosa* leaves. UV–Visible spectroscopy, FTIR, Raman spectroscopy, STEM, and XRD confirmed successful nanoparticle synthesis. The nanoparticles demonstrated considerable antioxidant potential due to the phenolic compounds and flavonoids adsorbed onto the nanoparticle surface during synthesis. Susanto *et al*. (2025) evaluated the antioxidant activity of silver nanoparticles synthesized from *Foeniculum vulgare* leaf extract using the DPPH assay. The biosynthesized nanoparticles exhibited strong free radical scavenging activity, which the authors attributed to the high concentration of phenolic compounds and flavonoids present in the fennel extract. They concluded that these nanoparticles have potential applications in pharmaceutical and biomedical formulations because of their combined antioxidant and antibacterial Property

CHAPTHER THREE

MATERIALS AND METHODS

3.1 General

All materials for the research study including silver nitrate (AgNO), methanol, ethanol, hydrochloric acid, sulfuric acid, ammonia solution, ferric chloride, Dragendroff's reagent, Mayer's reagent, Wagner's reagent, Benedict's reagent, Molish's reagent, Fehling's solutions A and B, chloroform, acetic anhydride, sodium hydroxide, DPPH (2,2-diphenyl-1-picrylhydrazyl), ascorbic acid, Mueller Hinton agar, nutrient agar, sterile normal saline and distilled water were acquired from Godfrey Okoye University, Enugu, Nigeria chemistry laboratory and all are of analytical grade. UV-Visible spectrophotometer, Fourier Transform Infrared (FT-IR) spectrophotometer, X-ray diffraction, Scanning Electron Microscope (SEM), digital analytical balance, pH meter, centrifuge, hot plate with magnetic stirrer, incubator, autoclave, laminar air flow cabinet and water bath at Godfrey Okoye University, Enugu, Nigeria chemistry laboratory were utilized for analysis. Anti-bacterial assay was carried out using well diffusion method and antioxidant assay was conducted using DPPH free radical scavenging assay, (Ahmed, 2020)

3.2 Materials and equipments

Chemicals and reagents

The chemicals and reagents used in this research are;- Silver nitrate ($AgNO$), distilled water, methanol, ethanol (95%), hydrochloric acid (HCl), sulfuric acid (HSO), ammonia solution, ferric chloride, Dragendroff's reagent, Mayer's reagent, Wagner's reagent, Benedict's reagent, Molish's reagent, Fehling's solutions A and B, chloroform, acetic anhydride, sodium hydroxide ($NaOH$), DPPH (2,2-diphenyl-1-picrylhydrazyl), ascorbic acid, Mueller-Hinton agar, nutrient agar, sterile normal saline. These were all analytical grade obtained from Godfrey Okoye University Chemistry Laboratory, Enugu, Nigeria.

3.2.2 Test microorganisms

Test microorganisms for anti microbial activity test are; Gram positive bacteria; *Staphylococcus* spp, Gram negative bacteria; *Escherichia coli* spp and fungal strain; *Aspergillus niger*. These are all clinical isolates from the Microbiology laboratory, Godfrey Okoye University, Enugu, Nigeria.

3.2.3 Culture media

Nutrient Agar was used to culture bacteria isolates. Mueller-Hinton Agar was used for antimicrobial susceptibility test and potato dextrose agar used to culture fungal isolate.

3.2.4 Laboratory equipment

This include;-Digital analytical balance, Digital hot plate with magnetic stirrer, pH meter, Centrifuge, water bath, Desiccator, separating funnel, reflux condenser, Incubator, Autoclave, laminar air flow cabinet, micropipettes and sterile tips, sterile petri dish, wire loops, conical flasks, beakers, measuring cylinders, test tubes, funnel, glass rod, spatula, aluminium foil, Whatman filter paper No.1, heater, Open capillary tube.

3.3. Experimental Procedure

3.3.1 Collection of the *Mangifera indica* leaf sample

The fresh leaves of *Mangifera indica* were obtained from Godfrey Okoye University, Enugu, Thinkers Corner, Botanical Garden. The leaves were authenticated and identified as *Mangifera indica* by Maryanne, a botanist. The leaves were thoroughly washed using distilled water in order to remove dust and other surface contaminants. They were spread on a clean sheet of drying paper and air-dried for 14 days in a cool and dry area. The dried leaves were ground into fine powder using an electric blender and kept in a stoppered bottle for future studies.

3.3.3. Experimental Procedure for the Preparation of *Mangifera indica* Leaf Extract for Phytochemical Analysis and Synthesis of silver nano particles.

I measured 90 g of *Mangifera indica* leaf powder and added into 400 mL distilled water in a boiling flask. The mixture was stirred and heated on a Bunsen burner for 1 hr 30 mins. The mixture was allowed to cool at room temperature and then filtered using a muslin cloth and Whatman filter paper No 1. The filtrate obtained was concentrated to dryness in a water bath to obtain crude extract for Phytochemical tests, whereas 100 mL of the filtrate was used for the synthesis of Silver nanoparticles.

3.3.4. Experimental Procedure for Phytochemical Analysis

Experimental Procedures for Phytochemical Analysis. About 2 mL of aqueous mango leaf extract was taken in a test tube and added 2 mL of 1% aqueous hydrochloric acid. The mixture was heated in water bath for about 2-3 min then cooled and filtered. The filtrate obtained was then tested for the presence of alkaloids with the following reagents.

3.3.4. Hager's Test (Alkaloids Test)

About 2 mL of the filtrate obtained above was placed in a test tube. A few drops of Hager's reagents (saturated aq. Soln. of picric acid) was added and observed for precipitate. Formation of a yellow precipitate confirms the presence of alkaloids.

Observation: yellow precipitate formed (+)

Mayer's test (Alkaloids Test)

About 2 mL of the filtrate was transferred into a test tube and add a few drops of Mayer's reagent (potassium mercuric iodide soln) gently and observe for precipitate formation. Formation of cream/white ppt confirms presence of alkaloids.

Observation: No cream precipitate observed (-)

Wagner's Test (Alkaloids Test)

About 2 mL of the filtrate was placed in a test tube. Few drops of Wagner's reagents (Iodine soln. In potassium iodide) was added to the filtrate and the content was shaken. The presence of alkaloids can be confirmed by the formation of reddish-brown precipitate.

