“GREEN SYNTHESIS OF SILVER NANOPARTICLES FOR ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY”

Thesis submitted in partial fulfilment of the requirements for the award

of the degree of

Masters of Science in biotechnology

Under the supervision of

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by

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DECLARATION

I hereby declare that the work presented in this report entitled **“Green Synthesis of Silver nanoparticles for antimicrobial and antioxidant activity”** in partial fulfillment of the requirements for the award of the degree of “Masters in Biotechnology” submitted in the Department of Biotechnology & Bioinformatics, “Jaypee University of Information Technology Waknaghat”, is an authentic record of my own work carried out over a period from Feb 2024 to May 2024 under the supervision of **Dr. Abhishek Chaudhary** (Assistant Professor). The matter embodied in the report has not been submitted for the award of any other degree or diploma.

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SUPERVISOR'S CERTIFICATE

This is to certify that the project work titled **"Green Synthesis of Silver nanoparticles for antimicrobial and antioxidant activity"** by **Shashank singh** during his end semester in fulfillment for the award of the degree of Master's in Biotechnology of Jaypee University of Information Technology, Solan, has been carried out under my supervision. This work has not been submitted partially to any other University or Institute for the award of any degree or appreciation.

Signature of Supervisor- ____

Name of Supervisor**- Dr. Abhishek Chaudhary**
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Abstract

Silver Nanoparticles are frequently utilised in the pharmaceutical industry as a drug delivery system or as an antimicrobial agent. This work used the Green synthesis process to create silver Nanoparticles in which plant extract of *Prunus cerasoides* acts as capping agent and reducing agent. The synthesized nanoparticles were subjected to UV-VISIBLE spectroscopy that confirmed the formation of NPs at absorption peak from 380 to 400nm and shoulder peak around 520-530nm.

Agar well diffusion method (AST) confirmed antimicrobial activity against bacterial & fungal strain, Antioxidant Assays like DPPH radical scavenging test and ABTS method tests were also performed under lab conditions to confirm desired properties in synthesized nanoformulations. Therefore, the primary goal of the current work was to synthesize a green synthesis-based nanoparticles that fights microbial attack and shows antioxidant properties which can be further studied for cancer cells inhibition with improved drug loading efficiency and bioavailability of the natural compounds.

Chapter 1

Introduction

Silver Nanoparticles are created using *Prunus cerasoides* leaves extract are very promising, dependable, safe and long lasting antibacterial and antifungal molecules against clinically significant species of bacteria and fungus that use multitarget mechanisms such as antioxidant enzyme inactivation, reactive oxygen species(ROS) mediated cellular damage and inhibition of acyl-homoserine-lactone synthase necessary for quorum sensing and biofilm formation, therefore passing all the mechanisms of bacterial antibiotic resistance & silver Nanoparticles could potentially interact with GPCRs or their downstream signalling components, leading to disruption in signal transduction & affecting fungal growth & behaviour therefore show fungal antibiotic resistance.

silver nanoformulations might thus be viewed as a promising & long-term antibacterial & antifungal therapeutic alternative for dealing with the current problem of antibiotic resistance. These nanoformulations can be used as anticancer as well as antimicrobial agents due to their anti-cancer properties & anti-microbial properties.

According to Global Cancer facts & figures 5th edition 2024 by American Cancer Society Journal, The yearly facts & figures report offers that approximately 20 million cancer cases were newly diagnosed & 9.7 million people died from the diesease worldwide. By 2050,the no. of cancer cases is predicted to increase to 35 million based solely on projected population growth. The overall cancer incidence & mortality rate per 100,000 person-years is 213 cases & 110 deaths in males & 186 cases & 77 deaths in females.

Chemotherapy is still a very effective method for treating cancer, but in frequently comes with serious risks & side effects. Scientists are looking into herbal remedies, nutrients & natural medicine marks the common primary challenges in conventional cancer treatment [3]. Nanoscale drug carriers may be able to minimize the severe adverse effects associated with specific molecular anti-cancer therapy selective absorption.

The required ligand is typically chemically conjugated to the nanocarrier's constituent components before synthesis or conjugated to nanocarriers that have already been created utilising reactive modifiers or coupling reagents [4].

A better understanding of the molecular alterations that lead to the onset & spread of Microbes is essential for its prevention & treatment. Targeting certain mechanisms allows for the prevention of spread & advancement of microbial activity despite having any negative side effects.

In addition to the anti-microbial medication that are chemically created, numerous antimicrobial compounds are being isolated from the *Prunus cerasoides* leaves which is a powerful flavonoid & has antimicrobial & anti-inflammatory properties [5] . Furthermore, biogenic AgNPs offer potential advantages in terms of biocompatibility due to the presence of biomolecules from the plant extract and represent a more sustainable and eco-friendly production method compared to traditional chemical synthesis. However, translating this potential into clinical reality necessitates addressing challenges related to safety, standardization of production methods, and development of effective delivery systems. Exploring the diverse applications of these nanoformulations in cancer therapy, wound healing, and biosensor development holds immense promise for the future of healthcare. silver nanoformulations synthesized using *Prunus cerasoides* leaves represent a revolutionary approach to combating antimicrobial resistance. Their potent activity, unique mechanisms of action, and potential for targeted delivery make them a promising class of therapeutic agents. Addressing the challenges of safety, standardization, and delivery systems will be crucial in realizing their full potential. Additionally, exploring their diverse applications in cancer therapy, wound healing, and biosensor and antioxidant activity development holds immense promise for the future of healthcare.

This study will concentrate solely on the antimicrobial and antioxidant use,of silver nanoparticles which have been made by green synthesis using *Prunus cerasoides* leave extract.

CHAPTER 2

REVIEW OF LITERATURE

Brief description of silver nanoparticles

Silver nanoparticles are tiny particles of silver that range in size from 1 to 100 nanometers. These nanoparticles have unique properties that make them incredibly valuable for various applications, including medicine, electronics, and even clothing. Due to their small size and large surface area to volume ratio, silver nanoparticles exhibit exceptional antibacterial, antiviral, and antifungal properties. This makes them highly effective in the fight against pathogens and microbes. In the field of medicine, silver nanoparticles are used in wound dressings, medical coatings, and as drug delivery systems [6]. Their antibacterial qualities help prevent infection and aid in the healing process. In electronics, silver nanoparticles are used in conductive inks, sensors, and as components in electronic devices. Their high conductivity makes them ideal for these applications. Another area where silver nanoparticles are making a big impact is in the textile industry. They are used in fabrics to create antimicrobial clothing that can help prevent the growth of odor-causing bacteria and fungi. This is especially useful in sportswear and healthcare settings where hygiene is crucial. Despite their many promising applications, there are some concerns about the potential toxicity of silver nanoparticles. Studies have shown that these nanoparticles can accumulate in the environment and in living organisms, raising questions about their long-term effects on human health and the ecosystem. Researchers are actively investigating ways to mitigate these risks and ensure the safe use of silver nanoparticles [7]. In conclusion, silver nanoparticles are a fascinating and versatile material with a wide range of applications across various industries. Their unique properties make them highly valuable, but it is essential to continue studying their potential impacts on health and the environment to ensure their safe use.

Silver nanoparticles made by *Prunus cerasoides* leaf extract with quercetin-3-rhamnoglucoside present on the surface of the nanoparticles have been a topic of interest in the scientific community due to their potential benefits. These nanoparticles have shown promising results in various fields such as medicine, agriculture, and environmental remediation [8].

The presence of quercetin-3-rhamnoglucoside on the surface of silver nanoparticles enhances their antimicrobial properties. Quercetin is a flavonoid known for its antioxidant and anti-inflammatory effects, while aminoglycoside is a glycoside that can enhance the stability and bioavailability of the nanoparticles. When combined, these compounds create a potent antimicrobial agent that can be used to combat a wide range of pathogens [9].

In the field of medicine, silver nanoparticles with quercetin-3-rhamnoglucoside have shown potential in the treatment of various infections. The nanoparticles can effectively kill bacteria, viruses, and fungi, making them ideal for use in wound dressings, medical implants, and drug delivery systems. Additionally, the presence of quercetin-3-rhamnoglucoside can help reduce inflammation and promote tissue regeneration, further enhancing the healing process.

In agriculture, silver nanoparticles made from *Prunus cerasoides* leaf extract have been shown to have pesticidal properties. The nanoparticles can be used to control pests and diseases in crops, reducing the need for harmful chemical pesticides. Furthermore, the presence of quercetin-3-rhamnoglucoside can enhance the nanoparticles' ability to protect plants from oxidative stress and promote their growth and development [10].

In environmental remediation, silver nanoparticles with quercetin-3-rhamnoglucoside can be used to remove contaminants from water and soil. The nanoparticles can effectively adsorb heavy metals, organic pollutants, and other harmful substances, making them a valuable tool for cleaning up polluted environments.

Overall, silver nanoparticles made by *Prunus cerasoides* leaves extract with quercetin-3-rhamnoglucoside present on the surface have great potential for various applications. Further research is needed to fully understand their benefits and optimize their use, but the initial results are very promising [11].

Prunus cerasoides

Wild Himalayan cherry: A Natural Wonder

<u>Plant Description</u>

The Wild Himalayan cherry, scientifically named *Prunus cerasoides*, is a medium-sized tree that can grow up to 30 metres tall. It has shiny, circular bark and long, toothed leaves. This tree is famous for its bright pink and white flowers that bloom twice each year—first in the fall and then in the spring. The oval, yellow fruits of the tree turn red when they ripen, adding to the beauty of this species.

Taxonomic classification

Kingdom : Plantae

Subkingdom : Tracheobionta (Vascular plants)

Infrakingdom : Streptophyta (Land plants)

Superdivision : Spermatophyta (Seed plants)

Division : Magnoliophyta (Flowering plants)

Subdivision : Spermatophytina (Spermatophyes)

Class : Magnoliopsida (Dicotyledons)

Subclass : Rosidae

Superorder : Rosanae

Order : Rosales

Family : Rosaceae (Rose family)

Subfamily : Amygdaloideae

Genus : Prunus

Subgenus : Cerasus

Species : *P. cerasoides*

Traditional Uses and Plant Chemistry

*Prunus cerasoides*is respected in different cultural and religious traditions in India. Known by names like padam, pajja, and pahhiya, it is considered important among Hindus in areas like Himachal Pradesh and Uttarakhand. People use the tree's leaves during Maha Shivaratri to make decorations paired with wild citrus fruits for prayer altars, and its wood plays a significant role in village weddings, symbolizing strength and happiness for newlyweds [13].

Apart from its cultural value, *Prunus cerasoides* is also appreciated for its healing abilities. It has been traditionally used to relieve conditions like back pain and sprains.

The plant's chemical study shows it has useful substances like terpenes, flavonoids, and phenols that help fight germs and reduce swelling. It also has compounds that can release cyanide when eaten, so caution is needed. These compounds are sometimes used in medicine to help breathing and digestion, but can be dangerous in large doses. The fruits and seeds of *Prunus cerasoides* usually have small amounts of these toxins, which should be safe to eat if they taste sweet or slightly bitter [14].

<u>Phytochemistry</u>

Quercetin-3-rhamnoglucoside, kaempferol were isolated from leaves whereas naringenin-5-O-α-L-rhamnopyranoside,.4´-O-methyl-liquiritigenin-7-O-α-L-rhamnopyrano-side, .naringenin 4´-methylether7-xyloside,.β-sitosterol-3-O-D-galactopyranoside from seeds. A compound characterized as 2, 4, 4'-dihydroxy-6-methoxy chalcone-4-O-[β-D-glycopyranosyl (1→4)] α-L-rhamnopyranoside was isolated from fruits [15].

