Camellia sinensis Mediated Biogenic Synthesis of Silver Nanoparticles for Antibacterial Application

Project report submitted in partial fulfillment of the requirement for the degree of Bachelor of Technology In

Biotechnology

By

Palak Bhardwaj

(191801)

Darpan Miglani

(191808)

UNDER THE SUPERVISION OF

DR. Abhishek Chaudhary

Jaypee University of Information Technology,

Waknaghat,173234, Himachal Pradesh, INDIA

DECLARATION

I hereby declare that this project has been done by me under the supervision of **Dr. Abhishek Chaudhary, Assistant Professor (Grade-II)** Jaypee University of Information Technology. I also declare that neither this project nor any part of this project has been submitted elsewhere for award of any degree or diploma.

Supervised by:

Dr. Abhishek Chaudhary

Assistant Professor (Grade-II)

Department of Biotechnology and Bioinformatics

Jaypee University of Information Technology

Submitted by:

Palak Bhardwaj (191801)

Darpan Miglani (191808)

Department of Biotechnology and Bioinformatics

Jaypee University of Information Technology

CERTIFICATE

This is to certify that the work which is being presented in the project report titled ***Camellia sinesis* mediated biogenic synthesis of silver nanoparticles for antibacterial application** in partial fulfillment of the requirements for the award of the degree of Beach in Biotechnology and submitted to the Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat is an authentic record of work carried out by Palak Bhardwaj, Darpan Miglani during the period from July 2022 to May 2023 under the supervision of Dr. Abhishek Chaudhary, Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat.

Palak Bhardwaj
(191801)

Darpan Miglani
(191808)

The above statement made is correct to the best of my knowledge.

Dr. Abhishek Chaudhary

Assistant Professor (Grade-II)

Department of Biotechnology and Bioinformatics

Jaypee University of Information Technology

ACKNOWLEDGEMENT

Firstly, I express my heartiest thanks and gratefulness to almighty God for his divine blessing that made it possible to complete the project work successfully.

I am really grateful and wish my profound indebtedness to Supervisor **Dr. Abhishek Chaudhary** Assistant Professor (Grade-II)**,** Department of Biotech, Jaypee University of Information Technology, Waknaghat. Deep Knowledge & keen interest of my supervisor in the field of **Nanobiotechnology** to carry out this project. His endless patience, scholarly guidance, continual encouragement, constant and energetic supervision, constructive criticism, valuable advice, reading many inferior drafts and correcting them at all stages have made it possible to complete this project.

I would like to express my heartiest gratitude to Dr. Abhishek Chaudhary**,** Department of Biotech, for his kind help to finish my project.

I would also generously welcome each one of those individuals who have helped me straightforwardly or in a roundabout way in making this project a win. In this unique situation, I might want to thank the various staff individuals, both educating and non-instructing, which have developed their convenient help and facilitated my undertaking.

Finally, I must acknowledge with due respect the constant support and patience of my parents.

Palak
Bhardwaj
(191801)

Darpan
Miglani
(191808)

TABLE OF CONTENT

LIST OF ABBREVIATIONS

AgNPs – Silver nanoparticles

TEM: Transmission Electron Microscopy

XRD: X-ray Diffraction

FTIR: Fourier Transform Infrared Spectroscopy

UV-Vis: Ultraviolet-Visible Spectroscopy

SEM: Scanning Electron Microscopy

AgNO3: Silver Nitrate

NPs: Nanoparticles

UV: Ultraviolet

IR: Infrared

LIST OF FIGURES

ABSTRACT

Due to their abundant polyphenol content and potent antioxidant properties, green and black tea have lately attracted interest as reducing and stabilising agents in the manufacture of silver nanoparticles. There are potential uses for this economical and environmentally beneficial strategy in medicine, catalysis, and environmental restoration. The process of nanoparticle generation, parameters influencing the synthesis, and numerous characterization approaches are all covered in detail in this paper's thorough overview of the silver nanoparticle synthesis using tea. The most used technique for characterizing tea-mediated silver nanoparticles is UV-Vis spectroscopy because of its ease and sensitivity. The production of silver nanoparticles using tea as a catalyst is a sustainable method, which is crucial for nanotechnology in the twenty-first century.

Keywords: Nanoparticle synthesis, silver nanoparticles, biological synthesis, tea leaves, biosynthesis, UV-Vis spectroscopy.

CHAPTER 1

1.1 INTRODUCTION

Nanotechnology is the study of the atomic, molecular, and macromolecular levels of matter on a scale between one nanometer and one hundred nanometers. Biotechnology: Biotechnology is the use of biological systems, processes, or organisms to create goods that will enhance the standard of living for people. Nanobiotechnology is where these two worlds come together. It employs nanotechnology to study and build biological Nano systems; it builds technological, useful Nano systems out of biological components and structural blueprints. Nanomaterials are microscopic particles with sizes between 1 and 100 nm. The physical, chemical, and biological characteristics of materials differ fundamentally and usefully from those of individual atoms and molecules or bulk matter at the nanoscale. The subject of creating and developing gadgets known as nanobiotechnology is one that is expanding quickly.

The creation of NPs with various chemical make-ups, sizes and morphologies, and regulated disparities is a significant field of research in nanobiotechnology. A new era in material science has begun with the discovery of nanobiotechnology, which has emerged as a fundamental branch of modern nanotechnology with several practical applications. It is a multidisciplinary strategy that emerged from the exploratory use of NPs in biological systems and incorporates the fields of biology, biochemistry, chemistry, engineering, physics, and medicine. The development of safe, non-toxic, and environmentally acceptable methods for the synthesis and assembly of metal nanoparticles (NPs) with the inherent capacity to decrease metals by metabolic processes is another crucial application of nanobiotechnology.[1]

In the field of biotechnology and biomedicine, the biogenic method is increasingly used to synthesize nanomaterials. In this process, organisms like bacteria, fungus, and plants serve as both capping and reducing agents. Nanomaterials can also be divided into categories based on their dimensions, including those that are zero dimensional (0D), one dimensional (1D), two dimensional (2D), and three dimensional (3D)

(i) Zero-dimensional nanomaterials: all dimensions (x, y, and z) are at the nanoscale, with no dimension larger than 100 nm. nanospheres and nanoclusters, for example.