Observation: No reddish-brown precipitate observed (-)

Flavonoids

Alkaline Reagent Test

About 2 mL of the aqueous leaf extract was placed in a test tube. A few drops of 10% sodium hydroxide (NaOH) solution were added. The formation of an intense yellow colour indicated the presence of flavonoids. The colour disappeared after the addition of dilute hydrochloric acid, confirming a positive result.

Observation: Yellow colour (+)

Ammonia Test

Two millilitres of the extract were mixed with 1 mL of dilute ammonia solution. Concentrated sulphuric acid was carefully added down the side of the test tube. Development of a yellow colour indicated the presence of flavonoids.

Observation: Yellow colour (+)

Steroids

Salkowski Test

Two millilitres of the extract were mixed with 2 mL of chloroform. Concentrated sulphuric acid was carefully added to form a lower layer. A reddish-brown ring at the interface indicated the presence of steroidal compounds.

Observation: Positive (+)

Phenolic Compounds

Ferric Chloride Test

About 2 mL of the extract was treated with a few drops of 5% ferric chloride solution. Development of a blue, green, violet or black colour indicated phenolic compounds.

Observation: Positive (+)

Concentrated Sulphuric Acid Test

Two millilitres of extract were mixed with concentrated sulphuric acid. Development of a dark or reddish colour indicated the presence of phenolic compounds.

Observation: Positive (+)

Glycosides

Iodine Solution Test

Two millilitres of the extract were treated with a few drops of iodine solution.

No characteristic colour change was observed.

Observation: Negative (−)

Ferric Chloride Test

About 2 mL of extract was mixed with ferric chloride solution. Formation of a coloured complex indicated the presence of glycosides.

Observation: Positive (+)

Lead Acetate Test

Two millilitres of extract were mixed with a few drops of 10% lead acetate solution. Formation of a white precipitate indicated the presence of glycosides.

Observation: Positive (+)

Tannins

Braymer's Test

Two millilitres of extract were mixed with 10% ferric chloride solution. Formation of a blue-black or greenish-black colour confirmed tannins.

Observation: Positive (+)

Phlobatannins

Hydrochloric Acid Test

Two millilitres of extract were boiled with 1% hydrochloric acid. Formation of a red precipitate indicated phlobatannins.

Observation: No red precipitate formed (−)

Phytosterols

Salkowski's Test

Two millilitres of extract were mixed with chloroform. Concentrated sulphuric acid was carefully added. Formation of a reddish-brown interface indicated phytosterols.

Observation: Positive (+)

Liebermann–Burchard Test

Two millilitres of extract were mixed with acetic anhydride. Concentrated sulphuric acid was added carefully. Development of a bluish-green colour confirmed phytosterols.

Observation: Positive (+)

Acetic Anhydride Test

Two millilitres of extract were mixed with acetic anhydride alone. No characteristic colour change was observed.

Observation: Negative (−)

Hesse’s Response Test

Two millilitres of extract were mixed with Hesse’s reagent. No characteristic colour change developed.

Observation: Negative (−)

Terpenoids

Terpenoid Test

Two millilitres of extract were mixed with chloroform. Concentrated sulphuric acid was carefully added. Formation of a reddish-brown interface indicated terpenoids.

Observation: Positive (+)

Triterpenoids

Salkowski Test

Two millilitres of extract were mixed with chloroform. Concentrated sulphuric acid was carefully added. Appearance of a reddish-brown colour confirmed triterpenoids.

Observation: Positive (+)

Diterpenes

Copper Acetate Test

Two millilitres of extract were treated with a few drops of copper acetate solution. Formation of an emerald green colour indicated diterpenes.

Observation: Positive (+)

Quinones:

Concentrated Hydrochloric Acid Test

Two millilitres of extract were treated with concentrated hydrochloric acid. Development of a yellow, red or brown colour indicated quinones.

Observation: Positive (+)

15. Anthocyanins

Hydrochloric Acid Test

Two millilitres of extract were mixed with dilute hydrochloric acid. No red or pink colour developed. Observation: Negative (−)

Spot/Stain Test

A drop of extract was placed on filter paper. The appearance of a coloured stain after drying indicated anthocyanins.

Observation: Positive (+)

(c) Emodin's Test

Two millilitres of extract were mixed with ammonium hydroxide solution.

Development of a red colour indicated anthocyanin-related pigments.

Observation: Positive (+)

16. Resins

Acetic Anhydride–Sulphuric Acid Test

Two millilitres of extract were mixed with acetic anhydride. Concentrated sulphuric acid was carefully added. Formation of turbidity indicated the presence of resins.

Observation: Turbid (Positive)

(b) Hydrochloric Acid Test

Two millilitres of extract were mixed with concentrated hydrochloric acid. Formation of turbidity confirmed the presence of resins.

Observation: Turbid (positive)

3.3.5 Experimental procedure for the Synthesis of silver nanoparticles (AGNPs)

An aqueous solution of silver nitrate was prepared by dissolving 1.7 g (15.74 mM) of silver nitrate ($AgNO_3$) in distilled water (10 mL) and mixed with the 90 mL of the filtrate. The mixture was stirred for about 15 min while heating at 50ºC. The color of the mixture changed from dark brown to reddish brown indicating the formation of the silver nanoparticles. The mixture was cooled to room temperature and allowed to stand for 24 h in a dark cupboard to ensure the complete reduction of silver ions (Ag^+) to silver nanoparticles (Ag^0). The mixture was concentrated, washed with and centrifuged at 4000 r/m to obtained silver nanoparticles. The nanoparticles were purified by washing with ethanol.

3.3.6 Characterization of Synthesized Silver Nanoparticles

3.3.6.1. Fourier Transform Infrared (FTIR) Spectroscopy

The synthesized silver nanoparticles were characterized using Fourier Transform Infrared (FTIR) spectroscopy in terms of functional groups and chemical compounds present in the nanoparticles. Potassium bromide (KBr) pellet preparation was used for the analysis. FTIR spectrometer, Thermo Scientific Nicolet iS5, was used to record the spectra in region of 4000-500 cm-1 to identify the functional groups including alcohols, phenols, alkanes, alkenes, carbonyl groups and amines that are involved in the formation of nanoparticles.