<u>Quercetin</u>

Quercetin and quercitin-3-rhamnoglucoside are two powerful bioactive compounds found in *Prunus cerasoides* leaves. These compounds have attracted a lot of attention in the scientific community due to their potential health benefits.

Quercetin is a flavonoid that has been studied for its antioxidant and anti-inflammatory properties. It has been shown to have a positive impact on various aspects of health, including cardiovascular health, immune function, and even mental health. Quercetin is also known for its ability to help regulate blood sugar levels and improve overall metabolism [16].

On the other hand, quercitin-3-rhamnoglucoside is a glycoside form of quercetin, which means it is bound to a sugar molecule. This form of quercetin is believed to have a higher bioavailability compared to quercetin alone, making it potentially even more effective in providing health benefits.

Quercetin-3-rhamnoglucoside details-

Quercetin-3-rhamnoglucoside, a flavonoid compound found in various plant sources, has garnered significant attention for its potential health benefits and therapeutic properties. This part of the chapter delves into the chemical structure, properties, and natural occurrence of Quercetin-3-rhamnoglucoside, shedding light on its pharmacological activities, bioavailability, and metabolic processes within the human body. Additionally, the chapter explores the potential therapeutic applications of this compound in disease management, discusses its safety profile, and highlights current research efforts and future directions in the field. [17].

Chemical Structure and Properties of Quercetin-3-rhamnoglucoside

Structural Composition

Quercetin-3-rhamnoglucoside, also known as rutin, is a flavonoid compound composed of quercetin and the sugar rhamnose. This combination gives it unique chemical properties and biological activities.

Physical and Chemical Properties

Quercetin-3-rhamnoglucoside is a yellow crystalline powder with a bitter taste. It is soluble in water and ethanol, making it easily absorbable in the body. Its chemical structure plays a crucial role in its antioxidant and anti-inflammatory properties [18].

Dietary Sources and Natural Occurrence

Plant Sources

Quercetin-3-rhamnoglucoside is commonly found in various plants, including buckwheat, citrus fruits, onions, and tea. These natural sources make it easily accessible through a balanced diet rich in fruits and vegetables.

Distribution in Foods

Foods like apples, cherries, and broccoli contain quercetin-3-rhamnoglucoside in varying amounts. Its presence in these foods contributes to their health-promoting properties, making them valuable additions to a well-rounded diet [19].

Pharmacological Activities and Health Benefits

Antioxidant Properties

Quercetin-3-rhamnoglucoside exhibits potent antioxidant effects, scavenging free radicals and protecting cells from oxidative stress. This property contributes to its role in reducing inflammation and supporting overall health.

Anti-inflammatory Effects

Studies have shown that quercetin-3-rhamnoglucoside possesses anti-inflammatory properties, helping alleviate inflammation-related conditions such as arthritis and allergies. Its ability to modulate inflammatory pathways makes it a valuable compound in promoting wellness [20].

Bioavailability and Metabolism in the Body

Absorption and Distribution

Once consumed, quercetin-3-rhamnoglucoside is absorbed in the small intestine and distributed throughout the body via the bloodstream. Its bioavailability is influenced by factors like food matrix and individual metabolic processes.

Metabolic Pathways

In the body, quercetin-3-rhamnoglucoside undergoes enzymatic reactions that break it down into metabolites with various biological activities. Understanding its metabolic pathways is essential in harnessing its full potential for health benefits & Potential Therapeutic Applications in Disease Management [21].

Cardiovascular Health

Quercetin-3-rhamnoglucoside, Studies suggest that this compound could help in maintaining cardiovascular health by supporting healthy blood pressure levels and promoting overall heart function. So, we should add foods consisting of quercetin-3-rhamnoglucoside to our healthy diet.

Immune Modulation

keeping our immune system in good condition is very necessary , quercetin-3-rhamnoglucoside helps us to maintain our immunity. Research hints at its ability to modulate the immune response, potentially helping us fend off pesky invaders and stay in fighting form. [22]

Safety Profile and Adverse Effects

Potential Drug Interactions

While quercetin-3-rhamnoglucoside may be a good option for many health related issues and for their treatment, it's always wise to check for potential interactions with other medications.

Mixing different compounds can sometimes lead to unexpected outcomes, so it's best to consult with your healthcare provider to ensure a harmonious blend of treatments [23].

Current Research and Future Directions

<u>Ongoing Studies</u>

Ongoing studies are exploring its potential benefits in various health realms, from chronic disease management to overall well-being. Stay tuned as researchers delve deeper into the potential of this intriguing compound [24].

<u>Potential Areas for Further Exploration</u>

As we scratch the surface of quercetin-3-rhamnoglucoside's capabilities, exciting possibilities emerge for further exploration. Could it hold the key to combating other health issues? Is there a hidden potential waiting to be uncovered? The future looks bright for this compound, promising a thrilling journey of discovery ahead. In conclusion, Quercetin-3-rhamnoglucoside stands out as a fascinating compound with immense potential for improving human health. Its diverse pharmacological activities, coupled with its natural occurrence in various dietary sources, make it a subject of ongoing research and exploration. As scientists continue to unravel the mysteries surrounding this compound, its promising therapeutic applications in disease management and wellness maintenance pave the way for a brighter, healthier future. Stay tuned for more developments in the realm of Quercetin-3-rhamnoglucoside as we strive to unlock its full potential for the benefit of all [25].

Antimicrobial properties of *Prunus cerasoides*

Prunus cerasoides, the wild Himalayan cherry or sour cherry, has captured the attention of researchers due to its potential as a natural source of antimicrobial compounds. Traditional medicine practices in the Himalayas have long utilized its leaves for various ailments, hinting at their potential health benefits. This exploration delves into the scientific evidence supporting the antimicrobial activity of *Prunus cerasoides* leaves, unveiling the mechanisms at play and exploring future directions for research.

Prunus cerasoides leaves are a treasure trove of bioactive compounds, including flavonoids, terpenoids, and phenolics. These compounds are not merely bystanders; they play a crucial role in the plant's defense mechanisms against microbial invaders. Modern scientific research is unraveling the specific contributions of these compounds to the overall antimicrobial activity of the leaves.

Flavonoids: These potent antioxidants are nature's frontline defense against microbes. Studies have shown that specific flavonoids present in *Prunus cerasoides* leaves, such as quercetin and kaempferol, exhibit significant antimicrobial activity against a broad spectrum of bacteria [3]. Their mechanism of action involves disrupting bacterial cell membranes. The cell membrane acts as a barrier, controlling the flow of essential nutrients and waste products. Flavonoids can alter the structure and permeability of this membrane, causing leakage of cellular contents and ultimately cell death [26].

Terpenoids: This diverse group of naturally occurring compounds boasts a wide range of biological activities, including antimicrobial properties. Specific terpenoids identified in *Prunus cerasoides* leaves, such as limonene and α-pinene, have been shown to interfere with essential bacterial processes [5]. These processes can include protein synthesis, which is crucial for bacterial growth and replication. By disrupting these processes, terpenoids can hinder bacterial growth and survival.

Phenolics: These ubiquitous plant metabolites possess a range of biological activities, including antimicrobial effects. Phenolic compounds in *Prunus cerasoides* leaves, such as phenolic acids and tannins, can inhibit bacterial enzymes and disrupt essential cellular processes [6]. Bacterial enzymes play a vital role in various metabolic pathways. By inhibiting these enzymes, phenolics can cripple essential bacterial functions, contributing to the overall antimicrobial potential of the leaves.

In vitro studies, conducted under controlled laboratory conditions, have provided compelling evidence for the antimicrobial activity of *Prunus cerasoides* leaf extracts. These studies often employ various techniques to quantify the effectiveness of the extracts against different bacterial strains.

Agar Well Diffusion Assay: This common technique involves creating wells in an agar plate seeded with bacteria. Extracts are then added to the wells, and the plates are incubated. The presence of a clear zone of inhibition around the well indicates the extract's ability to inhibit bacterial growth [27].

Broth Microdilution Assay: This quantitative technique determines the minimum inhibitory concentration (MIC) of the extract. Different dilutions of the extract are added to wells containing bacterial cultures, and the lowest concentration that inhibits visible bacterial growth is considered the MIC [28]. These studies have demonstrated that extracts from *Prunus cerasoides* leaves exhibit activity against both Gram-positive and Gram-negative bacteria, including common pathogens like *Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa*

Understanding the mechanisms by which the bioactive compounds from *Prunus cerasoides* leaves exert their antimicrobial effects is crucial for optimizing their use and potentially overcoming limitations associated with conventional antibiotics. Several potential mechanisms are being explored:

Membrane Disruption: As mentioned earlier, flavonoids and other compounds present in the leaves might disrupt the bacterial cell membrane. This disruption can lead to leakage of essential cellular contents and ultimately cell death.

Inhibition of Enzyme Activity: Phenolic compounds can target and inhibit bacterial enzymes critical for various metabolic processes. By hindering these processes, the extracts can hinder bacterial growth and survival

Interference with Biofilm Formation: Biofilms are communities of bacteria encased in a protective matrix. These biofilms can be difficult to eradicate with conventional antibiotics. Some studies suggest that extracts from *Prunus cerasoides* leaves might interfere with biofilm formation, making bacteria more susceptible to other antimicrobial agents [11].

The potency of the antimicrobial activity of *Prunus cerasoides* leaves is not a static phenomenon. Several factors can influence the observed effects, necessitating a more nuanced understanding.

Plant Origin and Harvest Time: The geographical location, growth conditions, and even the specific time of harvest can impact the concentration and composition of bioactive compounds in the leaves. Studies suggest that leaves harvested during peak growing seasons might exhibit higher levels of antimicrobial activity due to increased production of these compounds [23]. Additionally, variations in soil composition and exposure to sunlight can also influence the chemical profile of the leaves [24].

Bacterial Strain Specificity: While research indicates broad-spectrum activity against both Gram-positive and Gram-negative bacteria, the specific susceptibility of different bacterial strains can vary. This variation could be attributed to differences in cell wall structures, enzyme profiles, and efflux pump mechanisms employed by different bacteria to expel harmful compounds [26]. Further research needs to explore this strain-specific susceptibility to identify bacterial targets most vulnerable to the antimicrobial effects of *Prunus cerasoides* leaves.

Synergistic Effects: The presence of multiple bioactive compounds within the leaves opens doors for potential synergistic effects. Research is ongoing to identify synergistic combinations of compounds within the leaves and explore their potential for developing more potent antimicrobial formulations [29].

While the research into the antimicrobial properties of *Prunus cerasoides* leaves holds immense promise, some key limitations need to be addressed:

Limited In Vivo Studies: The majority of research to date has been conducted in vitro, meaning it is performed under controlled laboratory conditions. Although these studies provide valuable insights, they may not fully translate to the complexities of a living organism, where factors like absorption, metabolism, and potential interactions with other medications can come into play. In vivo studies, conducted on live animals, are crucial for determining the efficacy and safety of these extracts in a more realistic setting [28]

Standardization and Quality Control: As research progresses towards potential applications, developing standardized extraction methods and quality control protocols becomes essential. This ensures consistency in the production of these extracts, guaranteeing a specific level of bioactive compounds and ensuring reproducible antimicrobial activity [29].