(ii) One-dimensional (1D): When two dimensions are present at the nanoscale but another exists at a larger scale, the result is a nanostructure that resembles a needle. ex-nanotubes, nanowires, and nanorods

(iii) Two-dimensional (2D): When one dimension is at the nanoscale and the other two are not, the result is a plate-like structure for 2-D nanomaterials. a nanometer-thick nanofilm, nanolayer, or nanocoating, for example.

(iv) three dimensional (3D): These nanomaterials have arbitrary dimensions more than 100 nm and are made up of various configurations of nanoclusters. They are not restricted to the nanoscale.

Quantum effects govern the behavior and characteristics of particles at the nanoscale, making nanotechnology the most promising field of study in terms of controlling and comprehending matter between dimensions of 1 nm and 100 nm.[2]

When a bulk substance is broken down into tiny particles having one or more dimensions (length, thickness, or width) in the nanometer range, the individual particles show surprising characteristics that are distinct from the bulk substances. It is well known that atoms and molecules in small size particles behave entirely differently from those in bulk materials. Due to their small size and unique qualities, such as a high surface area to volume ratio and high heat transfer, metallic nanoparticles have found uses in optical, thermal, magnetic, sensorics devices, and catalysis. Due to their distinct physical and chemical characteristics from bulk materials, nanoparticle production has attracted a lot of research. For the production of silver nanoparticles, both top-down and bottom-up approaches have been suggested. Chemical reduction, nonchemical reduction, micro emulsion techniques, electrochemistry, hydrothermal, sol gel synthesis, polyol process, biological synthesis, and microwave aided techniques are the main methods for producing silver nanoparticles via chemical means. The physical processes of laser ablation, vapor phase synthesis, mechanical milling, and pulsed wire discharge are used to create silver nanoparticles. The creation of metallic nanoparticles chemically is easy to do, very flexible, and environmentally beneficial. Silver nanoparticles can be made using the electrochemical approach at room temperature without the use of any chemicals.[3]

1. High purity and the lack of need for harmful chemicals, high pressure, or vacuum systems for producing powder using electrolysis are its key benefits.
2. In his renowned presentation from 1959, Richard Feynman detailed molecular machines with atomic precision. Invented by There's Plenty of Room in the Bottom, nanotechnology.

3. When Taniguchi adopted the word "nanotechnology" in his work on onion-sputter machining in 1974, the field of study officially began. In 1979, Drexler created the idea of molecular technology at MIT. In 1981, the first technical paper on atomic-level molecular engineering was released.

Due to the distinct characteristics of nanoparticles, which make them extremely useful in a wide range of applications, including electronics, medicine, and energy, nanoparticles have undergone extensive research in recent years. The nanoparticles made of silver, which is one of the most extensively studied types. These nanoparticles have outstanding catalyst, electrical, and optical properties, making them very desirable for use in a variety of technological applications. As a result, scientists have been looking into fresh strategies for creating these tiny particles with an eye on creating more environmentally friendly and sustainable processes.[4]

Using tea extract is one of the most promising ways to make silver nanoparticles. Tea is a widely consumed beverage that has been shown to have anti-inflammatory, antimicrobial, and antioxidant properties. Nevertheless, it has also been discovered that tea contains a number of substances, such as antioxidants and polyphenols, or which have been demonstrated to function as reduction agents in the formation of nanoparticles.

A relatively recent field of study that has grown in popularity recently is tea-mediated synthesis of nanoparticles. In the procedure, tea extracts are used to create nanoparticles as a reducing and stabilizing agent. Due to the substantial number of polyphenols and flavonoids, black and green tea samples have been the most thoroughly studied.

A significant amount of epigallocatechin gallate, also known as (EGCG), a type of polyphenol with powerful antioxidant properties, has been found in green tea in particular. It has been demonstrated that EGCG can reduce the ions of silver to form nanoparticles, as well as the particles that result have outstanding stability and biocompatibility. Additionally, it has been demonstrated that the green tea-mediated production of nanoparticles is a more economical and environmentally friendly option than the traditional methods of nanoparticle production.[5]

Contrarily, black tea includes theaflavins and tarbagans, two polyphenols that have been demonstrated to have potent reducing effects. The synthesis of nanoparticles using black tea has been demonstrated to be very effective, and the end product nanoparticles have excellent optical and stability characteristics. In comparison to other synthesis techniques, black tea-mediated nanoparticle synthesis has been noticed to have been less sensitive to the pH changes.[6]

Contrary to more traditional approaches, tea extracts have several advantages as reducing and strengthening agents in the production of nanoparticles. First, the production of nanoparticles is more affordable because tea extracts are widely available and reasonably priced. Secondly, tea-mediated nanoparticle synthesis is a green technique that does not use hazardous chemicals, which is good for the environment and for people's health. Last but not least, tea extracts have numerous biological characteristics that result in the leading nanoparticles that are extremely bio-compatible, which is crucial for biomedical applications.

In conclusion, the utilization of extracts from tea as reducing and stabilizing agents in the production of silver nanoparticles is an exciting branch of study that has a chance to completely change the area of nanotechnology as a in comparison to traditional methods, the use of tea extracts has several benefits, including affordability, environmental friendliness, and biocompatibility. The results so far are extremely encouraging and suggest that this method may soon be a viable alternative to traditional methods of nanoparticle synthesis. However, more research is required to optimize the procedure of tea-mediated nanoparticle synthesis.[7]

In addition to the previously mentioned benefits, tea-mediated nanoparticle synthesis allows for extensive control over the final nanoparticles' size, shape, and composition. The amount of the tea the extract, pH, and duration of reaction can all be easily adjusted, among other things, to fine-tune the process. The production of nanoparticles with the precise properties needed for a given application requires this level of control at every stage.Additionally, it has been discovered that the synthesis of nanoparticles using tea has a high degree of reproducibility, with the resulting particles having excellent size and shape uniformity. In order to produce nanoparticles in large quantities for use in commercial applications, reproducibility is crucial.Other kinds of nanoparticles, such as gold and oxide of zinc nanoparticles, were also discovered to be successfully synthesized using tea as a catalyst. This expands the uses for tea-mediated nanoparticle synthesis beyond silver nanoparticles.