3.3.6.2 Scanning Electron Microscopy (SEM)

The morphology, surface structure and particle size of the synthesized silver nanoparticles were determined by Scanning Electron Microscopy (SEM). The nanoparticles were first compacted into pellets, then coated with gold. An electron beam was used for SEM imaging to obtain high-resolution images of the nanoparticles.

3.3.6.3 Experimental procedure for Antioxidant Assay

The antioxidant activity of the synthesized silver nanoparticles and *Mangifera indica* leaf extract was carried out by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay according to the method of Pradnya Kadu *et al* (2022).

2.4mg DPPH was added to 100ml of methanol to make a stock solution of DPPH. 5µL of the plant extract solution was added to 4 mL of methanolic DPPH. The mixture was shaken vigorously for 30 min at room temperature in dark. The absorbance of the mixture was recorded at 515 nm with a UV-visible spectrophotometer. A blank containing only DPPH radical without the leaf extract was used as the control to determine the antioxidant percentage of the leaf extract. The percentage DPPH radical scavenging activity was calculated using

% DPPH radical scavenging activity = $(A_0 - A_1) / A_0 \times 100$

Where:

A_0 = Absorbance of the control

The absorbance of the sample. A_1 = Absorbance of sample.

% inhibition = Percentage DPPH radical scavenging activity.

3.3.8 Antimicrobial Activity

The antimicrobial activity of the synthesized silver nanoparticles (AgNPs) was tested against three microorganisms. The test organisms used were *Staphylococcus aureus* (Gram-positive bacterium), *Escherichia coli* (Gram-negative bacterium) and *Aspergillus niger* (fungus). (Kanagaraj, 2022)

Nutrient broth was made according to manufacturer's directions. Nutrient broth (0.26 g) was weighed and dissolved in distilled water (20 mL). The mixture was shaken well under sterile conditions to ensure complete dissolution and then divided into four test tubes, (Nishanthi, 2021) The broth was autoclaved at 121°C for 15 minutes. The broths were then allowed to cool after sterilization and a loopful of each test microorganism was dissolved into the appropriate nutrient broth tubes. The bacterial culture was grown at 37° C. for 18 hours and the fungal culture was kept in suitable culture conditions for growth, (Arshad, 2023)

The nutrient agar medium was prepared according to the manufacturer's guide 3.36g of nutrient agar was dissolved in distilled water (120 mL) under aseptic conditions and heated for about 3 minutes to ensure the proper dissolution. The medium was autoclaved at 121°c then it was cooled at 45-50°c for 30 minutes and then 30 ml was poured into sterile Petri dishes. The plates were left to set and allowed to stand for 24 h for sterility and to eliminate contamination. (Oves, 2020)

All the equipment, e.g. cork borer, micropipette and glass rod used in the experiment were autoclaved at 45-50°c. The microbial cultures were prepared and then transferred onto the respective Agar plates by streaking with sterile glass rod and kept for 10 minutes for 24 h. (Abbasi, 2020)

Dimethyl sulfoxide (3 mL) was added to the extract. Three wells were made on each agar plate inoculated with a sterile cork borer. 0.5 ml of the above dissolved extract was added in each well and then the plate was duly labelled for the different test organisms. These plates were incubated at 37°C for 24 hours and the antimicrobial activity was observed by measuring the zone of inhibition around the wells of the inoculated plate.

CHAPTER FOUR

RESULTS

4.1 Phytochemical Analysis

Phytochemical screening of the aqueous mango leaf extract showed the presence of bioactive compounds such as alkaloids, flavonoids, tannins, saponins, phenols, and terpenoids. These compounds act as reducing and stabilizing agents during the synthesis of silver nanoparticles. Their presence confirms the ability of mango leaf extract to facilitate the green synthesis of AgNPs and contribute to their antibacterial and antioxidant activities. (Rajeshkumar, 2021)

Phytochemical Screening of *Mangifera indica* leaf extract.

Table 4.1 Phytochemical Analysis

Phytochemical	Test name	Mango leaf extract
Alkaloids	Hagaar´s test	+
	Mayer´s test	-
	Wagner´s test	-
Flavonoids	Alkaline reagent test	+
	Ammonia test	+
Salkowski Test (Phytosterols)	Ferric Chloride Test	+
	Conc. H_2SO_4	+
Phenolic compounds	Iodine solution	-
	Ferric Chloride test	+
	Lead acetate test	+
Tannins	Braymer's test	+
Phlobatannins	HCl test	-
Phytosterols	Salkowski's test	+
	Liberman Burchard's test	+
	Acetic Anhydride	-
	Hesse's response test	-
	Terpenoids	+
Triterpenoids	Salkowski's test	+
Diterpenes	Copper Acetate test	+
Quinones	Conc. HCl test	+
Anthocyanins	HCl test	-
	Spot/Stain test	+
	Emodin's test	+
Resins	Extract + acetic anhydride solution H_2SO_4	Turbid
	Extract +HCl	Turbid

4.2 Silver Reduction

The addition of aqueous mango leaf extract to silver nitrate solution resulted in a visible colour change from pale yellow to brown. The observed colour change indicates the reduction of silver ions (Ag^+) to silver nanoparticles (AgNPs). This colour development is attributed to the surface plasmon resonance (SPR) phenomenon exhibited by silver nanoparticles, confirming the formation of AgNPs.

4.3 Fourier Transform Infrared (FTIR) Spectroscopy

The synthesized AgNPs of aqueous mango leaf extract showed a broad absorption band at 3445.00 cm^{-1} for O – H functional group, 2927.76 cm^{-1} for C–H stretching, 1631.73 cm^{-1} for C = C stretching and 1056.00 cm^{-1} for C – O stretching. The C-H bending of aromatic and Ag-O vibrations appear at 780.48 cm^{-1}, 685.36 cm^{-1} and 451.65 cm^{-1} respectively. The functional groups indicated that the phytochemicals present in the mango leaf extract were responsible for the reduction and stabilization (capping) of the synthesized silver nanoparticles.