Safety Considerations: While the traditional use of *Prunus cerasoides* leaves suggests a relatively safe profile, further toxicological studies are necessary to assess potential side effects or interactions with other medications. Additionally, research on potential allergic reactions or contraindications for specific individuals is crucial for safe and responsible use [30].

Despite these limitations, the future of research into the antimicrobial properties of *Prunus cerasoides* leaves is brimming with exciting possibilities:

Developing Novel Antimicrobials: The identification of the most potent bioactive compounds and their mechanisms of action can pave the way for the development of novel, plant-based antimicrobials. These natural alternatives could address the growing threat of antibiotic resistance and offer promising therapeutic options [31].

Enhancing Food Safety: Extracts from *Prunus cerasoides* leaves could potentially be employed in the food industry to extend the shelf life of food products and prevent spoilage caused by microbial growth. This application would benefit both consumers and food producers by reducing food waste and ensuring food safety [32].

Combination Therapies: The potential synergistic effects of *Prunus cerasoides* leaf extracts with conventional antibiotics could be explored. Such combinations could offer a more potent and potentially less toxic approach to combating bacterial infections [33].

In conclusion, the exploration of the antimicrobial properties of *Prunus cerasoides* leaves reveals a promising natural treasure trove. By addressing limitations, exploring future directions, and harnessing the power of these leaves, we can contribute to the fight against microbial foes and unlock a new frontier in natural antimicrobial solutions. This journey requires continued research collaboration, bridging the gap between in vitro and in vivo studies, and ensuring safety and responsible use. As we delve deeper, the potential of *Prunus cerasoides* leaves to safeguard humans.

Antimicrobial Activity of Silver Nanoparticles

The relentless battle against microbial pathogens necessitates a constant search for novel antimicrobial solutions. In this ongoing fight, silver nanoparticles (AgNPs) have emerged as a potent weapon, offering a powerful defense against a broad spectrum of bacteria. This exploration delves into the fascinating world of AgNPs, unveiling their unique properties, potent antimicrobial mechanisms, and exciting future applications in the fight against infectious diseases.

Silver's antimicrobial properties have been recognized for centuries. Ancient civilizations used silver vessels and utensils to store food and water, harnessing its natural ability to inhibit microbial growth and prevent spoilage. Modern science has now shed light on the underlying mechanisms of this phenomenon. Silver ions (positively charged silver atoms released from the metal) are the primary contributors to its antimicrobial activity. These ions can interact with bacterial cell membranes in several ways, leading to their disruption and ultimately cell death [31]

Antimicrobial properties of Silver Nanoparticles

While bulk silver possesses antimicrobial properties, silver nanoparticles (AgNPs) take this power to a whole new level. These nanoparticles are microscopic particles of silver ranging from 1-100 nanometers in diameter. Due to their incredibly small size, AgNPs possess a vastly increased surface area compared to bulk silver. This translates to a significant enhancement in their antimicrobial activity. Here's why:

- Enhanced Contact with Bacteria: The increased surface area of AgNPs allows them to come into greater contact with bacterial cells. This increased contact probability maximizes the potential for interaction with the cell membrane, a crucial step in their antimicrobial action.
- Greater Ion Release: The smaller size of AgNPs facilitates a more significant release of silver ions compared to bulk silver. These ions are the primary weapons in the fight against bacteria, and their increased availability translates to a more potent antimicrobial effect.
- Penetration Potential: Due to their small size, AgNPs can potentially penetrate the bacterial cell wall, a feat not readily achievable with bulk silver. This deeper penetration allows the silver ions to directly interact with intracellular components, further disrupting essential bacterial processes [32].

The potent antimicrobial activity of AgNPs is attributed to a multi-pronged attack on bacterial cells. Here are some of the key mechanisms at play:

- Membrane Disruption: Silver ions can interact with the bacterial cell membrane, causing disruptions in its structure and function. This disruption can lead to leakage of essential cellular contents and ultimately cell death.
- Inhibition of Enzyme Activity: Silver ions can bind to and inhibit essential bacterial enzymes critical for various metabolic processes. By hindering these enzymes, AgNPs can disrupt bacterial growth and replication.
- DNA Damage: In some cases, silver ions can interact with bacterial DNA, causing damage and hindering its replication. This can prevent the bacteria from reproducing and ultimately lead to cell death.
- Reactive Oxygen Species (ROS) Generation: Silver nanoparticles can induce the generation of ROS within bacterial cells. These ROS are highly reactive molecules that can damage essential cellular components, further contributing to cell death.

- Quorum Sensing Inhibition: Bacteria communicate with each other through a process called quorum sensing. AgNPs might interfere with this communication, hindering bacterial ability to coordinate activities like biofilm formation or virulence factor production [34].
- Disruption of Respiratory Chain: Some studies suggest that AgNPs might disrupt the bacterial electron transport chain, a crucial pathway for generating energy. This disruption can hinder bacterial growth and survival [35].

The antimicrobial activity of silver nanoparticles is not a singular phenomenon. It is a complex interplay of various mechanisms, influenced by factors like size, shape, and surface chemistry. By delving deeper into these nuances, we can optimize AgNPs for enhanced efficacy and explore new frontiers in combating bacterial infections. As research progresses, silver nanoparticles hold immense promise for revolutionizing our fight against microbial foes and safeguarding public health.

Building upon the foundation laid out previously, this exploration delves deeper into the intricacies of the synergistic effect between silver nanoparticles (AgNPs) synthesized using *Prunus cerasoides* leaf extract and their enhanced antimicrobial activity. We will explore the potential mechanisms of synergy, characterization techniques for AgNPs, and future research directions for optimizing this promising approach.

The potential synergistic effect between AgNPs and the bioactive compounds in *Prunus cerasoides* leaf extract is not a simple phenomenon. It likely involves a complex interplay of various mechanisms:

- **Enhanced Stability**: The bioactive compounds, particularly flavonoids, might act as capping agents. These capping agents bind to the surface of AgNPs, preventing them from aggregating and maintaining their size and shape. Smaller, well-dispersed AgNPs possess a larger surface area, which is crucial for effective interaction with bacterial cells and optimal antimicrobial action [36].
- **Targeted Delivery**: The bioactive compounds within the leaf extract might possess specific affinities for bacterial cell surfaces. By selectively binding to the AgNP surface, these compounds could act as a Trojan horse, facilitating the delivery of the nanoparticles directly to the target bacteria. This targeted delivery can significantly enhance the overall efficacy of the AgNPs [37].

- **Membrane Disruption Synergy**: The combined action of AgNPs and the bioactive compounds could lead to a more potent disruption of the bacterial cell membrane. Flavonoids and other compounds might weaken the membrane structure, making it more susceptible to the disruptive effects of the AgNPs. This synergistic membrane disruption can lead to a more rapid and effective bactericidal effect [38].
- **Inhibition of Repair Mechanisms**: Some bacterial strains can employ repair mechanisms to counteract damage caused by antimicrobials. The bioactive compounds might inhibit these repair mechanisms, rendering bacteria more vulnerable to the destructive effects of the AgNPs [39].

Significant exploration is needed to translate the potential of this synergistic approach into practical applications:

- **In Vitro and In Vivo Studies**: Rigorous in vitro studies are essential to quantify the synergistic antimicrobial activity of AgNPs synthesized with the leaf extract against a broad spectrum of bacterial strains. Subsequent in vivo studies are crucial to assess their efficacy and safety in a more realistic setting .
- **Mechanism Elucidation**: Further research is needed to definitively identify and understand the specific mechanisms by which the bioactive compounds from *Prunus cerasoides* leaves contribute to the synergistic antimicrobial effect of the AgNPs.
- **Biocompatibility Assessment**: A comprehensive assessment of the biocompatibility of these AgNPs is essential to ensure their safe use in potential therapeutic or industrial applications .
- **Application Exploration**: The potential applications of this synergistic approach can be explored in various fields, including wound healing dressings, medical device coatings, food preservation, and water purification technologies .
- The ability of bioactive compounds in *Prunus cerasoides* leaf extract to bind to the surface of AgNPs plays a central role in the synergistic effect. Several binding mechanisms might be at play:
- **Electrostatic Interactions:** The bioactive compounds might possess functional groups with specific charges (positive or negative) that can interact electrostatically with the charged surface of the AgNPs. This electrostatic attraction can facilitate the binding of the compounds to the nanoparticle surface [40].

- **Hydrogen Bonding**: Bioactive compounds containing hydroxyl (-OH) or amine (-NH2) groups can form hydrogen bonds with oxygen atoms present on the surface of the AgNPs. These hydrogen bonds can create a stable association between the compounds and the nanoparticles.
- **π-π Stacking**: Certain aromatic compounds present in the leaf extract might interact with the AgNPs through π-π stacking interactions. This involves the interaction between the electron clouds of aromatic rings, leading to a stable binding between the compounds and the nanoparticle surface.
- **Metal-Ligand Interactions**: Some bioactive compounds, especially those containing phenolic groups, can form coordination complexes with the silver ions on the surface of the AgNPs. These metal-ligand interactions can create a strong and specific binding between the compounds and the nanoparticles [41].

By understanding these potential binding mechanisms, researchers can tailor the extraction process from *Prunus cerasoides* leaves to enrich specific bioactive compounds with desired functionalities that can optimize their binding to the AgNPs and enhance synergy.

Antioxidant property of Quercetin

Through its impact on glutathione (GSH), the activity of enzymes, pathways for signaling, and the formation of ROS i.e., the reactive oxygen species brought on due to ecological and toxic conditions, quercetin's antioxidant action is primarily demonstrated. By preserving the equilibrium of oxidative processes, quercetin has high antioxidant activity. By controlling GSH levels,quercetin boosts the body's potential for antioxidant defense. This is due to the fact that superoxidedismutase, also known as (SOD), quickly absorbs oxygen and converts it into hydrogen peroxideonce free radicals from oxygen are produced inside the body. Furthermore, this enzyme catalysisleads to the conversion of hydrogen peroxide to nontoxic water. GSH is required as a donor of hydrogen in this process. Quercetin promotes GSH production, according to research done on animals and cells. Free radicals are molecules that react with other substances very fast in order to grab their electron and become stable. When a molecule is attacked, it loses an electron and becomes a free radical, setting off a series of events that damage living cells.It has been shown from the literature that the hydroxyl groups at positions 3, 5, 7, 3′, and 4′ of the rings A and B having double bond, that exists between the 2nd and 3rd carbons, and the group of carbonyls on the fourth carbon play a major part in the antioxidant capabilities of quercetin's most oxidative injury in vivo is caused by ROS, which quercetin is able to scavenge, quercetin can protect against oxidative damage, including radiation-induced UVB wounds, pulmonary damage and its related oxidant damage illnesses. Skin is extremely resilient & capable of withstanding many environmental stresses, but UVB exposure causes an imbalance in the body's natural systemic antioxidants and a brief rise in Reactive oxygen species, that worsens inflammation and the production of free radicals while also having an impact on cellular functions.

According to studies, quercetin not only protects mitochondria from ROS-induced damage but also scavenges ROS, preventing the depolarization of the mitochondrial membrane as well as the movement of the cell membrane. It follows that by repressing this imbalance, quercetin appears to be able to prevent UVB-induced skin damage [42].