The creation of nanoparticles using tea extracts offers a sustainable method of doing so. This method is eco-friendly because tea leaves are plentiful, renewable, and biodegradable.Tea-mediated nanoparticle synthesis has some benefits, but it also has some drawbacks. The challenge of controlling the measurement and shape of the leading nanoparticles is one of the main difficulties. As a result, the nanoparticles' characteristics may differ, which could have an impact on how well they perform in various applications.[8]

As a result, the use of tea extracts in the synthesis of silver nanoparticles presents a viable, affordable, and eco-friendly substitute for traditional methods of nanoparticle synthesis. Numerous benefits, such as high reproducibility, control over nanoparticle properties, and broad applicability, come with tea-mediated nanoparticle synthesis. The process needs to be improved, and the limitations of tea-mediated nanoparticle synthesis need to be addressed, so more research is required.[9]

CHAPTER 2

2.1 OBJECTIVES

objective of our report is as follows -

- to get acquainted with the world of nano biotech
- to understand the fundamentals of quantum physics
- to analyze and understand the properties and applications of nanoparticles
- to assess the use of nano-biotech in day-to-day life.

CHAPTER - 3

3.1 LITERATURE REVIEW

The design, manufacture, and manipulation of nanoparticles with dimensions that range from close to 1-100 nm in a single dimension are the main research areas in nanotechnology. The development of this new technique has opened up new fundamental and practical frontiers by enabling the formation of nanoscale materials and the investigation of their peculiar physicochemical and optoelectronic features. Numerous industries, including those in the fields of energy science, optics, catalysts, reprography, single electron semiconductors, light emitters, nonlinear optical equipment, and photoelectrochemical applications, as well as those in health care, cosmetics, agricultural products, safety in the environment, mechanics, optics, biomedical sciences, the chemical sector, electronics, space sectors drug-gene delivery, and mechanical and optical engineering can all benefit from this technology. [10]

In the areas of catalysis, medicine, water treatment, and solar energy conversion, nanomaterials are thought to be the answer to a number of technological and environmental problems. The continuously rising demand for nanomaterials must be accompanied by environmentally friendly synthesis techniques in the context of global efforts to reduce hazardous waste.

The synthesis of materials and the construction of devices are being fundamentally altered by nanotechnology. By using a "bottom-up approach," nanoscale building blocks can be incorporated into functional assemblies and then into multifunctional devices. Because they differ from bulk materials in terms of optoelectronic, magnetic, and mechanical properties, research into the synthesis of nanoscale materials is very interesting [11].

Having sizes between 1 and 100 nanometers, nanoparticles are very small particles. Nanoparticles are extremely appealing for a wide range of applications in a variety of fields, including medicine, food manufacturing, agriculture, and electronics, due to their special physical and chemical properties. Because of their exceptional antibacterial, antioxidant, and catalytic properties, silver nanoparticles have drawn the most attention among the different nanoparticles. The use of potentially harmful chemicals is a part of the conventional processes for producing silver nanoparticles, which could have negative effects on the environment and human health. In order to create these nanoparticles, green and environmentally friendly processes are required. A distinct approach has recently been investigated: using fresh and used tea leaves to create silver nanoparticles. This review aims to provide a concise summary of the research on the synthesis of silver nanoparticles from fresh and used tea leaves as well as on possible applications.[12]

Green synthesis of silver nanoparticles from tea leaves

Because tea leaves contain a variety of reducing and stabilizing agents, the green production of nanoparticles of silver from fresh and used tea leaves has received extensive research. The tea plant, *Camellia sinensis*, is an abundant source of bioactive substances like catechins, flavonoids, and polyphenols that have anti-inflammatory, antimicrobial, and antioxidant properties. For the production of silver nanoparticles, these bioactive substances function as reducing agents. In addition, tannins and polysaccharides in tea leaves serve as stabilizing substances that stop the collection of nanoparticles.

A straightforward one-pot technique was used by Gnana Sangeetha et al. (2015) to produce nanoparticles of silver from fresh Camellia sinensis tea leaves. Fresh tea leaves were boiled for 10 minutes in distilled water to make the tea extract. The silver nitrate solution was then mixed with the extract, and the process was allowed to sit for thirty minutes at room temperature on a magnetic stirrer. When silver nanoparticles formed, the solution's color transformed from pale yellow to brown. Transmission electron microscopy (TEM), infrared spectroscopy using the Fourier transform (FTIR), X-ray diffraction (XRD), and energy-dispersive X-ray spectroscopy (EDX) were used to characterize the synthesized nanoparticles. The strong peak of absorption at 430 nm in the UV-visible spectrum, which is typical of silver nanoparticles, was visible. The reduction and stabilization of silver nanoparticles are caused by a variety of functional groups, including amine, carboxylic acid, and hydroxyl, which were confirmed by the FTIR analysis. The synthesized nanoparticles were found to be crystalline, according to an XRD analysis, and spherical nanoparticles with an average size of 30 nm were visible in TEM images. Silver was found in the nanoparticles, according to the EDX analysis. When tested against a variety of pathogenic bacteria, including *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*, the synthesized silver nanoparticles displayed excellent antimicrobial activity.[13]

A straightforward and environmentally friendly technique was used by Kaviya et al. (2011) to create silver nanoparticles from used tea leaves. The tea leaves were procured from a neighborhood tea shop and thoroughly cleaned with distilled water to get rid of any impurities. To make the tea extract, the washed tea leaves were next placed in a 10-minute boil with distilled water. After that, the extract was combined with a silver nitrate solution, and the two components were left to sit on a magnetic stirrer for four hours at room temperature. The formation of silver nanoparticles was indicated by the solution's color changing from light yellow to dark brown. UV-visible spectroscopy, XRD, and TEM were used to characterize the synthesized nanoparticles. The prominent peak of absorption at 440 nm in the UV-visible spectrum, which is typical of silver nanoparticles, was visible. The XRD analysis supported the synthetic nanoparticles' crystalline nature, and TEM images revealed nanoparticles that were spherical with an average size of 50 nm. The created silver nanoparticles effectively combatted a variety of pathogenic bacteria, including *Bacillus subtilis, Klebsiella pneumoniae*, and *Staphylococcus aureus*.[14]