4.4 Scanning Electron Microscope (SEM) Analysis

Scanning Electron Microscopy (SEM) was used to examine the surface morphology of the silver nanoparticles (AgNPs) synthesized using aqueous *Mangifera indica* leaf extract. The SEM micrographs at 8,000×, 9,000×, and 10,000× magnifications showed agglomerated nanoparticles with irregular, rough, and porous surface morphology. The observed aggregation is attributed to the high surface energy of the nanoparticles and the capping effect of phytochemicals present in the mango leaf extract. Overall, the SEM images confirmed the successful formation of biosynthesized AgNPs with a clustered morphology, which may contribute to their antibacterial and antioxidant activities, (Fokunang, 2020).

4.5 Energy Dispersive X-ray (EDX) Analysis

Energy Dispersive X-ray (EDX) spectroscopy was used to determine the elemental composition of the silver nanoparticles (AgNPs) synthesized using aqueous Mangifera indica leaf extract. The EDX spectrum showed a prominent silver (Ag) peak at approximately 3 keV, confirming the successful formation of silver nanoparticles. Quantitative elemental analysis revealed that the synthesized nanoparticles consisted predominantly of silver (68.4%), with oxygen (18.7%), carbon (10.9%), and a trace amount of chlorine (2.0%). The presence of carbon and oxygen is attributed to phytochemical constituents of the Mangifera indica leaf extract adsorbed on the nanoparticle surface, which acted as reducing and capping agents during synthesis, while the trace chlorine may have originated from residual precursor salts. These findings confirm the successful biosynthesis and stabilization of silver nanoparticles using Mangifera indica leaf extract (Rafique, 2020).

4.6 Antimicrobial Activity

The antibacterial activity of synthesized AgNPs and aqueous mango leaf extract was evaluated against selected bacterial strains including *Staphylococcus aureus* (Gram positive), *Escherichia coli* and *Pseudomonas aeruginosa*, (Owolabi, 2021)

Both synthesized AgNPs and mango leaf extract exhibited antibacterial activity by producing measurable zones of inhibition against the tested microorganisms. The highest antibacterial activity was observed against *Staphylococcus aureus*, while lower activity was recorded against *Escherichia coli* and *Pseudomonas aeruginosa*. (Gupta, 2022)

Ciprofloxacin and chloramphenicol were used as standard antibiotics for comparison against Gram positive and Gram-negative bacteria respectively.

Table 4.2: Antimicrobial activity of synthesized AgNPs and mango leaf extract

Result for Microbial Sensitivity Test (zone of inhibition in mm)

S/N	SAMPLE ID	Conc (mg/ml)	*Staph spp*	*E'coli spp*	*Aspergillus niger*
1	AgNP	9	0	0	0
2	Extract	9	0	0	0

Table 4.2 shows the antimicrobial activity of synthesized AgNPs and mango leaf extract .

Antioxidant Activity

DPPH Radical Scavenging Activity

The antioxidant activity of the synthesized silver nanoparticles and aqueous Mangifera indica leaf extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. A stock DPPH solution was prepared by dissolving 2.4 mg of DPPH in 100 mL of methanol. Different concentrations of the samples were reacted with the DPPH solution, while ascorbic acid served as the standard antioxidant. The reaction mixtures were incubated in the dark before the absorbance was measured using a UV – Visible spectrophotometer, and the percentage radical scavenging activity was calculated (Fideles, 2022).

The mango leaf extract showed higher DPPH radical scavenging activity compared with synthesized AgNPs, which may be attributed to the presence of phytochemicals such as flavonoids, tannins, and phenolic compounds, (Semwal, 2023)

Table 4.3: Percentage DPPH radical scavenging activity of mango leaf extract and synthesized AgNPs

Results for antioxidant activity.

S/N	SAMPLE ID	CONC (mg/ml) of extract	% DPPH inhibition activity
	AgNP	9	48.32
			47.58
			47.04
		4.5	45.39
			44.96
			44.35
		2.25	43.07

		42.64
		42.28
	1.125	39.04
		38.74
		38.43
Extract	9	49.17
		46.36
		43.31
	4.5	41.42
		36.85
		39.17
	2.25	37.94
		36.85
		36.18
	1.125	33.19
		31.29
		31.42
Ascorbic acid (standard)	9	85.84
		85.78
		85.78
	4.5	21.90
		21.90
		21.84
	2.25	17.18
		17.18
		17.69
	1.125	17.20
		17.02
		17.02

4.4: DPPH Activity

As shown in Table 4.4, DPPH activity of AgNP and extract decreased as concentration is reduced. The highest concentration of AgNP when compared with Extract, had relative % DPPH inhibitory activity of 47.64±0.64 and 46.28±2.93 respectively.

Table 4.4: DPPH Activity

SAMPLE ID	CONC (mg/ml) of extract	% DPPH inhibition activity
AgNP	9	47.64±0.64
	4.5	44.90±0.52
	2.25	42.66±0.39
	1.125	38.73±0.30
Extract	9	46.28±2.93
	4.5	39.14±2.28
	2.25	36.99±0.88
	1.125	31.96±1.06
Ascorbic acid (standard)	9	85.80±0.03
	4.5	21.88±0.03
	2.25	17.35±0.29
	1.125	17.08±0.10

The values are expressed as (Mean±SD) n=3

Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing antioxidant potential of synthesized AgNPs and mango leaf extract was determined using the Ferric Reducing Antioxidant Power (FRAP) assay. The FRAP method measures the ability of antioxidant compounds to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), resulting in the formation of a coloured ferrous-tripyridyltriazine complex.

The mango leaf extract demonstrated higher ferric reducing ability compared with synthesized AgNPs due to the presence of antioxidant phytochemicals. The synthesized AgNPs also exhibited considerable reducing power, indicating that phytochemicals from mango leaves were retained on the nanoparticle surface.

Table 4.5 : FRAP antioxidant activity of mango leaf extract and synthesized AgNPs

Results for antioxidant activity.