Green synthesis of nanoparticles

In the realm of science, nanotechnology more so in green synthesis of silver nanoparticles (AgNPs) has opened a new era with its vast application in health care, environment, and technology. This innovative method employs phytochemicals from plant extracts to offer an eco-friendly, cost-effective and sustainable alternative to conventional techniques which use toxic chemicals and are often environmentally hazardous1. The green synthesis pathway applies natural reducers present in *Prunus cerasoides* leaves; this is an example of green chemistry12. The process not only helps to alleviate associated problems in traditional nanoparticle synthesis but also performs at par with global transition on green processing and synthesis thereby underlining the benefits that accrue from green chemistry12.

In this study, we delve into various advantages of AgNPs prepared using green chemistry which include their high antimicrobial activities against different pathogens as well as other roles they play such as; antibacterial applications or cut flower preservation enhancement24. By prioritizing the synthesis of nanoparticles using plant extracts, this investigation not only aligns with the environmental ethos of minimizing chemical waste.

In the comparison of other approaches, the present study is unique in that it places emphasis on the synthesis of nanoparticles by plant extracts. The research strategy employed in this study not only supports a sustainable environmental policy but also demonstrates how nanoparticles' efficiency and safety can be improved by reducing their chemical waste during production124. In our subsequent analysis and applications, green synthesis of Nanoparticles with *Prunus cerasoides* seems to be at the forefront of nanotechnology's sustainability agenda [43].

Green synthesis

Silver, a highly toxic metalloid, can be synthesized using green methods. Such methods are environment-friendly unlike others which utilize poisonous chemicals and as such, produce dangerous end products. The normal ways of coming up with AgNPs often lead to high polydispersity and low yield 12. In response to these limitations, the scientific world has resorted to more sustainable approaches that are considered harmless. They include exploiting bacteria, fungi and plant extracts as biological systems for synthesizing nanoparticles. This technique is praised for being inexpensive, simple and trustworthy besides being environmentally friendly compared to chemical way of synthesizing materials.

To address the environmental issues, biological synthesis of AgNPs employing bacteria and fungi or plant extracts has been developed, which combines the benefit of controlling size and shape of particles in order to have what is necessary for medical and technological applications 968. The procedure employs natural reducing agents such as secondary metabolites in plants that can reduce silver ions to nanoparticles without the need for any stabilizer; therefore, facilitating a simple synthetic route with lower overall expenses.

Traditional methods for AgNP synthesis often rely on toxic chemicals, leading to environmental hazards.

Green synthesis offers an eco-friendly alternative using plant extracts as reducing agents.

This approach is cost-effective, simple, and aligns with sustainable practices.

Benefits of Green Synthesized AgNPs

High antimicrobial activity: Green synthesized AgNPs exhibit strong antibacterial properties against various pathogens.

Reduced environmental impact: By minimizing chemical waste, this method promotes a cleaner environment.

Improved safety and efficiency: Green synthesis can potentially enhance the safety and efficacy of AgNPs compared to traditional methods [44].

Overall Significance

Green synthesis of AgNPs using plant extracts presents a promising approach for producing effective and environmentally friendly nanoparticles with vast applications in healthcare, environment, and technology.

While the antimicrobial properties of green-synthesized AgNPs are well-documented, their potential extends far beyond fighting pathogens. Here, we delve into the diverse applications that emerge from this sustainable approach [38].

Healthcare Frontiers:

Wound healing: Green-synthesized AgNPs exhibit excellent wound healing properties due to their antibacterial activity and ability to stimulate cell proliferation [10]. This offers a promising avenue for treating chronic wounds, diabetic ulcers, and burns.

Antiviral potential: Studies suggest that AgNPs can inhibit the replication of various viruses, including HIV and influenza [11]. Further research in this area could lead to the development of novel antiviral therapeutics.

Cancer theranostics: Green-synthesized AgNPs hold promise in cancer theranostics, a field that combines therapy and diagnosis. By attaching specific molecules to the AgNPs, researchers can target them to deliver drugs directly to cancer cells [12]. Additionally, the inherent imaging properties of AgNPs can be used for cancer detection.

Water purification: Green-synthesized AgNPs demonstrate the ability to remove contaminants like bacteria, viruses, and heavy metals from water sources [13]. This opens doors for developing eco-friendly water purification methods for both industrial and domestic applications.

Textile antimicrobial finishing: Incorporating green-synthesized AgNPs into textiles can impart long-lasting antimicrobial properties, reducing odor and preventing the growth of bacteria on clothing and other fabrics [14]. This approach holds immense potential for the textile industry.

Bioremediation: Green-synthesized AgNPs can be used to degrade organic pollutants and heavy metals in contaminated environments [15]. This paves the way for innovative bioremediation strategies to clean up polluted soil and water bodies.

Technological Advancements:

Sensors: The unique electrical and optical properties of AgNPs make them suitable for developing highly sensitive sensors for detecting various chemicals and biological agents [16]. This has applications in environmental monitoring, food safety testing, and medical diagnostics.

Catalysis: Green-synthesized AgNPs can act as efficient catalysts in various chemical reactions, offering a sustainable alternative to traditional catalysts that are often toxic or expensive [17]. This approach can potentially revolutionize industrial processes and green chemistry.

Conductive materials: Due to their excellent conductivity, green-synthesized AgNPs can be used in the development of next-generation electronic devices, solar cells, and conductive inks [18]. This paves the way for advancements in various technological fields.

Despite the remarkable progress, green synthesis of AgNPs faces certain challenges. Scaling up the production process for industrial applications remains an ongoing effort. Additionally, ensuring the consistent size, shape, and properties of AgNPs requires further research and standardization.

Future directions in this field involve exploring a wider variety of plant extracts for AgNP synthesis. Investigating the mechanisms by which these plant extracts reduce silver ions and stabilize the nanoparticles is crucial for optimizing the process. Additionally, research on the potential cytotoxicity of green-synthesized AgNPs is essential for ensuring their safe implementation in various applications.

Green synthesis of AgNPs using plant extracts emerges as a powerful tool for nanotechnology, offering a sustainable and effective alternative to conventional methods. With its vast potential in healthcare, environmental protection, and technological advancements, this approach holds immense promise for shaping a brighter future. As research continues to refine and optimize green synthesis techniques.

Anticancer properties of silver nanoparticles

Silver nanoparticles have garnered significant attention in the field of cancer therapy due to their promising anticancer properties. With their unique physicochemical characteristics, silver nanoparticles have shown potential in targeting and destroying cancer cells through various mechanisms. This article provides an overview of the role of silver nanoparticles in cancer treatment, exploring their mechanisms of action, research evidence supporting their efficacy, potential applications, and considerations regarding safety and toxicity in therapeutic settings. Understanding the evolving landscape of silver nanoparticles in anticancer strategies is essential for advancing the development of innovative and effective therapeutic interventions [25].

Definition and Characteristics of Silver Nanoparticles

Silver nanoparticles are tiny particles of silver that are 1 to 100 nanometers in size. They possess unique properties due to their small size, large surface area-to-volume ratio, and high surface energy. These characteristics make them highly effective in various applications, including cancer therapy.

Rationale for Exploring Anticancer Properties

The use of silver nanoparticles in cancer therapy has gained attention due to their potential anticancer properties. Their ability to selectively target and kill cancer cells while sparing normal cells makes them a promising candidate for novel cancer treatments. Researchers are exploring the mechanisms by which silver nanoparticles can inhibit tumor growth and improve cancer treatment outcomes [18].

Mechanisms of Action of Silver Nanoparticles in Cancer Cells

Induction of Oxidative Stress

Silver nanoparticles exert their anticancer effects by inducing oxidative stress in cancer cells. This leads to an imbalance in reactive oxygen species levels, causing damage to cellular components and ultimately triggering cell death pathways in cancer cells.

Inhibition of Cell Proliferation

Another mechanism by which silver nanoparticles combat cancer is by inhibiting the proliferation of cancer cells. They disrupt crucial cellular processes involved in cell division, leading to the suppression of tumor growth and metastasisent.

Potential Applications of Silver Nanoparticles in Cancer Treatment

Targeted Drug Delivery Systems

Silver nanoparticles can be utilized in targeted drug delivery systems for cancer therapy. By functionalizing the surface of silver nanoparticles with specific ligands, drugs can be effectively delivered to cancer cells, maximizing therapeutic efficacy and minimizing off-target effects.

Enhancement of Imaging Techniques

In addition to their therapeutic applications, silver nanoparticles can enhance imaging techniques used in cancer diagnosis and treatment monitoring. Their unique optical properties enable them to serve as contrast agents for various imaging modalities, such as MRI, CT scans, and fluorescence imaging, improving visualization of tumors and facilitating more accurate cancer management. Safety and Toxicity Considerations of Silver Nanoparticles in Cancer Therapy

Silver nanoparticles have emerged as a promising candidate in the field of cancer therapy due to their unique properties. However, concerns regarding their safety and toxicity must be carefully considered before widespread use [45].

Biological Fate and Accumulation

Once administered, silver nanoparticles can follow various biological pathways, including distribution to different organs and tissues. Understanding how these nanoparticles accumulate in the body is crucial for assessing their potential impact on overall health.

Potential Adverse Effects and Mitigation Strategies

While silver nanoparticles show promise in combating cancer, there is a need to address potential adverse effects, such as cytotoxicity and genotoxicity. Implementing mitigation strategies, such as surface modifications or targeted delivery systems, can help reduce the risk of toxicity in cancer therapy applications. In conclusion, the exploration of silver nanoparticles as a potential treatment modality for cancer holds great promise. Continued research and development in this field are crucial for harnessing the full therapeutic potential of these nanoparticles. As advancements are made in understanding their mechanisms of action and optimizing their use, silver nanoparticles have the potential to revolutionize cancer therapy and improve patient outcomes. The future looks bright for utilizing silver nanoparticles as a valuable tool in the fight against cancer.

Characterization of Nanoparticles

Nanoparticles have gained significant attention in recent years due to their unique properties and potential applications in various fields such as medicine, electronics, and environmental science. In order to fully understand and utilize these nanoparticles, it is crucial to characterize them effectively. This article will delve into the characterization of nanoparticles, focusing on the distinction between primary and secondary characterization techniques, as well as the importance of high-level characterization methods [46].

The Importance of Characterisation

Characterization of nanoparticles is essential to determine their size, shape, surface properties, and composition. These characteristics play a crucial role in influencing the behavior and interactions of nanoparticles in different environments. By accurately characterizing nanoparticles, researchers can optimize their synthesis, tailor their properties for specific applications, and ensure their safety and efficacy.

Primary Characterization Techniques

Primary characterization techniques focus on measuring the fundamental properties of nanoparticles. These techniques include:

Transmission Electron Microscopy (TEM): TEM allows for high-resolution imaging of nanoparticles, providing valuable information about their size, shape, and structure on a nanometer scale.

Dynamic Light Scattering (DLS): DLS is used to measure the hydrodynamic size of nanoparticles in solution, providing insights into their aggregation state and stability.

X-ray Diffraction (XRD): XRD is employed to determine the crystalline structure of nanoparticles, aiding in the identification of different phases and compositions.

Secondary Characterization Techniques: Secondary characterization techniques delve deeper into the properties and behavior of nanoparticles, providing more comprehensive information. These techniques include:

Fourier Transform Infrared Spectroscopy (FTIR): FTIR is utilized to analyze the chemical composition and surface functional groups of nanoparticles, offering insights into their reactivity and surface properties.

Scanning Electron Microscopy (SEM): SEM allows for detailed imaging of nanoparticles at a micrometer scale, providing information on their morphology, size distribution, and surface topography.