In a straightforward and environmentally friendly procedure, Masamune et al. (2013) produced silver nanoparticles from used tea leaves. To remove any impurities, the tea leaves were thoroughly cleaned with distilled water after being purchased from a neighborhood tea shop. The tea extract was then made by boiling the previously-washed tea leaves in water that had been distilled for 10 minutes. Following the addition of silver nitrate solution, the extract was combined, and the mixture was left to sit on a magnetic stirrer for 24 hours at room temperature. Silver nanoparticles formed, as evidenced by the solution's color changing from light yellow to dark brown. FTIR, XRD, UV-visible spectroscopy, and TEM were used to analyses the synthesized nanoparticles. As expected for silver nanoparticles, the UV-visible spectrum displayed a strong absorption peak at 430 nm. The FTIR analysis verified the presence of a number of functional groups, including amine, carboxylic acid, and hydroxyl, which are in charge of the reduction and stabilization of silver nanoparticles. The XRD analysis showed that the synthesized nanoparticles were crystallized, and the TEM images showed spherical nanoparticles with an average size of 20 nm. Against gram-positive and gram-negative bacteria like *Staphylococcus aureus, Escherichia coli*, and *Salmonella typhi*, the synthesized silver nanoparticles demonstrated good antibacterial activity.[15]

For the synthesis of silver nanoparticles, tea leaves are a rich source of bioactive compounds that can act as reducing and stabilizing agents. In contrast to traditional chemical techniques, the green synthesis of nanoparticles from tea leaves is straightforward, environmentally friendly, and economical. The synthetic nanoparticles have demonstrated promising properties like antioxidant, antimicrobial, and cytotoxicity against cancer cells, making them appealing for a variety of applications in various industries like medicine, agriculture, and energy. The characterization of the synthesized nanoparticles using various analytical techniques, including UV-visible spectroscopy, FTIR, XRD, and TEM, has revealed important details about their morphology, crystallinity, and physicochemical characteristics. It is possible to create more effective and stable nanoparticles by conducting additional research on the optimization of synthesis parameters like concentration, temperature, and ph. Before using nanoparticles in medicine and other fields, research on their toxicity and biocompatibility is also required.[16]

There have been other plant extracts investigated for the green synthesis of nanoparticles, in addition to the green synthesis of silver nanoparticles from tea leaves. For example, a number of plant extracts, including Aloe vera, neem, and Tulsi, have been used to create silver nanoparticles with interesting properties. Because they are more environmentally friendly, biocompatible, and have lower toxicity than chemical processes, plant extracts are becoming more and more popular as reducing and stabilizing agents in the synthesis of nanoparticles.

The use of nanoparticles in medicine has also drawn a lot of interest because of their distinctive qualities, including high surface area, enhanced reactivity, and tunable size and shape. For the treatment of conditions like cancer, diabetes, and bacterial infections, nanoparticles have been used for drug delivery, imaging, and therapy. Potentially providing a secure and efficient substitute to traditional chemical techniques for the creation of nanomedicines is the use of tea leaf-derived green-synthesized nanoparticles.[17]

But it's important to take into account how nanoparticles might harm both the environment and human health. If nanoparticles are not properly disposed of or if they are released into the environment, they can be toxic and cause environmental pollution. Therefore, before using nanoparticles in medicine or other fields, it is crucial to conduct studies on their toxicity and biocompatibility.The environmentally friendly synthesis of silver nanoparticles from tea leaves is an exciting area of study that may provide a sustainable and environmentally friendly method for the creation of nanomaterials. Using a variety of analytical techniques, nanoparticles can be characterized to learn more about their characteristics and potential uses. Additionally, the use of nanoparticles produced through green synthesis in the medical field and other industries may provide a secure and efficient substitute for traditional chemical processes. However, more research is required to fine-tune the synthesis parameters, evaluate the toxicity and biocompatibility of nanoparticles, and investigate their potential uses in various industries.[18]

The synthesis process' standardization and reproducibility should be taken into account when creating nanoparticles from tea leaves or any other plant extract. The properties of synthesized nanoparticles may vary depending on how plant extracts are processed, how they grow, and other environmental and abiotic factors. Therefore, it is essential to standardize the extraction and synthesis protocols in order to guarantee the reproducibility of nanoparticles that have been synthesized.A further advantage of using tea waste for the synthesis of nanoparticles is that it can lessen waste and encourage sustainability. Polyphenols and other bioactive compounds found in waste from the tea industry, such as tea bags, stems, and leaves, can be used to create nanoparticles. The use of tea waste in the synthesis of nanoparticles may offer a sustainable and economical method for the manufacture of nanomaterials.[19]

More effective and precise control over the size, shape, and characteristics of synthesized nanoparticles can also be achieved through the development of novel methods for their synthesis. For example, using microfluidics to create nanoparticles can provide exact control over the conditions of the reaction and result in the creation of more uniform and stable nanoparticles. The combination of microfluidics and green synthesis techniques may provide a scalable and sustainable method for making nanoparticles. The green synthesis of nanoparticles from tea leaves provides a sustainable and environmentally friendly method for creating nanomaterials with promising properties and potential applications in a variety of fields. Reproducibility and sustainability can each be improved through the standardization of the synthesis process and the use of tea waste. Additionally, the creation of novel synthesis methods may allow for a more precise and effective control over the characteristics of synthesized nanoparticles. To fully explore their potential in various applications, more investigation is required into the optimization, toxicity, and biocompatibility of tea leaf-derived nanoparticles.[19]

The created tea leaf nanoparticles can potentially be used in the field of water treatment in addition to the uses already mentioned. For the effective adsorption of organic pollutants and heavy metal ions from contaminated water sources, nanoparticles can be used. The high surface area and distinctive characteristics of nanoparticles can improve the efficacy and selectivity of adsorption for particular pollutants. Additionally, the green synthesis approach's affordability and eco-friendliness can provide a long-lasting and economical solution for the treatment of water.Additionally, the synthesis of nanoparticles using tea leaves and other plant extracts can help rural areas' local economies grow. In many countries, tea production is a significant component of the agricultural sector. Using tea waste to create nanoparticles may provide tea farmers with an additional source of income. Furthermore, the green synthesis method can provide a decentralized and community-based method for the production of nanomaterials, supporting equitable and sustainable development.