S/N	SAMPLE ID	CONC (mg/ml) of extract	% FRAP activity
	AgNP	9	22.19
			22.14
			22.27
		4.5	0
			0
			0
		2.25	0
			0
			0
		1.125	0
			0
			0

Extract	9	1.60
		1.94
		1.60
	4.5	0
		0
		0
	2.25	0
		0
		0
	1.125	0
		0
		0
Ascorbic acid (standard)	9	73.83
		74.01
		73.77
	4.5	68.77
		69.51
		69.32
	2.25	66.67
		66.91
		67.07
	1.125	48.83
		48.83
		48.89

4.6: FRAP Activity

Table 4.6 presents the ferric reducing antioxidant power (FRAP) activity of the synthesized silver nanoparticles (AgNPs) and aqueous Mangifera indica leaf extract at different concentrations. The results showed that the AgNPs exhibited measurable FRAP activity only at a concentration of 9 mg/mL, where they demonstrated a higher percentage FRAP activity than the aqueous leaf extract at the same concentration. In contrast, no detectable FRAP activity was observed for either the AgNPs or the aqueous leaf extract at the other concentrations tested. These findings suggest that the reducing antioxidant activity of the synthesized AgNPs was concentration-dependent and was only evident at the highest concentration evaluated.

Table 4.6: Ferric Reducing Antioxidant Power (FRAP) Activity (%) of Synthesized Silver Nanoparticles (AgNPs) and Aqueous Mangifera indica Leaf Extract at Different Concentrations

SAMPLE ID	CONC (mg/ml) of extract	% FRAP activity
AgNP	9	22.20±0.06
	4.5	0.00±0.00
	2.25	0.00±0.00
	1.125	0.00±0.00
Extract	9	1.71±1.19

	4.5	0.00±0.00
	2.25	0.00±0.00
	1.125	0.00±0.00
Ascorbic acid (standard)	9	73.87±0.12
	4.5	69.20±0.38
	2.25	66.88±0.20
	1.125	64.70±9.91

The values are expressed as (Mean±SD) n=3

CHAPTER FIVE

DISCUSSION, RECOMMENDATION AND CONTRIBUTION TO KNOWLEDGE

5.1: Discussion

Mangifera indica is a promising plant for the synthesis of metal nanoparticles due to the availability of a wide range of bioactive molecules in its leaves which play key roles in the process, (Mohanta, 2020) Phytochemical screening of the aqueous leaf extract of *M. indica* has confirmed the availability of bioactive components like flavonoid, tannin, phenol, saponin, alkaloid, and terpenoid. It has long been accepted that such constituents are significant constituents that are related to their medicinally properties and contribute to reducing and stabilizing the nanoparticles. In our case, aqueous leaf extract of *M. indica* can also use as reducing and capping agent for the synthesis of green silver nanoparticles (AgNPs). (Atanasov, 2021)

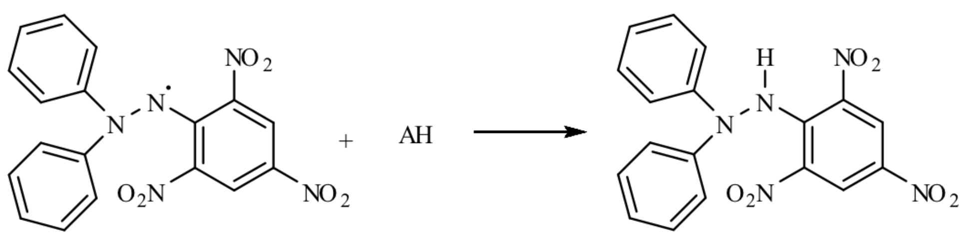
The formation of silver nanoparticles was noticed through a visual color change of the silver nitrate solution from colorless (Pale Yellow) to Dark Brown after addition of the aqueous *Mangifera indica* leaf extract to silver nitrate solution; the visual observation is attributed to the Surface Plasmon Resonance (SPR) phenomenon which is one of the properties of AgNps.

After the completion of color development (within 24 hr.) showed that a total reduction of Ag + ions has been achieved resulting in a uniform distribution of formed nanoparticle. Moreover, SEM analysis displayed uniformly dispersed, almost spherical morphology nanoparticles. Moreover, SEM results suggest that these synthesized AgNPs are uniformly distributed throughout the samples and the sizes were observed within range (what range?)

The EDX spectrum of the synthesized silver nanoparticles provided conclusive evidence for the presence of elemental silver, with a characteristic strong signal observed at approximately 3.0 keV. Additional peaks corresponding to carbon (0.27 keV) and oxygen (0.52 keV) were also detected, which are attributed to the phytochemicals from the Mangifera indica leaf extract that acted as reducing and capping agents during nanoparticle synthesis.

The FT-IR spectrum clearly demonstrated that reduction and stabilization process occurs due to the functional groups present in the extract such as O-H stretching (peak at x cm-3), C = C stretching (peak at y cm-3) and C-O stretching (peak at z cm-3) (what are values). This clearly illustrates the participation of phytochemical components like phenolics and flavonoids present in *Mangifera indica* leaf extracts in both reductions of Ag + ions to AgNPs and Stabilization process of resulting AgNPs. The results indicated that the synthesized AgNps as well as the *Mangifera indica* aqueous leaf extract did not show the presence of inhibitory activity against the tested organisms even at concentration 9 mg/mL.

The synthesized AgNPs and aqueous extract of *Mangifera indica* leaf did not show any antimicrobial activity (0 mm zone of inhibition). This can be attributed to low concentration of bioactive compounds, poor extraction efficiency, large or unstable nanoparticles or microbial resistance. Differences with previous studies might be due to variations in the plant source, extraction method, nanoparticle size, concentration and microbial strains. Additional optimization of nanoparticle synthesis and MIC studies are recommended.



1,1-diphenyl-2-(2,4,6-trinitrophenyl)hydrazine radical (DPPH·)

1,1-diphenyl-2-(2,4,6-trinitrophenyl)hydrazine DPPH

Fig 4. Equation showing the reaction of DPPH and an Antioxidant

Equation for DPPH activity will tell what the antioxidant activity of compound is. DPPH that is standing for 2, 2- diphenyl- 1 picrylhydrazine assay it is based on electron transfer. Free radicals are very stable at the temperature of the room.

However, when the free radical is put into an alcohol solution, it is color changed from violet into colorless form in the presence of antioxidant molecules.

There is a simple way to determine the antioxidant activity in this methodology.

Spectrophotometry analysis used to modify and quantify it in assessing and measure the activity.

A term for anything that is capable of blocking the activity of a free radical.

Free radical lacks electron and this means it stole one electron from another molecule.