Energy-dispersive X-ray Spectroscopy (EDS): EDS is used in conjunction with SEM to analyze the elemental composition of nanoparticles, enabling researchers to identify the presence of specific elements within the particles.

Characterizing Nanoparticles: Primary, Secondary & High-LevelGreen Synthesis for Sustainable AgNPs Flavonoids on Silver Nanoparticles *Prunus cerasoides*for Green Synthesis of AgNPs.

Characterization of Nanoparticles: Primary vs. Secondary to High Level Nanoparticles have gained significant attention in recent years due to their unique properties and potential applications in various fields such as medicine, electronics, and environmental science. In order to fully understand and utilize these nanoparticles, it is crucial to characterize them effectively. This article will delve into the characterization of nanoparticles, focusing on the distinction between primary and secondary characterization techniques, as well as the importance of high-level characterization methods. The Importance of Characterization of nanoparticles is essential to determine their size, shape, surface properties, and composition. These characteristics play a crucial role in influencing the behavior and interactions of nanoparticles in different environments. By accurately characterizing nanoparticles, researchers can optimize their synthesis, tailor their properties for specific applications, and ensure their safety and efficacy. Primary Characterization Techniques Primary characterization techniques focus on measuring the fundamental properties of nanoparticles. These techniques include: Transmission Electron Microscopy (TEM): TEM allows for high-resolution imaging of nanoparticles, providing valuable information about their size, shape, and structure on a nanometer scale. Dynamic Light Scattering (DLS): DLS is used to measure the hydrodynamic size of nanoparticles in solution, providing insights into their aggregation state and stability. X-ray Diffraction (XRD): XRD is employed to determine the crystalline structure of nanoparticles, aiding in the identification of different phases and compositions. Secondary Characterization Techniques Secondary characterization techniques delve deeper into the properties and behavior of nanoparticles, providing more comprehensive information. These techniques include: Fourier Transform Infrared Spectroscopy (FTIR): FTIR is utilized to analyze the chemical composition and surface functional groups of nanoparticles, offering insights into their reactivity and surface properties. Scanning Electron Microscopy (SEM): SEM allows for detailed imaging of nanoparticles at a micrometer scale, providing information on their morphology, size distribution, and surface topography. Energy-dispersive X-ray Spectroscopy (EDS): EDS is used in conjunction with SEM to analyze the elemental composition of nanoparticles, enabling researchers to identify the presence of specific elements within the particles. High-Level Characterization Methods High-level characterization methods combine multiple techniques to provide a comprehensive analysis of nanoparticles. These methods offer a more holistic view of the nanoparticles' properties and behavior, allowing for in-depth understanding and manipulation. Some high-level characterization methods include: Atomic Force Microscopy (AFM): AFM is used to image nanoparticles at the atomic scale, providing information on their surface roughness, mechanical properties, and adhesion forces. In conclusion, the characterization of nanoparticles plays a crucial role in unlocking their full potential for various applications. By utilizing a combination of primary, secondary, and high-level characterization techniques, researchers can gain a deep understanding of nanoparticles' properties and behavior, leading to advancements in fields such as medicine, electronics, and environmental science [47].

High-Level Characterization Methods

These methods combine multiple techniques to provide a comprehensive analysis of nanoparticles, offering a more holistic view of their properties and interactions with the surrounding environment.

Atomic Force Microscopy (AFM): AFM images nanoparticles at the atomic scale, revealing information on their surface roughness, mechanical properties, and adhesion forces.

By employing a combination of these characterization techniques, researchers can gain a comprehensive understanding of nanoparticles, enabling them to optimize their synthesis, tailor their properties for specific applications, and ensure their safety and efficacy.

The realm of nanotechnology hinges on the meticulous characterization of nanoparticles. As these miniscule particles exhibit unique properties vastly different from their bulk counterparts, understanding their intricate details becomes paramount. This continuation delves deeper into the intricacies of characterization techniques, exploring advanced methods and considerations for a holistic understanding of nanoparticles.

Advanced Characterization Techniques:

Beyond the foundational techniques lie advanced methods that offer unparalleled insights into the complex world of nanoparticles. These powerful tools provide a deeper understanding of:

Magnetic Properties: For magnetic nanoparticles, techniques like vibrating sample magnetometry (VSM) become essential. VSM helps assess the magnetization behavior of the particles, crucial for applications in data storage, targeted drug delivery, and magnetic resonance imaging (MRI).

Optical Properties: Techniques like photoluminescence spectroscopy unveil the light emission properties of nanoparticles. This information is valuable for applications in solar cells, light-emitting diodes (LEDs), and biosensors [48].

Porosity and Surface Area: Brunauer-Emmett-Teller (BET) surface area analysis is employed to determine the surface area and pore size distribution of nanoparticles. This information is crucial for understanding the adsorption capacity and catalytic activity of nanoparticles.

In-Situ Characterization: Observing nanoparticles in their actual working environment is crucial. Techniques like environmental transmission electron microscopy (ETEM) allow researchers to observe nanoparticles under controlled conditions of temperature, pressure, and atmosphere [13].

Considerations for Characterization:

While the choice of characterization techniques is vast, careful consideration is necessary to ensure the obtained data accurately reflects the true nature of the nanoparticles. Here are some key factors to consider:

Sample Preparation: Sample preparation techniques can significantly impact the data obtained. Techniques like centrifugation or sonication might alter the aggregation state of the nanoparticles, influencing size and stability measurements.

Data Interpretation: Raw data from characterization techniques requires proper interpretation. Utilizing appropriate software and expertise is crucial to extract meaningful information from the complex data sets generated.

Interplay of Properties: Nanoparticles are not isolated entities. Their size, shape, surface chemistry, and composition all interact, influencing each other. A holistic approach considering all these factors is necessary for a complete understanding [26].

Emerging Characterization Techniques:

The field of nanotechnology is constantly evolving, and so are the characterization techniques used to probe these materials. Some emerging techniques with immense potential include:

Cryo-Transmission Electron Microscopy (Cryo-TEM): This technique allows imaging of nanoparticles in their frozen, hydrated state, providing a more realistic picture of their morphology and interactions with water molecules.

X-ray Photoelectron Spectroscopy (XPS): XPS provides detailed information about the elemental composition and electronic states of atoms on the surface of nanoparticles, offering insights into their surface chemistry and bonding.

Electron Energy Loss Spectroscopy (EELS): EELS, used in conjunction with TEM, allows for the analysis of the elemental composition and electronic structure of nanoparticles at the nanoscale.

Applications of Characterization:

The insights gleaned from characterization techniques play a pivotal role in various applications of nanoparticles:

Nanomedicine: Characterization is crucial for designing nanoparticles for targeted drug delivery, biosensing, and imaging applications. Understanding the size, surface properties, and biocompatibility of nanoparticles is essential for their safe and effective use in the human body.

Electronics: Characterization helps tailor the properties of nanoparticles for use in transistors, sensors, and solar cells. Techniques like XRD and TEM are crucial for ensuring the desired crystal structure and morphology of the nanoparticles for optimal performance [29].

Environmental Science: Characterization is vital for understanding the environmental impact of engineered nanoparticles. Techniques like DLS and zeta potential measurement help assess the aggregation behavior and potential toxicity of nanoparticles in the environment.

Characterization forms the cornerstone of nanotechnology, unlocking the vast potential of nanoparticles. By employing a combination of primary, secondary, and high-level characterization techniques, researchers can gain a comprehensive understanding of these fascinating materials. This knowledge is essential for optimizing their synthesis, tailoring their properties for specific applications, and ensuring their safety and efficacy. As the field of nanotechnology continues to evolve, novel characterization techniques will emerge, further propelling our understanding and manipulation of these tiny yet powerful materials, paving the way for advancements in medicine, electronics, energy, and beyond [30].

CHAPTER 3

MATERIAL AND METHOD

Materials- Silver Nitrate, *Prunus Cerasoides,* DPPH, ABTS from (Himedia Lab.), Muller Hinton Agar, Muller Hinton Broth, *E.coli*.(ATCC 25922*), S. aureus*(ATCC 23235), Deionized water (Millipore Q.) All solvents used in this study are of analytical grade.

Method of making plant extract

Fresh Leaves Preparation:

- Fresh leaves of *Prunus cerasoides* were collected.
- Their weight was measured (48 grams in this case).
- The leaves were thoroughly washed with tap water to remove any dirt or debris.
- They were then rinsed with distilled water to ensure complete cleaning.

Drying the Leaves:

The washed leaves were dried for 2-3 hours to remove surface moisture.

Preparation of the Extract:

To make a 10% w/v extract, 480 mL of distilled water was added to the 48 grams of dried leaves. This ensures a 10% weight by volume (w/v) concentration of the extract, meaning 10 grams of dried leaves per 100 mL of final extract solution.

Extraction and Storage:

The mixture of leaves and water was boiled for 45 minutes at 60°C. Boiling helps to extract the desired compounds from the plant material into the water solvent.The extract was then filtered using Whatman paper, which removes the solid leaf material from the liquid extract.Finally, the extract was stored at 4°C for further use. Cold storage helps to preserve the extract and minimize degradation.Overall, the process described follows typical steps for preparing a plant extract at a specific concentration. The prepared extract was light orange in colour.

Fig 1- Showing the extract prep. & tree *Prunus cerasoides*

Method of making nanoparticles

Preparation of Silver Nitrate Solution:

1 mM solution of silver nitrate ($AgNO_3$) is prepared. This means there is 1 millimole of $AgNO_3$ per liter of solution.

Mixing with Plant Extract:

The silver nitrate solution (1.25 mL) is mixed with the plant extract (15 mL) in a 1:15 ratio.

Adjusting pH:

The pH of the solution is measured and found to be around 6.

Sodium hydroxide (NaOH) solution is added dropwise using a pH meter to raise the pH to 11. The plant extract likely plays a role in reducing the silver ions, but the higher pH from NaOH further facilitates the reduction process.

Synthesis with Stirring:

The solution is transferred to a 40 mL beaker and a magnetic stir bar is added.

The solution is stirred on a magnetic stirrer for 25 minutes at 600 rpm (rotations per minute).

The stirring speed is then increased to 1300 rpm for the final 5 minutes. Stirring helps to ensure even mixing and promotes the reaction between the silver ions and the reducing agents in the plant extract.

The temperature is maintained at 40°celcius throughout the stirring process.

Formation of Silver Nanoparticles:

After completing the stirring steps, the solution likely contains silver nanoparticles.

Additional Notes:

The plant extract plays a dual role: reducing silver ions (Ag^+ to metallic silver (Ag) and potentially stabilizing the nanoparticles to prevent them from clumping together.

The effectiveness of this method depends on the specific plant extract used. Different plant extracts contain various biomolecules that can act as reducing and stabilizing agents, and their efficiency can vary.

Characterization techniques like UV-Vis spectroscopy or transmission electron microscopy (TEM) would be required to confirm the presence and size of the synthesized silver nanoparticles.