The possibility of scaling up and commercialization is a crucial factor to take into account when creating nanoparticles from tea leaves. The feasibility of mass production and the affordability of the synthesized nanoparticles can be influenced by the scalability of the synthesis process. Since there may be a market for the synthesized nanoparticles, it is essential to scale up the synthesis process and take this into account.[19,20]

In conclusion, the environmentally friendly and sustainable process of creating nanoparticles from tea leaves offers a method for creating materials that could find use in a variety of industries, including energy, water purification, agriculture, and medicine. A sustainable approach to water treatment may be provided by using nanoparticles as an efficient adsorbent to remove contaminants from water sources. Additionally, using tea waste to create nanoparticles can support regional economic growth and decentralize the manufacturing of nanomaterials. The commercialization of green-synthesized tea leaf nanoparticles can be facilitated by additional study on scalability, market demand, and synthesis process optimization. [20]

The absence of standardized protocols for the characterization of synthesized nanoparticles is one of the difficulties in the synthesis of nanoparticles from tea leaves. The ability of nanoparticles to perform well in a variety of applications depends on the size, shape, surface charge, stability, and composition of those particles, all of which must be determined through characterization. To ensure the comparability of results across various studies, standard protocols for the characterization of synthesized nanoparticles must be established.

Additionally, a significant problem that must be addressed in the creation of nanomaterials for a variety of applications is the potential toxicity of nanoparticles. Although the green synthesis method presents a sustainable and environmentally friendly alternative to traditional synthesis techniques, it is still necessary to assess the toxicity of the synthesized nanoparticles to ensure their safety for both human and environmental health. The results of several studies that looked into the toxicity of nanoparticles made from tea leaves and other plant extracts indicate that the toxicity can vary depending on the type, concentration, and route of exposure to the nanoparticles. Therefore, more investigation is required to determine the toxicity of created nanoparticles and create safe and efficient usage methods.[21]

The combination of green chemistry and nanotechnology can also provide a sustainable and all-encompassing method for the creation of nanomaterials. To minimize the negative effects on the environment and advance sustainability, nanoparticle synthesis and applications can be guided by green chemistry principles like waste reduction, use of renewable feedstocks, and non-toxic reagents. Innovative answers to societal issues like climate change, energy use, and health can also be found by combining green chemistry and nanotechnology.

In conclusion, the creation of nanoparticles from tea leaves offers a green and sustainable method for creating new nanomaterials with exciting potential uses. Critical issues that must be resolved in order to guarantee the security and effectiveness of synthesized nanoparticles include the absence of standardized protocols for the characterization of nanoparticles and the potential toxicity of nanoparticles. Furthermore, combining green chemistry with nanotechnology can provide creative, long-lasting solutions to the world's problems. The creation and commercialization of tea leaf-derived green nanoparticles can be facilitated by additional study on the optimization, toxicity, and integration of green chemistry principles.

The process of optimizing synthesis for the desired properties and uses of nanoparticles is a crucial factor in the production of nanoparticles from tea leaves. The properties and functionality of synthesized nanoparticles can be influenced by the synthesis parameters including temperature, pH, reaction time, concentration, and precursor type. To control the size, shape, surface charge, and composition of nanoparticles and improve their functionality in various applications, it is therefore necessary to optimize the synthesis process.[22]

Additionally, the addition of tea leaves to other plant extracts or biomolecules can have a synergistic effect on the creation of nanoparticles, improving their characteristics and potential uses. For instance, it has been demonstrated that combining tea leaves with silver nitrate and pomegranate extract improves the antibacterial activity of synthesized nanoparticles when compared to synthesis using only a single plant extract. In addition to increasing the effectiveness and selectivity of synthesized nanoparticles, the combination of several plant extracts can provide a wide range of bioactive compounds. The use of nanoparticles made from tea leaves can also be improved by functionalizing them with biomolecules like enzymes, antibodies, and peptides. Enhancing the efficacy and selectivity of synthesized nanoparticles in various biomedical applications is possible through the functionalization of nanoparticles, which can provide precise targeting and recognition of biological molecules. For instance, it has been demonstrated that functionalizing silver nanoparticles made from green tea extract with an anti-cancer antibody improves their ability to selectively target and kill cancer cells.[23]

Additionally, the environmentally sound creation of nanoparticles from tea leaves can provide a method for disposing of tea waste that is both sustainable and environmentally friendly. Tea waste can cause pollution and greenhouse gas emissions, which are both problems for the environment. Utilizing tea waste to create nanoparticles can lessen the environmental impact of tea waste and support the principles of the circular economy. In addition, by creating new uses for tea waste, tea farmers could open up new revenue streams and promote environmentally friendly farming methods, the creation of nanoparticles from tea leaves presents a sustainable and environmentally friendly method for the creation of nanomaterials with promising qualities and potential uses in a variety of industries. Enhancing the properties and applications of synthesized nanoparticles and advancing sustainability and circular economy principles can be done by optimizing the synthesis process, combining it with other plant extracts or biomolecules, functionalizing it, and using waste tea. The development and commercialization of green-synthesized nanoparticles made from tea leaves can be facilitated by additional research on the optimization, combination, functionalization, and utilization of tea waste.[24]

In the field of environmentally friendly nanomaterial synthesis, the creation of nanoparticles from tea leaves is a relatively new technique. Tea leaves offer special benefits because of their high concentration of bioactive substances like polyphenols, flavonoids, and alkaloids, which can act as reducing and capping agents for the synthesis of nanoparticles, whereas other plant extracts have been thoroughly studied for the synthesis of nanoparticles. Additionally, using tea waste for the synthesis of nanoparticles can provide a sustainable and environmentally friendly method for the disposal of tea waste and advance the ideas of the circular economy.

Additionally, the creation of nanoparticles from tea leaves can result in materials with special qualities and potential uses in a variety of industries. For instance, it has been demonstrated that nanoparticles made from black tea extract have excellent catalytic activity for the reduction of organic dyes and antibacterial activity for E. coli and S. aureus. The excellent antioxidant and anticancer activity of silver nanoparticles made from green tea extract makes them promising candidates for biomedical applications.[25]

Additionally, combining tea leaves with other plant extracts or biomolecules can have synergistic effects on the production of nanoparticles, improving their characteristics and potential uses. For instance, pomegranate extract, silver nitrate, and tea leaves combined have been shown to increase the antibacterial activity of synthesized nanoparticles compared to a single plant extract-based synthesis. In addition to increasing the effectiveness and selectivity of synthesized nanoparticles, the combination of several plant extracts can provide a wide range of bioactive compounds.