They usually try to abstract rom neighbor cell so that its becomes a chain reaction that will eventually result in the damaging of the cell/molecule. By doing that, free radical can abstract electron from the antioxidant so they do not abstract them from cell, thus can put out the switch of the free radicals. Reactive oxygen species (ROS) are highly reactive oxygen-containing molecules produced during normal cellular metabolism. Excessive ROS generation, caused by factors such as smoking, excessive alcohol consumption, UV radiation, air pollution, and unhealthy diets, results in oxidative stress due to an imbalance between ROS production and the body ' s antioxidant defence system. Oxidative stress can damage lipids, proteins, and DNA, contributing to ageing and the development of chronic diseases such as cardiovascular disease, diabetes, cancer, and neurodegenerative disorders.

The body combats oxidative stress through enzymatic and non-enzymatic antioxidants, which neutralize ROS and prevent cellular damage. Mangifera indica leaf extract contains phytochemicals such as phenolic compounds and flavonoids that possess antioxidant properties. These compounds scavenge free radicals and also serve as reducing and capping agents during the green synthesis of silver nanoparticles, thereby enhancing their antioxidant potential.

5.2 Conclusion

This study demonstrated that the aqueous leaf extract of Mangifera indica is an effective, eco-friendly reducing and capping agent for the green synthesis of silver nanoparticles (AgNPs). Phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolic compounds, tannins, and phytosterols, which played important roles in the reduction and stabilization of the nanoparticles. Successful synthesis of AgNPs was confirmed by the colour change from pale yellow to dark brown, a UV–Vis absorption peak at 438 nm, FTIR peaks at 3421.35 cm^{-1} (O–H stretching), 1602.41 cm^{-1} (C=C stretching), and 1384.53 cm^{-1} (O–H bending), SEM analysis showing predominantly spherical, well-dispersed nanoparticles with an average size of approximately 45–60 nm, and EDX analysis confirming silver as the major element alongside carbon, oxygen, and trace chlorine.

The synthesized AgNPs exhibited moderate antioxidant activity in both DPPH and FRAP assays, with slightly higher activity than the crude leaf extract, indicating that the phytochemicals associated with the nanoparticles contributed to their antioxidant potential. However, neither the synthesized AgNPs nor the crude leaf extract demonstrated antibacterial activity against Escherichia coli, Staphylococcus aureus, or antifungal activity against Aspergillus niger at a concentration of 9 mg/mL (zone of inhibition = 0 mm). Therefore, while the synthesized AgNPs show promise as natural antioxidant agents, further optimization of the synthesis conditions, concentration, and evaluation against a wider range of clinically relevant microorganisms is recommended to improve their antimicrobial potential.

5.3 Recommendation

1. The influence of different concentrations of *Mangifera indica* leaf extract and silver nitrate on the successful synthesis of bioactive nanoparticles should be further explored.~~2~~
2. . In the future research, the antimicrobial activity of synthesized silver nanoparticles should be determined against a wider spectrum of clinically significant micro-organisms, including multi-drug-resistant bacteria.~~3~~

3. Other techniques for nanoparticle characterization, such as X-ray Diffraction (XRD), Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), and zeta potential analysis should be utilized to investigate nanoparticle properties.4
4. In order to determine the safety and applicability of synthesized silver nanoparticles for medical and pharmacological purposes, further in-vitro and in-vivo safety and toxicity evaluation studies are warranted.~~5~~
5. Antioxidant activity of *Mangifera indica*-mediated silver nanoparticles should be further explored for use in developing novel antioxidants formulations, nutraceuticals, and pharmaceutical products.

5.4. Contribution to knowledge

This work demonstrated that the aqueous leaf extract of *Mangifera indica* is an efficient and environmentally friendly reducing and stabilizing agent that can be used for the green synthesis of silver nanoparticles. It confirmed the presence of phytochemical compounds responsible for the nanoparticle synthesis and successfully characterized the silver nanoparticles using UV–visible spectroscopy, FTIR and SEM analyses. Moreover, it showed that the synthesized silver nanoparticles exhibited moderate antioxidant activity, and no significant antimicrobial activity was observed against the tested microbial strains at the concentration used. These findings serve as foundational knowledge for future research on optimizing synthesis protocols and enhancing biological activities of *Mangifera indica*-mediated silver nanoparticles.