CHAPTER 4

RESULT AND DISCUSSION

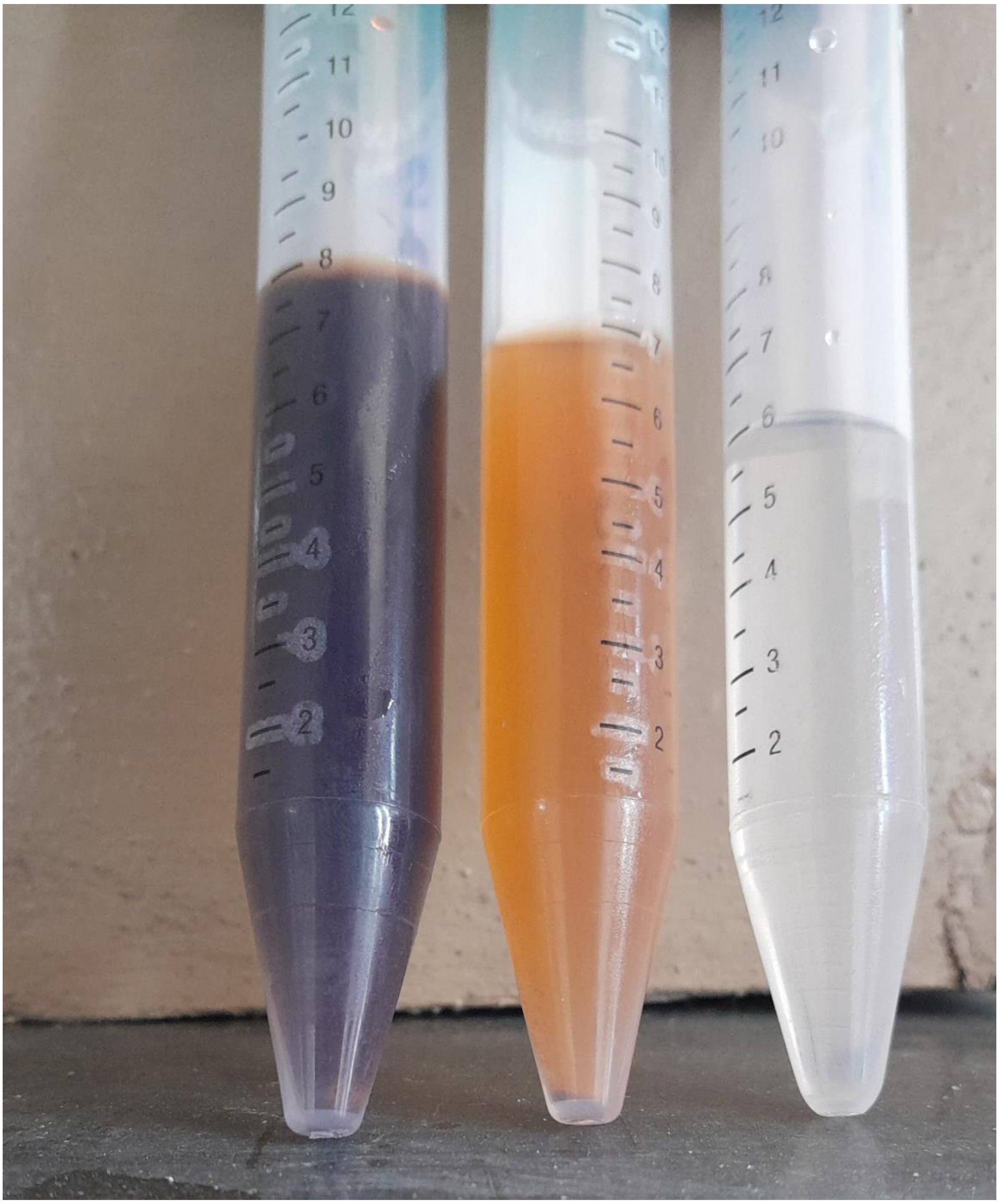

Figure 1represent nanoparticles, plant extract and salt solution

Optimization of concentration of AgNP

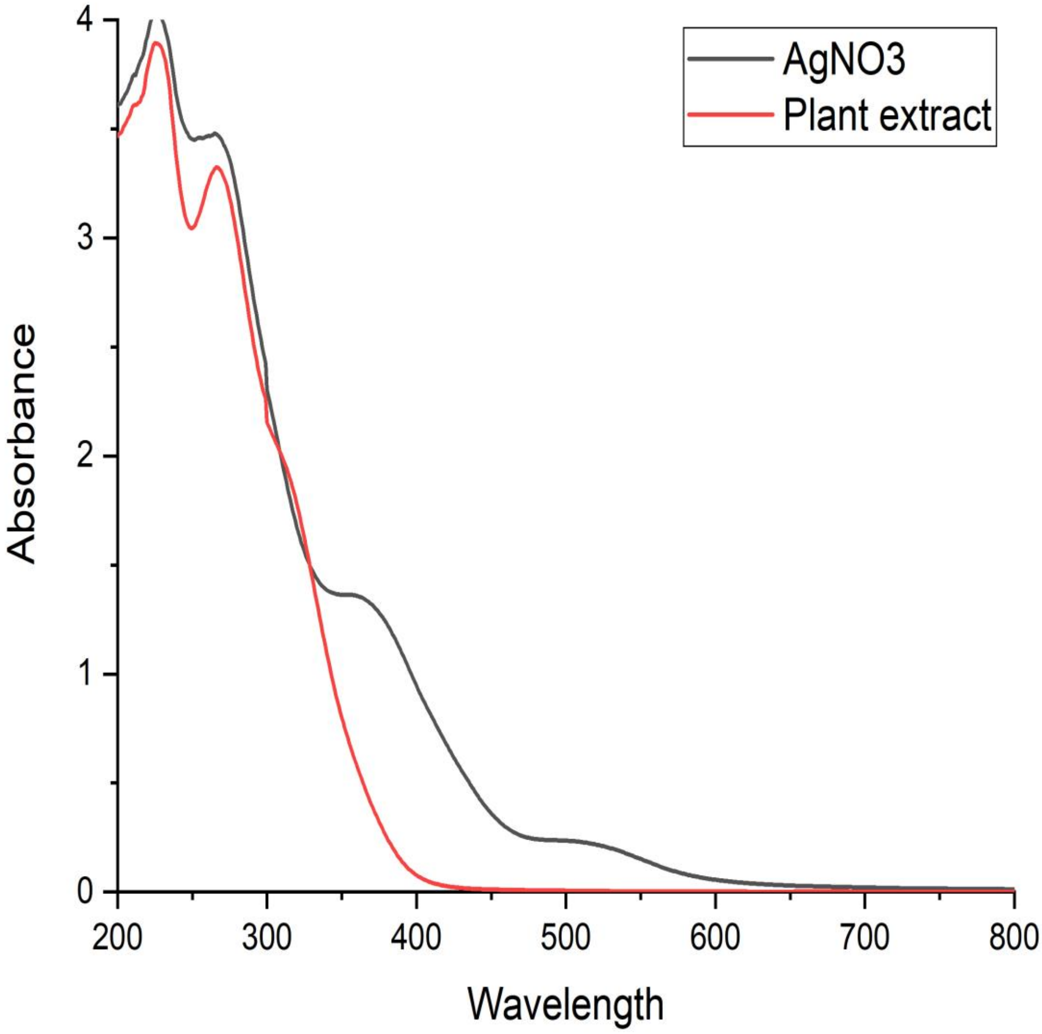

Figure 2 Showing the spectrum of $AgNO_3$ and Plant extract

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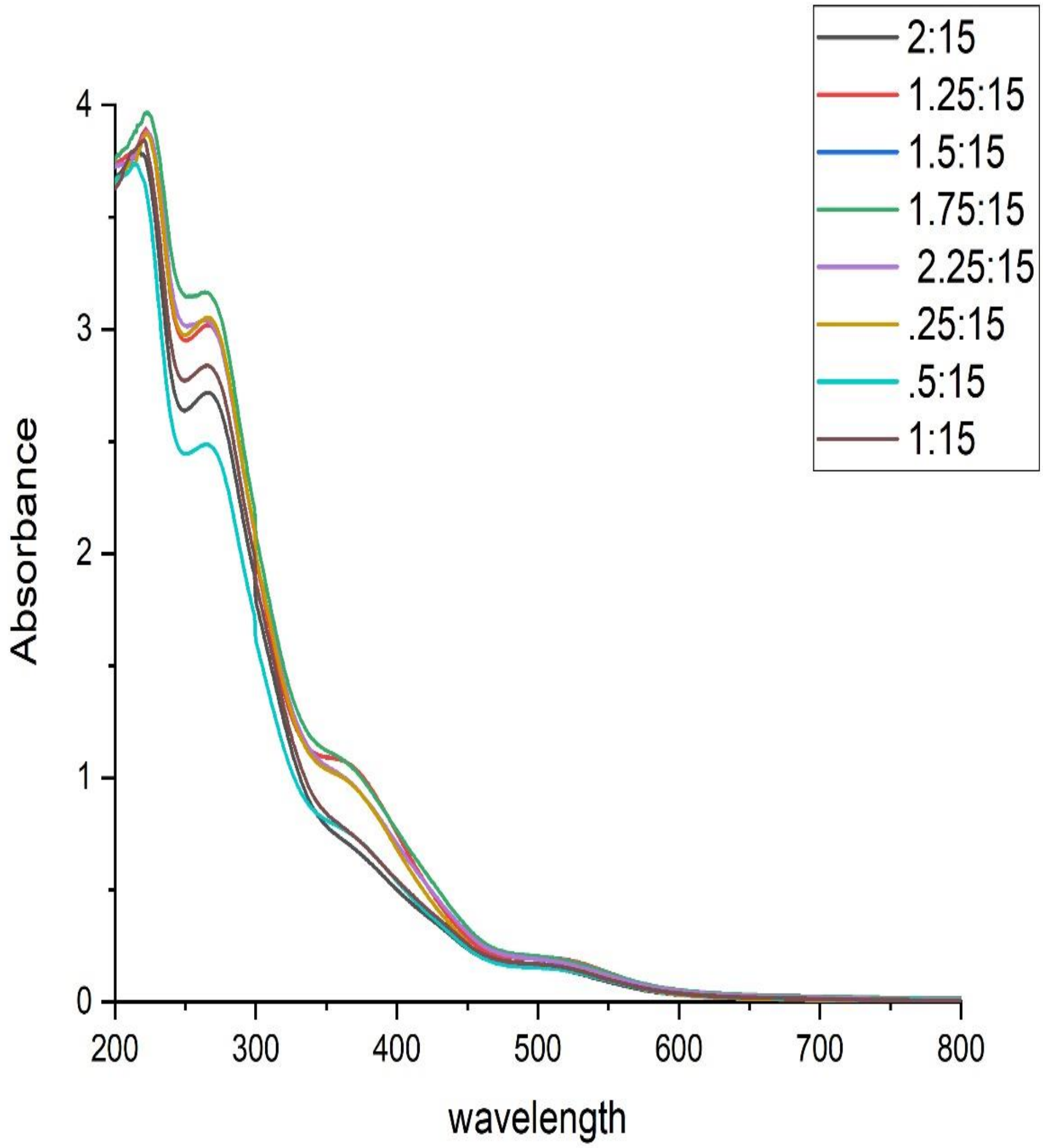

Figure 3 Showing the spectrum of various dilutions of AgNP, in which the best peak was observed at 1.25:15