Additionally, the incorporation of biomolecules like enzymes, antibodies, and peptides into tea leaf-derived nanoparticles can provide precise targeting and recognition of biological molecules, enhancing the efficacy and selectivity of the produced nanoparticles in a variety of biomedical applications. Contrary to traditional nanoparticle functionalization techniques, which rely on hazardous and non-renewable chemicals, this strategy provides a novel and sustainable substitute, the creation of nanoparticles from tea leaves offers a fresh and environmentally friendly method for creating materials with special qualities that could find use in a variety of industries. The circular economy principles can be supported by the use of tea waste in the synthesis of nanoparticles, and the effectiveness and selectivity of synthesized nanoparticles can be improved by combining them with other plant extracts or biomolecules. The commercialization of tea-based nanoparticles and the advancement of innovative, sustainable solutions to global problems can both be facilitated by further study on the optimization, combination, and functionalization of tea-based nanoparticles.[26]

The synthesis of nanoparticles from tea leaves has been extensively studied and reported in numerous scientific publications, including peer-reviewed journals and conference proceedings. The research has been conducted by various researchers and research groups from different countries, including India, China, Iran, and Korea.

As an illustration, a study by Krishnamoorthy et al. (2012) described the synthesis of silver nanoparticles from black tea extract and showed their catalytic activity in the reduction of organic dyes. Similar to this, a 2014 study by Iravani et al. reported the green synthesis of gold nanoparticles from green tea extract and showed their antibacterial activity against S. aureus and E. coli.[27]

Additionally, the creation of nanoparticles from tea waste has been documented in a number of studies, such as a study by Li et al. (2019) that described the creation of silver nanoparticles from waste green tea leaves and showed how effective they were at fighting S. aureus and E. coli bacteria.

The traditional and authentic knowledge of tea and its properties supports the use of tea leaves for the synthesis of nanoparticles. Traditional Chinese medicine has used tea for a variety of conditions including inflammation, digestion, and wound healing. Polyphenols and flavonoids, two of the bioactive components found in tea, have been shown to have anti-inflammatory, antibacterial, and antioxidant properties.

The use of tea for medicinal purposes is supported by reliable traditional knowledge, and the production of nanoparticles from tea leaves is supported by extensive research and scientific literature. The potential uses of tea-based nanoparticles in a range of industries, including biomedical and environmental applications, present innovative and promising answers to the world's pressing problems.[28]

CHAPTER 4

4.1 PROPERTIES OF SILVER NANOPARTICLES

A lot of interest has been paid to silver nanoparticles (AgNPs) in the areas of medical science, technology, and environmental remediation because of their distinctive physicochemical characteristics. As a result of their large surface area compared to volume ratio, AgNPs are highly reactive and have special optics, electrical power, and catalytic properties. The characteristics of AgNPs will be thoroughly covered in this article.

4.1.1 Size and Shape:

The physicochemical characteristics of AgNPs are significantly influenced by their size and shape. AgNPs can be made in many different sizes and shapes, including spherical, rod-like, triangular in shape and cubic. AgNPs can be made in a variety of shapes and sizes, with sizes ranging from 1 to 100 nm. The shape of the AgNPs can be altered by changing the temperature of the reaction, pH, and concentration of the reducing agent during the synthesis process. Since they have a greater area compared to volume ratio than larger AgNPs, the latter are more catalytically active and more reactive.[29]

4.1.2 Surface Area:

As a result of their large surface area to volume ratio, AgNPs are highly reactive and have special optical, electrical, and catalytic characteristics. AgNPs are suitable for a variety of applications, including catalysts, sensing, and drug delivery, because their surface area can be increased by manipulating their size and shape.[29]

4.1.3 Optical Properties:

Because of the outer surface plasmon resonance (SPR) phenomenon, AgNP's have distinctive optical characteristics. As a result of the free electrons in AgNP's oscillating as they react to the occurrence electromagnetic field, which causes light to be absorbed and scattered, the SPR phenomenon takes place. AgNP's are useful for a number of applications, including surface-enhanced Raman spectroscopy (SERS), optical sensing, and imaging because the SPR peak wavelength can be adjusted by adjusting AgNP's size and shape.[29]

4.1.4 Electrical Properties:

The free electrons on AgNP's surface are what give them their excellent electrical conductivity. AgNP's are useful for a variety of applications, including conducive inks, flexible electronic devices, and solar cells. By creating a network of AgNP's, the ability to conduct electricity of AgNP's can be further improved.[30]

4.1.5 Catalytic Properties:

Because of their large surface area and distinctive electronic characteristics, AgNP's have excellent catalytic activity. AgNP's are capable of catalyzing a variety of reactions, including reduction, oxidation, which as well as coupling reactions, and they can function as both homogeneous and heterogeneous catalysts. AgNP's can have their catalytic activity increased even more by adding different functional groups to their surface, including thiol, amines, and carboxylic acid groups.[30]

4.1.6 Antimicrobial Properties:

Due to their large surface area and distinctive electrical properties, silver nanoparticles, or AgNP's, are renowned for having exceptional antibacterial qualities. These nanoparticles are extremely efficient against many kinds of bacteria, viruses, and fungi because they can disrupt cell membranes, create reactive oxygen species (ROS), and effectively block enzyme function. AgNP's antibacterial effectiveness can be increased even more by surface-applying functional groups like amino acids and cationic polymers.[30]

4.1.7 Toxicity:

Due to their large surface area and specific electrical properties, silver nanoparticles (AgNP's) have special qualities that make them appropriate for a variety of applications. AgNP's may cause DNA damage, oxidative stress, and inflammation, which can result in cell death; however, their size, shape, chemical composition, and concentration all have an impact on how hazardous they are. the incorporation of hazardous, biocompatible chemicals. AgNP's are nonetheless valuable in applications including sensing, drug administration, and catalysis despite the possibility of harmful consequences. Although their antibacterial characteristics can be helpful in biological applications, careful evaluation of their toxicity is required.[31]