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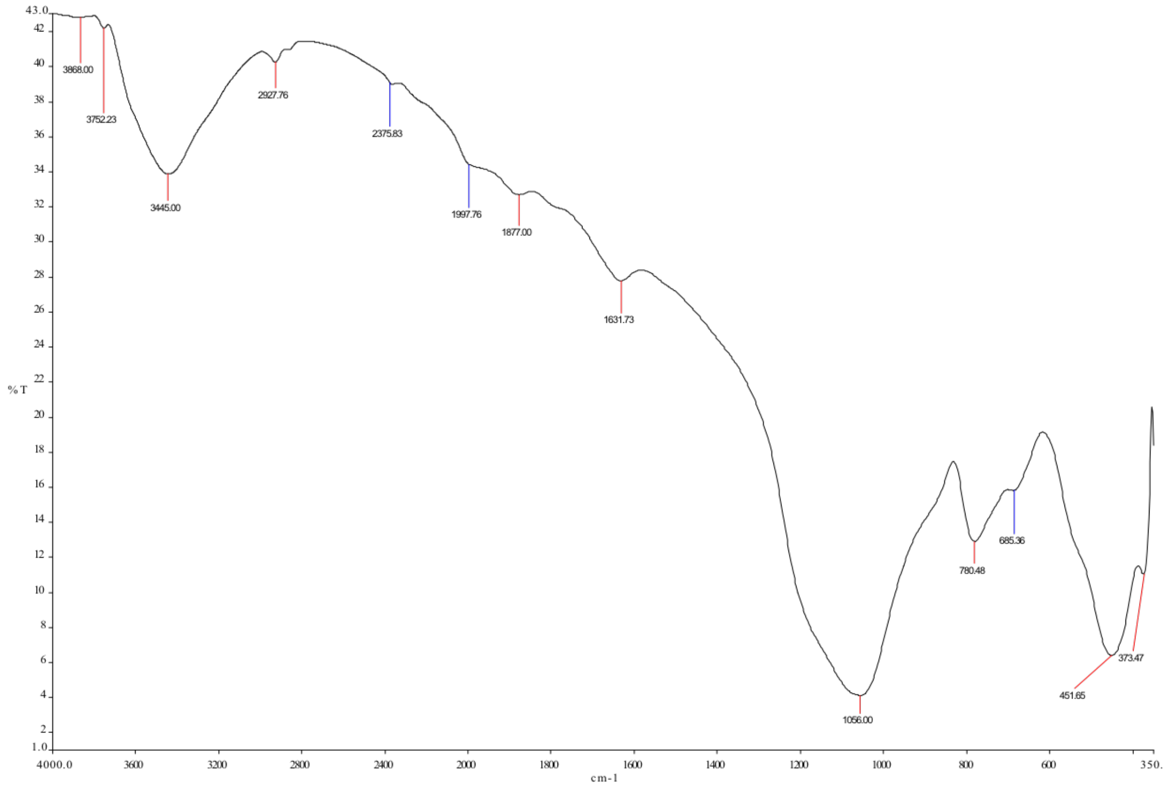
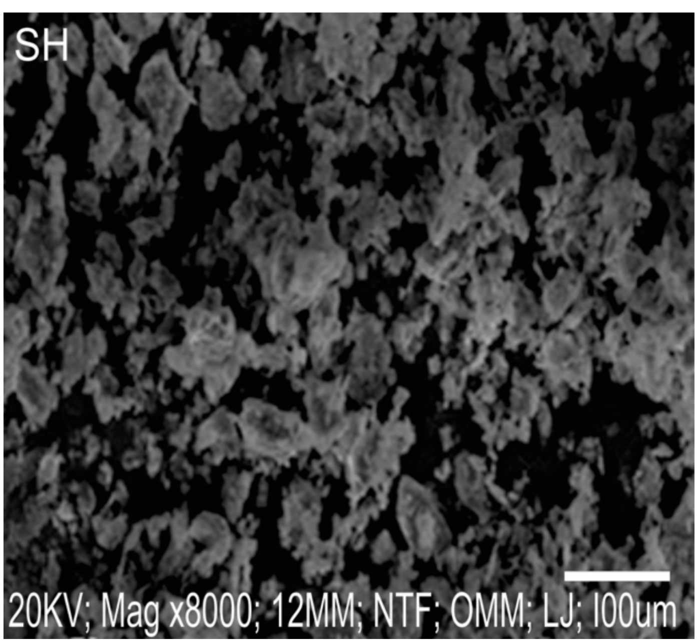
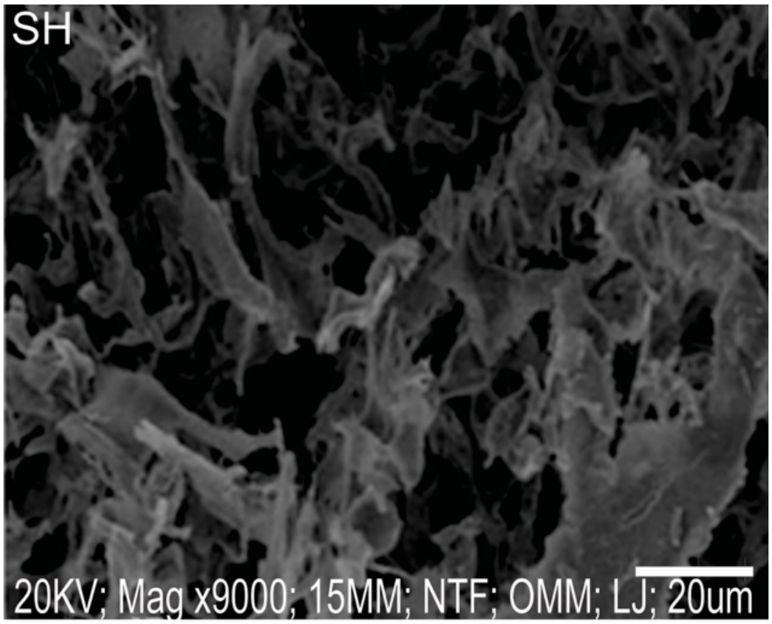
APPENDICES