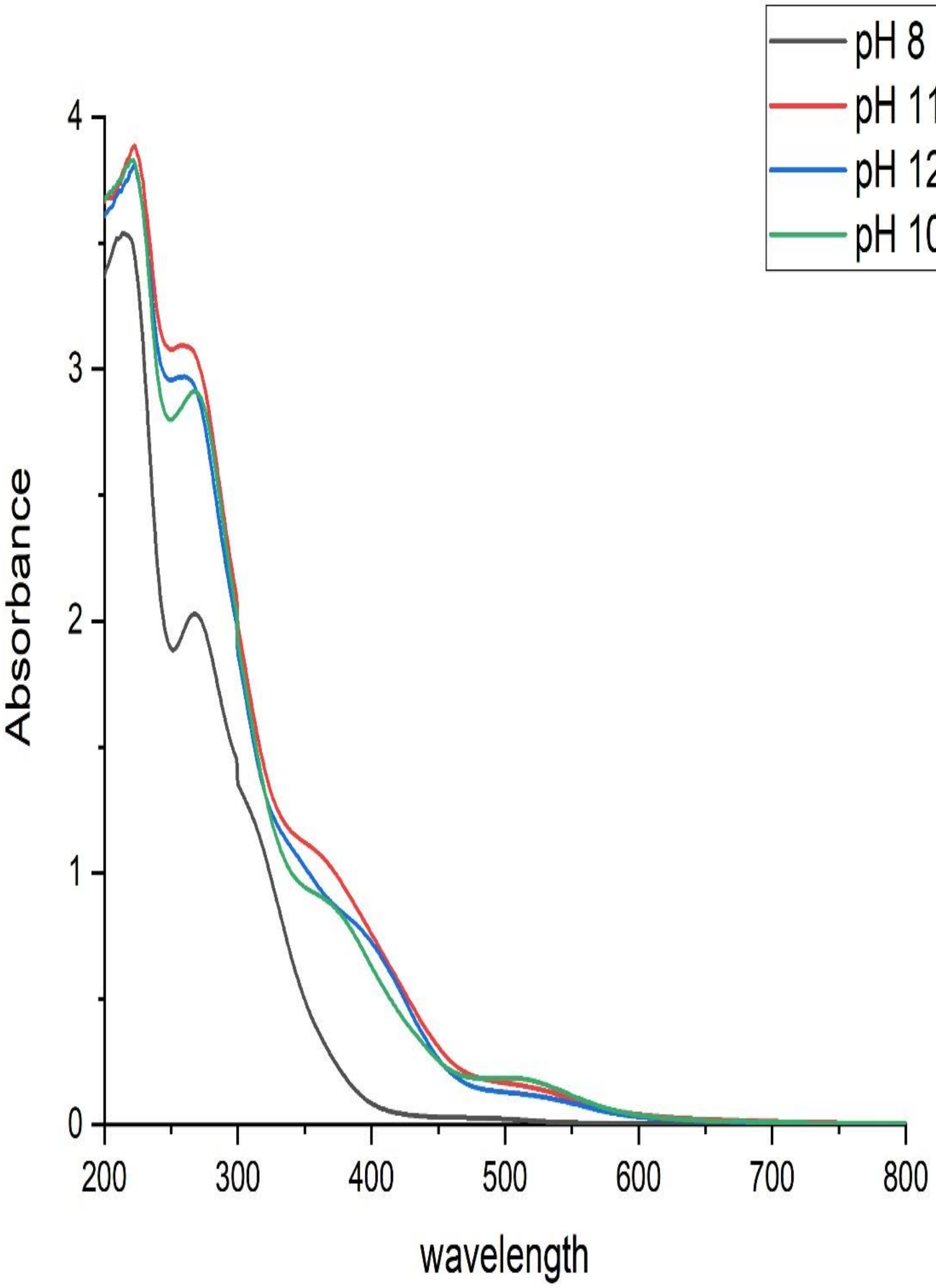

Figure 4Fig 4- Showing the spectrum of different pH of reactions, in which the best peak was visible at pH 11.

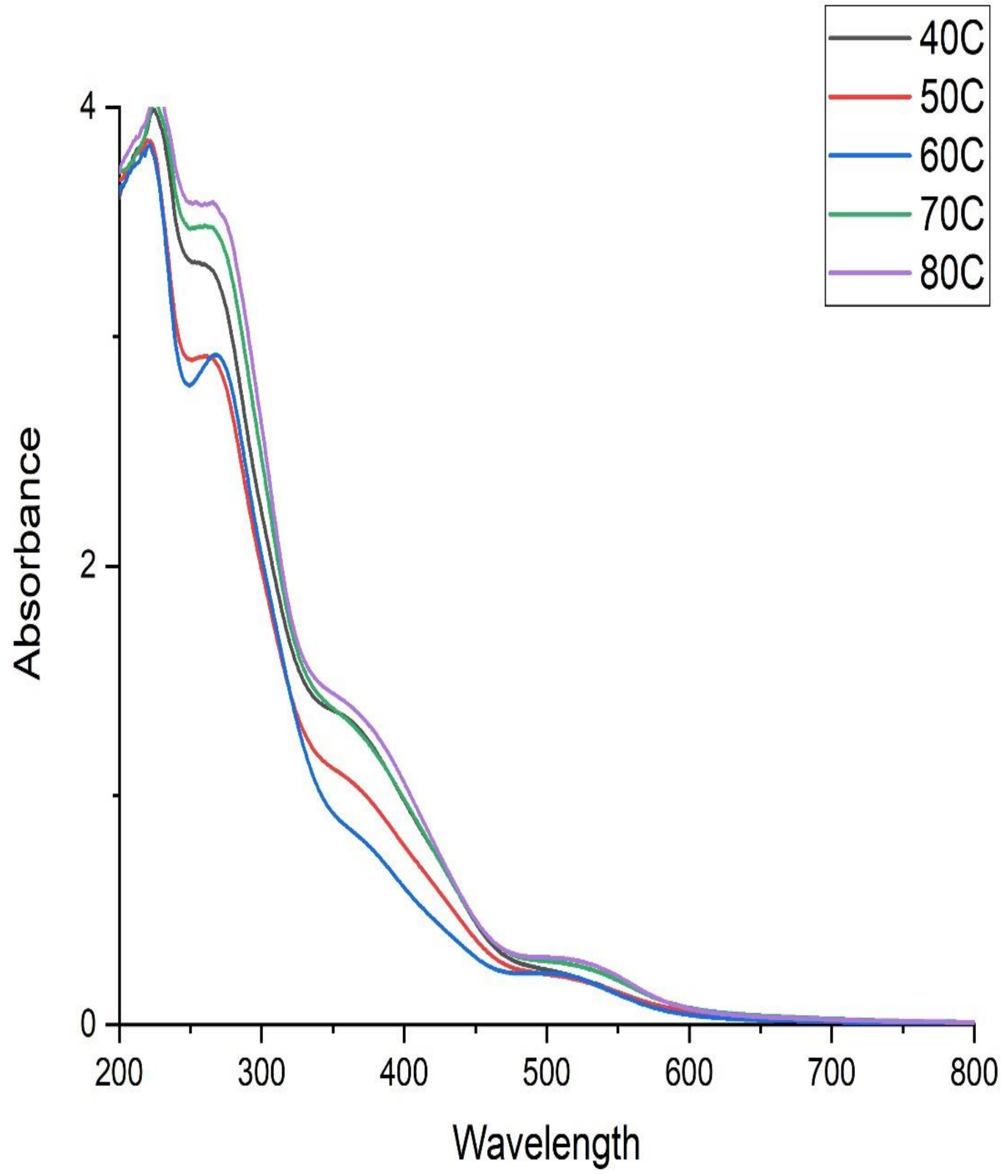

Figure 5- Showing the spectrum of various reaction kept at different temeratues, in which the best peak was visible at 40°C

DPPH Assay.

The free radical scavenging test to check the capacity of chitosan-based nanoformulations

was evaluated using the standard 2,2-diphenyl-1-Picrylhydrazyl DPPH assay. It is a free radical method based on electron transfer). The antioxidant's IC50 was determined. To determine the

radical scavenging activity (RSA) formula RSA (%) = [(AcontrolAsample)/Acontrol] /100 is used

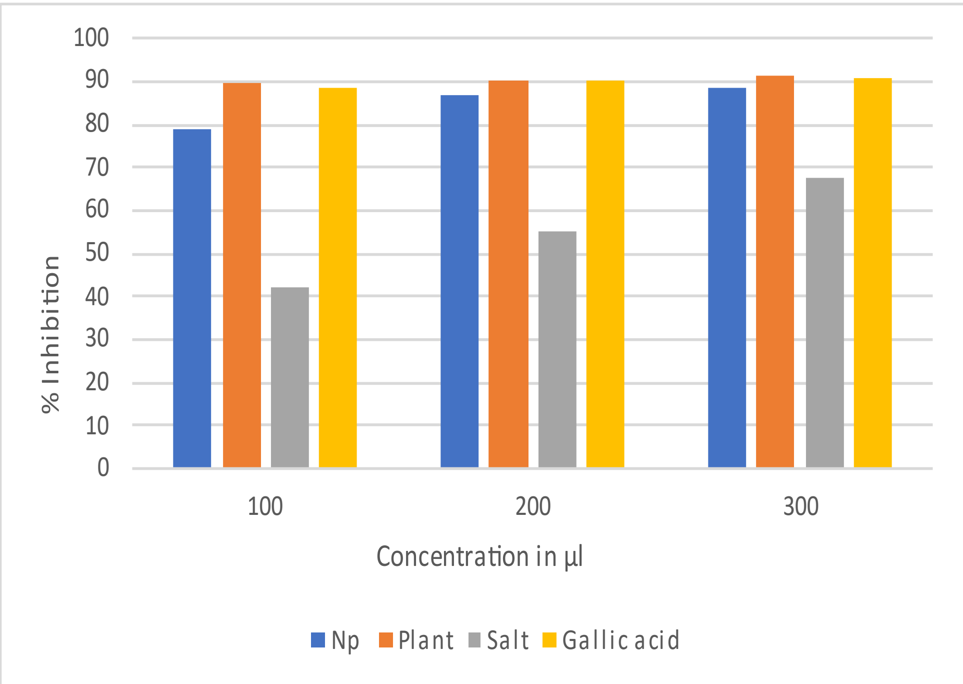

Fig 6- Showing the graph of DPPH free radical scavenging assay.

ABTS assay

The antioxidants are oxidized by the free radical ABTS. It is a cyan-colored (bluish-green)

reagent that becomes colorless when an antioxidant is applied. Antioxidant activity is assessed asa function of color change intensity. The 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic

acid) ABTS radical scavenging test was used to test the antioxidant activity of curcumin and

quercetin that had been encapsulated in chitosan. UV-Vis spectrometer was used to measure theabsorbance at 734 nm (Shimadzu, Japan). The antioxidant's IC50 was determined. [43]. To

determine the radical scavenging activity (RSA) formula RSA (%) = [(Acontrol

Asample)/Acontrol] /100 is used

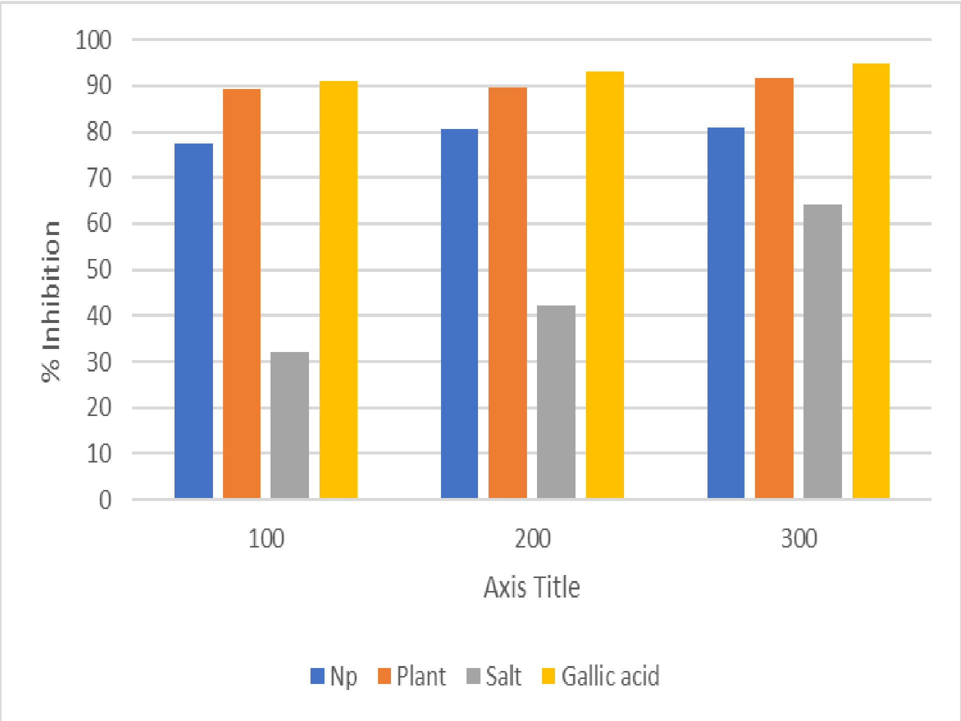

Fig 7- Showing the graph of various concentration of sample for ABTS assay.

Antibiotic Sensitivity Test (AST)

Antibiotic Sensitivity Test (AST) was performed using Kirby Bauer Method at different concentrations and the zone of inhibition was calculated using diameter.

Fig8- Showing AST in *E.coli*, in which S is for $AgNO_3$ salt, E is for extract and NP are synthesized nanoparticles, the zone of inhibition is observed in the nanoparticles and salt.

AST for Fungus

Aspergillus terreus

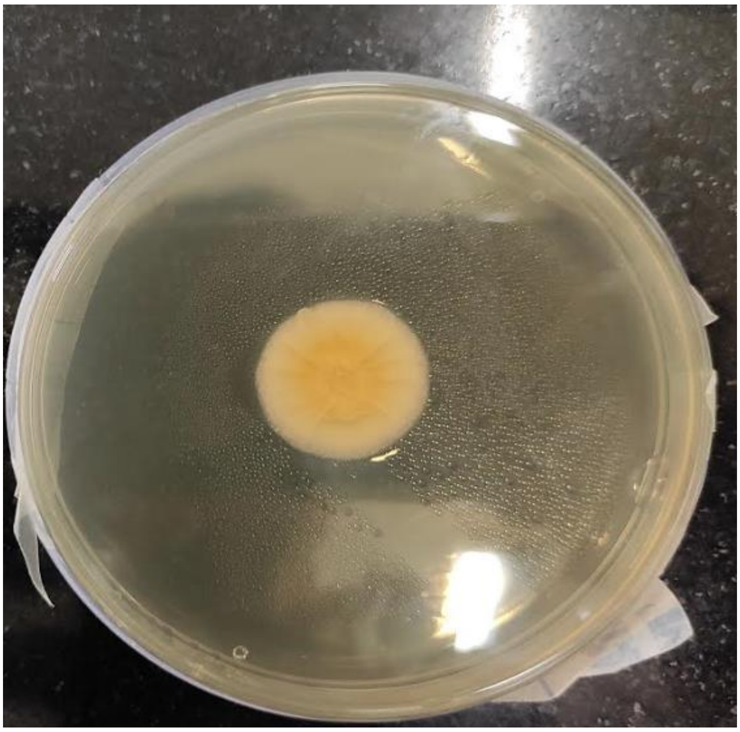

Fig 9- Showing zone of inhibition of nanoparticles

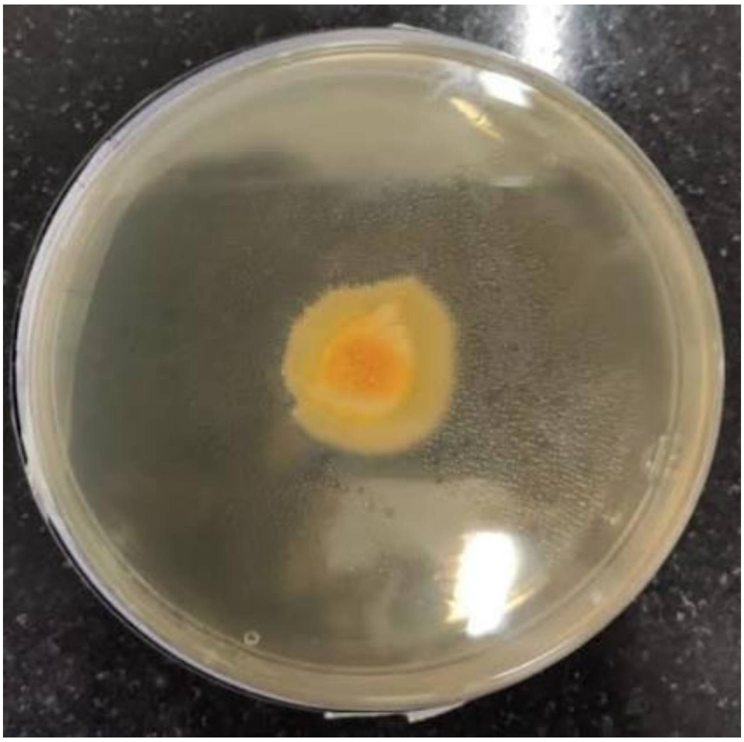

Fig 10- zone of inhibition shown by plant extract.

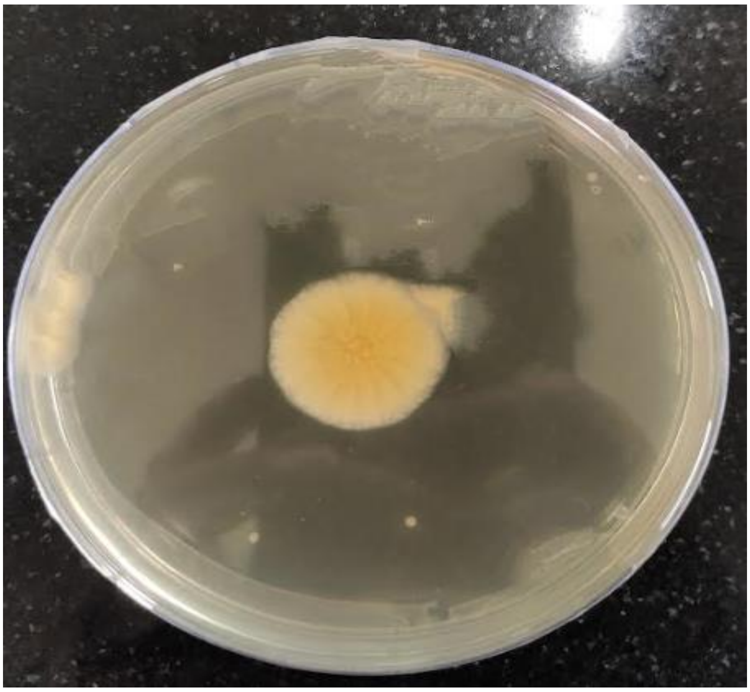

Fig 11- Showing zone of inhibition by silver nitrate salt.

Conclusion

The goal of developing the silver nanoparticles was to reduce issues with general medicines, such as poor solubility, low bioavailability,and degradation.

After the nanofurmulation successfully produced, synthesized, and physiochemically characterised, it had an impact on the drug's physical stability, cellular absorption, biodistribution, and release for antibacterial and antifungal properties.Nanoparticles small size, spherical shape, and zeta potential were useful for antimicrobial action. In this thesis, silver nanoparticles are made by green synthesis to treat and target bacterial and fungal strains.

The Noyes-Whitney equation states that a reduction in the size of a nanometer can greatly use in the increase of interfacial surface area, the rate of dissolution is increased and solubility in water is increased, which results in an improvement in bioavailability of medication. However, an increase in the combined surface area also improves a drug's pharmacological activity and boosts its reactability to particular molecular targets. Because they are hydrophobic by nature, the majority of chemotherapeutic drugs, including quercetin have limited bioavailability. Therefore, to counter the mentioned problem a nanoparticle drug delivery system is designed which not only increases reactability but also increases bioavailability.

Additionally, this organic bio-enhancer of plant extract has a broad range of pharmacological actions, including anti-cancer potential. Therefore, the above stated bioenhancers are used to tackle multidrug resistance, to enhance the bioavailability of quercetin by preventing intestinal and hepatic metabolism, and to produce a synergistic effect using silver nanoformulation

nanoformulation using plant extract as an encapsulating and reducing agent, all based on the forementioned facts. The biological efficacy of silver nanoparticles synthesized using *Prunus cerasoides* plant extract has been a subject of scientific interest due to the potential applications in various fields such as medicine, agriculture, and environmental remediation. Here's an overview of the potential biological efficacy:

Antimicrobial Properties: Silver nanoparticles are known for their antimicrobial activity. When synthesized using plant extracts like *Prunus cerasoides*, they may exhibit enhanced antimicrobial efficacy. Studies have shown that these nanoparticles can inhibit the growth of a wide range of bacteria, fungi, and even some viruses. This property makes them promising candidates for applications in wound dressings, medical implants, and food packaging to prevent microbial contamination.

Anti-inflammatory Effects: Some research suggests that silver nanoparticles synthesized from plant extracts may possess anti-inflammatory properties. These nanoparticles could potentially modulate the immune response, reducing inflammation and promoting tissue regeneration. This property could be beneficial in the treatment of inflammatory conditions and wound healing.

Antioxidant Activity: Plant extracts often contain compounds with antioxidant properties. Silver nanoparticles synthesized using these extracts may inherit some of these antioxidant properties, which can help in scavenging free radicals and reducing oxidative stress. This antioxidant activity could be advantageous for various biomedical applications, including in the treatment of oxidative stress-related diseases.

Biocompatibility and Toxicity: One crucial aspect of the biological efficacy of silver nanoparticles is their biocompatibility and potential toxicity. While silver nanoparticles have demonstrated promising biomedical applications, their safety profile is still under investigation. Studies have shown that the method of synthesis and the stabilizing agents used can influence the toxicity of silver nanoparticles. Thee fore, thorough toxicity studies are necessary to ensure their safe use in biomedical applications.

Environmental Impact: Beyond biomedical applications, the biological efficacy of silver nanoparticles synthesized from plant extracts also extends to environmental remediation. These nanoparticles can be utilized for wastewater treatment, pollutant degradation, and soil remediation due to their antimicrobial and catalytic properties. the particles were characterized using various parameters like UV-VISIBLE spectroscopy that confirmed the formation of nanoparticles having absorption spectra at around 350 to 400nm respectively.

The ph and thermal stability of the nanoformulations were also monitored using UV-VIS spectroscopy where the particles were found to be stable at pH 8-12 and temperature ranging between 40°c and 70°c. antimicrobial assays confirmed the effect of the nanoformulation by giving prominently increased zones of inhibition when compared with plant extract and salt solution.Strong antioxidant activity was observed using DPPH radical scavenging activity and ABTS method of the silver nanoparticles showing maximum antioxidant activity.

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