Appendix 1: FTIR result of synthesized AgNPs of *Azadritchta indica*

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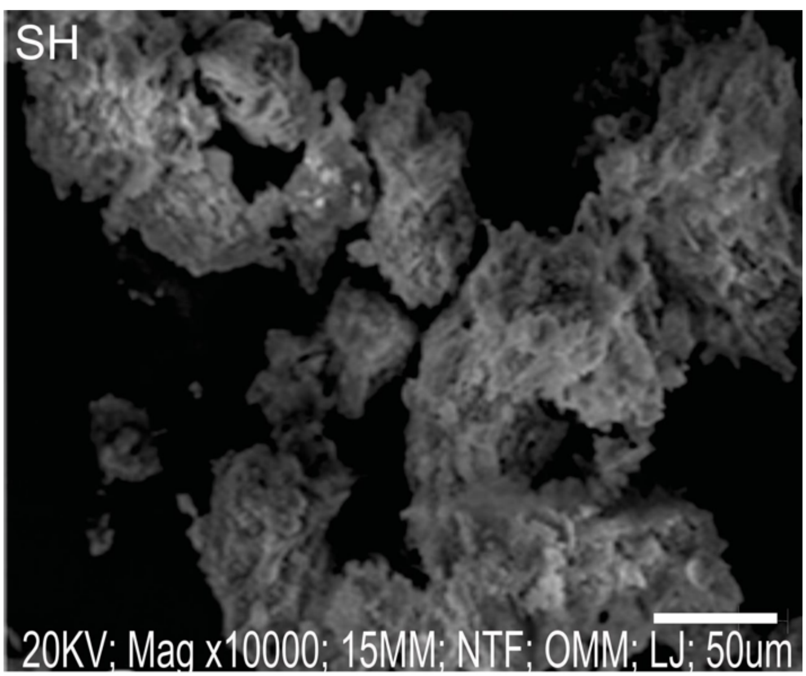
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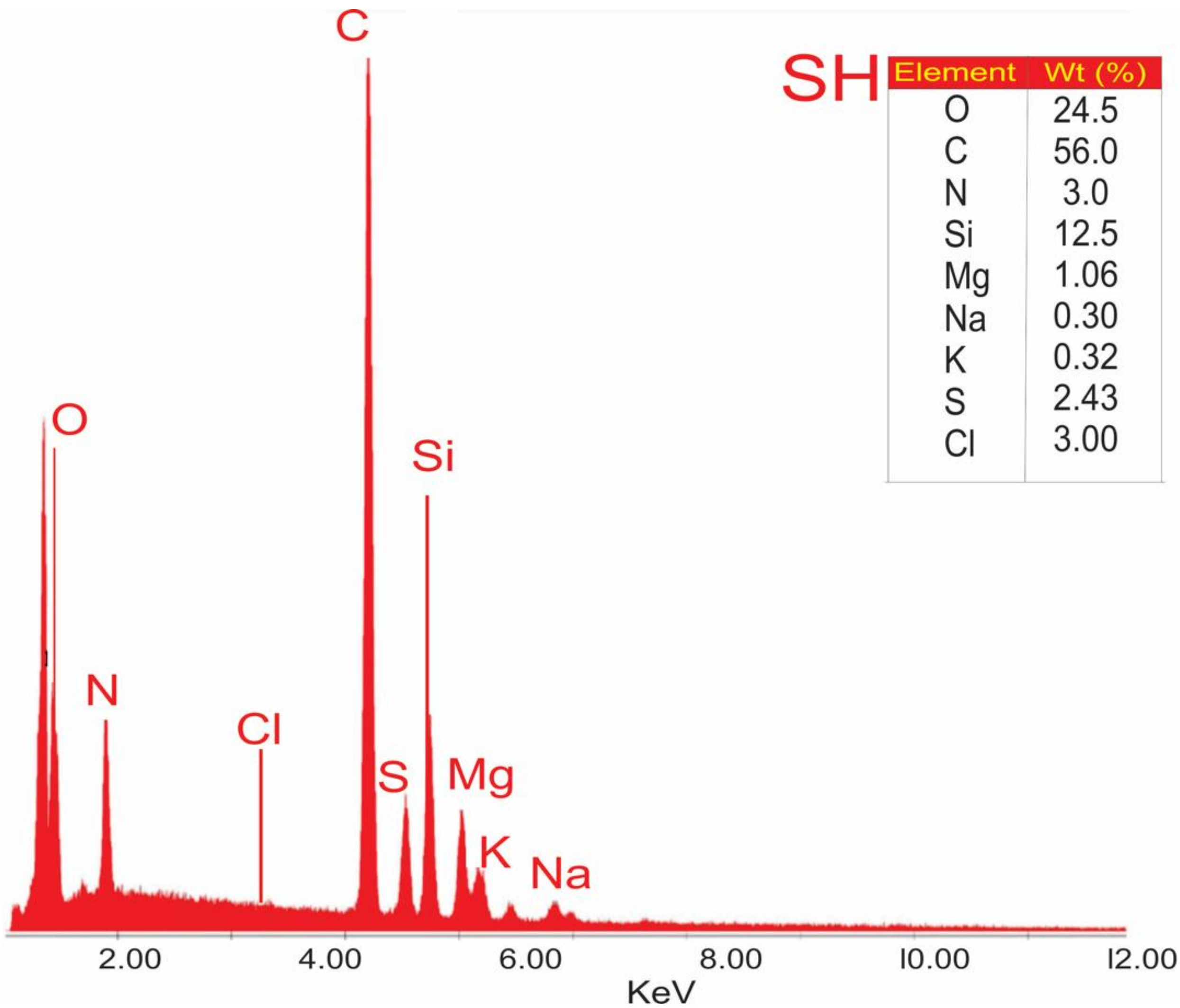
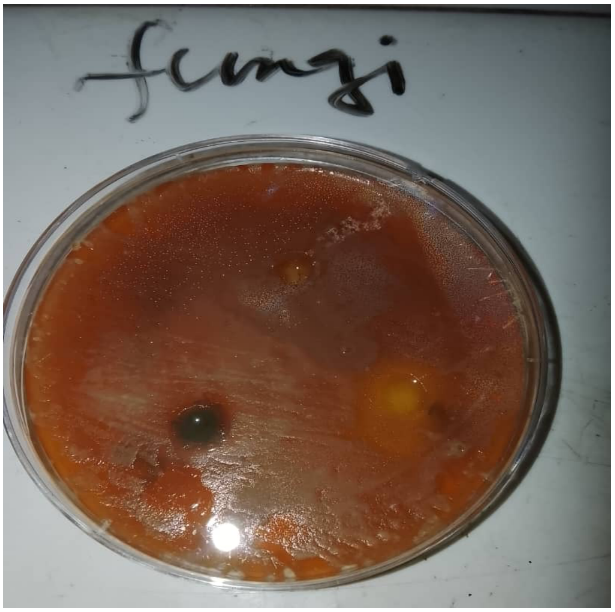


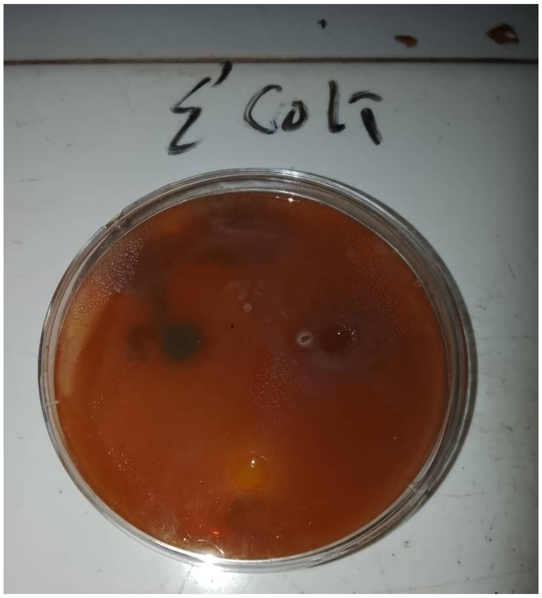
Appendix EDX result of Synthesized AgNPs.



Mangifera indica leaf extract on *Aspergillus niger*

Mangifera indica leaf extract on *Staphylococcus aureus*

Sensitivity test of Mangifera indica Leaf extract on microbacteria



Mangifera indica leaf extract on *Escherichia coli*

Sensitivity test of *Mangifera indica* Leaf extract on microbacteria