4.1.8 Magnetic Properties:

When subjected to a magnetic field, AgNP's display superparamagnetic behaviour, which means they may get magnetized and then lose their magnetization when the field is removed. Due to this property, they may be used for a variety of tasks, such as magnetic separation, medication administration, and hyperthermia therapy.[31]

4.1.9 Thermal Properties:

The high surface area and distinctive electronic characteristics of AgNP's result in their excellent thermal conductivity. AgNP's are advantageous for a variety of applications including thermal interface substances, heat transfer liquids, and electronics cooling because they can act as efficient heat sinks and dissipate heat quickly.[31]

4.1.10 Mechanical Properties:

Since they are so small and have a large surface area, AgNP's have excellent mechanical properties. Because the AgNP's can form powerful bonds with a variety of substances, they are useful for a wide range of applications, including strengthening polymers and enhancing the mechanical characteristics of materials.[31]

4.1.11 Surface Chemistry:

AgNP's physicochemical characteristics and interactions with living things are greatly influenced by their surface chemistry. AgNP's can have their surface functionalized with different amino, thiol, and carboxylic acid groups, which can change the surface charge, stability, and biocompatibility of the particles. AgNP's are useful for many biomedical applications because of their surface chemistry, which can be used to target only particular cells or tissues.[32]

4.1.12 Environmental Fate and Transport:

AgNP's tiny size and high reaction give them special ecological fate and transport characteristics. The mobility and bioavailability of AgNP's can be influenced by a variety of environmental factors, including pH, temperature, and ionic strength. AgNP's can adsorb onto a variety of environmental surfaces, including soil and sediment. [32]

4.2 COMPONENTS OF TEA

The varying types of tea, environmental factors, different processing techniques, and ways of propagation all contribute to changes in the chemical makeup of tea leaves. The composition of fresh tea flush, as depicted in the image, includes a variety of substances including polyphenol (including catechins), caffeine, amino acids, vitamins, flavonoids, polysaccharides, and fluorine. In the image below, the structural formulas for the four primary components of green tea—catechins, caffeine, theanine, and saponins—are depicted.

The most significant compounds in tea with regard to pharmacology are polyphenols and caffeine. The percentage of polyphenols in dried tea leaf matter, which ranges from 30 to 35 percent, affects the beverage's quality.[33]

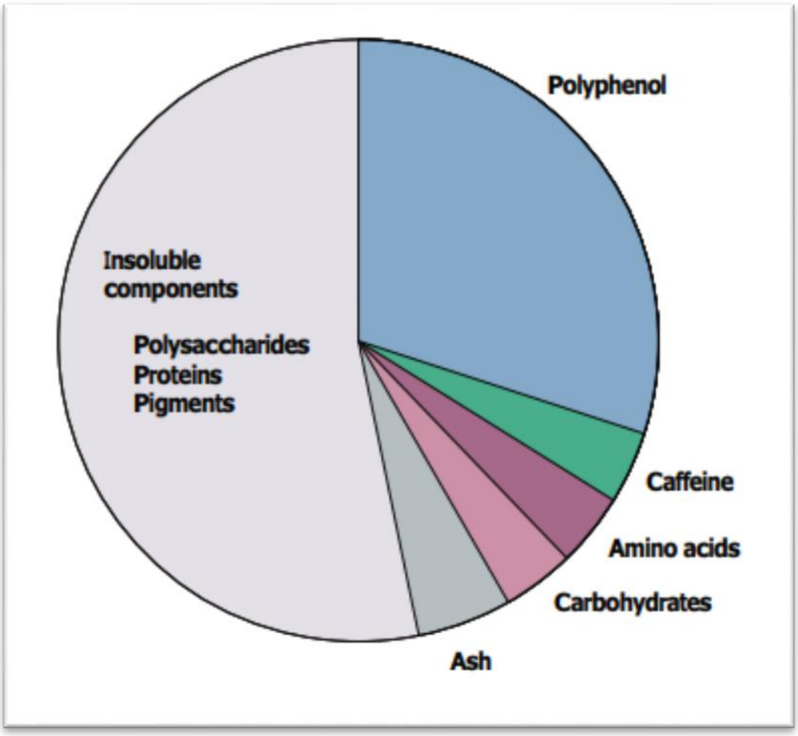


Figure 1 - Chemical components of Tea leaves (C*amellia sinensis*) [33]

The world over, tea is one of the most popular drinks. *Camellia sinensis* plant leaves are infused in hot water to create this beverage. The chemical makeup of tea is complex and varies depending on the type, where it is grown, the climate, the time of year, and the method of processing, among other variables.

4.2.1 Major Components of Black Tea

Polyphenols:

Up to 30% of the dry weight of black tea leaves are made up of polyphenols, a class of antioxidants that are prevalent in black tea. Theaflavins and tarbagans are two of black tea's primary polyphenols. These polyphenols, which are produced during the fermentation of tea leaves, help give black tea its distinctive color, aroma, and flavor. A complex of flavonoids with a bitter taste, theaflavins are reddish-brown in color. Large polymeric molecules with a sweet flavor, tarbagans are brown in color.[34]

Caffeine:

A stimulant that can increase alertness and concentration, black tea contains caffeine. Various elements, including the type of tea, the length of the brewing process, and the temperature of the water, can affect how much caffeine is present in black tea. Black tea contains an ingredient called theophylline, a xanthine alkaloid. It can boost respiratory health and has effects that are similar to those of caffeine. Black tea contains high levels of the amino acid L-theanine, which is specific to tea and only found in it. As a result of its calming effects on the brain, it can enhance relaxation and lessen stress.[34]

Minerals:

A healthy body requires a variety of critical minerals, including calcium, magnesium, and potassium, all of which may be found in small amounts in black tea. These minerals are essential for maintaining the health of bones and muscles, as well as for nerve and blood pressure management. Magnesium is necessary for the maintenance of healthy muscles and nerves, while calcium is crucial for keeping strong bones and teeth. Potassium promotes the healthy operation of muscles and neurons and aids in blood pressure regulation. Regularly drinking black tea might be a practical approach to include these nutrients to one's diet.[34]

4.2.2 Minor Components of Black Tea

Vitamins:

Essential vitamins including vitamin C, vitamin B2 (riboflavin), and vitamin B3 (niacin) are only found in trace levels in black tea. In addition to boosting the immune system, fostering good skin and hair, and assisting in the metabolism of carbohydrates, proteins, and fats, these vitamins play key roles in sustaining general health and wellbeing. The content of these vitamins in black tea is quite low compared to other dietary sources, so it's vital to keep in mind that it shouldn't be depended upon as the only source of these nutrients in a person's diet.[35]

Sugars:

Black tea contains trace amounts of sugars like sucrose, fructose, and glucose. Black tea contains a variety of amino acids, including lysine, theanine, and tryptophan.[35]

Volatile substances:

Geraniol, linalool, and citronellol are just a few of the volatile substances found in black tea that give it its distinctive aroma.[35]

4.2.3 Health Benefits of Black Tea

Cardiovascular Health:

According to studies, the polyphenols in black tea can lower blood pressure, improve lipid profiles, and lower the chance of developing heart disease. Strong antioxidant qualities found in these polyphenols can shield the body from the negative effects of oxidative stress and free radicals. Additionally, tooth caries and periodontal disease can be avoided because to black tea's antibacterial characteristics. Black tea's L-theanine and caffeine content can also improve cognitive abilities including memory, attentiveness, and attention span.[36]

4.2.4 Major Components of Green Tea

Catechins:

Catechins, strong antioxidants found in green tea, are present in high concentrations. Green tea contains epigallocatechin (EGC), epicatechin (EC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG), the most physiologically active of the four main catechins. Green tea has less caffeine than black tea. However, variables like tea kind, brewing time, and water temperature can affect how much caffeine is in green tea.The amino acid L-theanine, which is also found in green tea, has a calming impact on the brain comparable to that of black tea.[37]

Vitamins:

Vitamin C, vitamin B2, and vitamin E are all present in green tea in minute levels.[37]

Minerals:

Potassium, magnesium, and calcium are among the vital elements that green tea is abundant in.[37]

4.2.5 Minor Components of Green Tea

Carbohydrates:

Theanine, glutamic acid, and aspartic acid are just a few of the many amino acids that are abundant in green tea, which also includes trace quantities of carbohydrates including sucrose, glucose, and fructose. In addition, green tea includes volatile compounds that add to its scent, including geraniol, linalool, and nerolidol.[38]

4.2.6 Health Benefits of Green Tea

A group of antioxidants known as catechins, which are abundant in green tea, have been demonstrated to have a protective impact against oxidative stress and damage brought on by free radicals. Epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG) are the four primary catechins present in green tea. The most prevalent and powerful catechin in green tea, EGCG has several health advantages. Chronic illnesses including cancer, heart disease, and neurological disorders can all be prevented by catechins.[38]

Cardiovascular Health:

Catechins, a strong antioxidant found in green tea, help shield the body from the effects of oxidative stress and from free radical harm. Green tea catechins have also been associated to a number of health advantages, including boosting cardiovascular health by lowering blood pressure, increasing lipid profiles, and lowering the risk of heart disease. Green tea can help you lose weight by boosting your metabolism and promoting fat oxidation. By halting the development and division of cancer cells, catechins in green tea have also proven to have anti-cancer capabilities. Both black and green teas have a variety of chemical components that affect its colour, scent, and flavour; the main components are polyphenols, caffeine, theanine, vitamins, and minerals.[38]

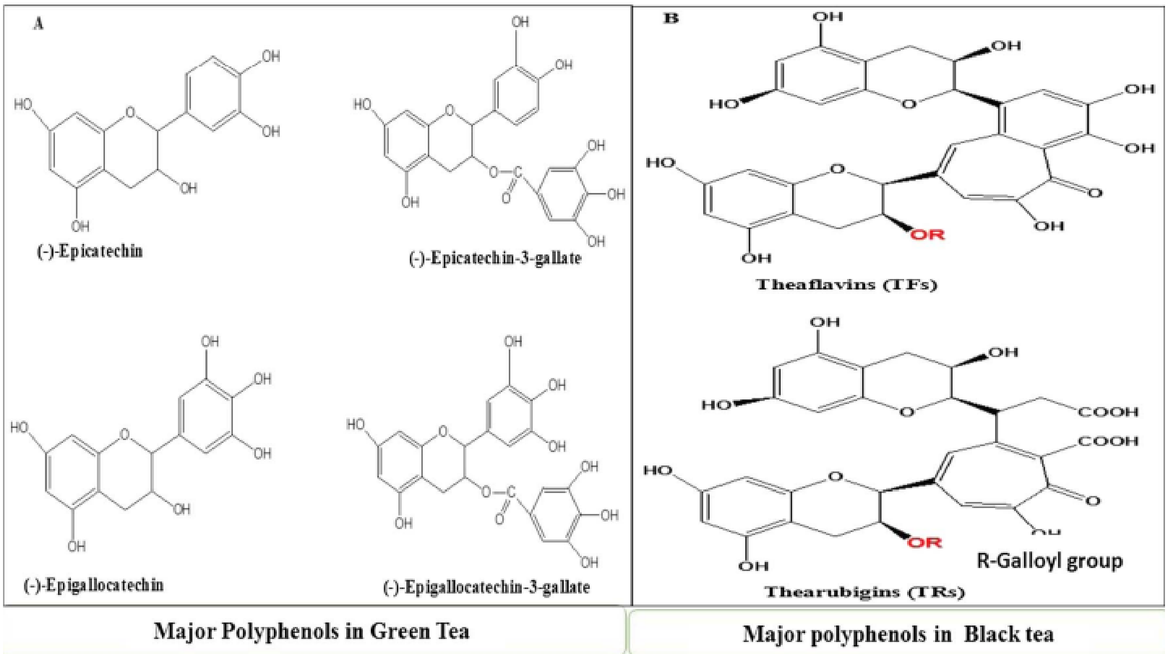


Figure 2 -. Polyphenols in tea The major poly-phenol in green tea [38]

Figure 3 : Tea used for the experiment

4.2.7 Sources:

The *Camellia sinensis* or *Thea sinensis*, which is the scientific name for the tea plant, is indigenous to tropical and temperate Asia, South America, and Africa. It is a member of the Thecae family, which is mostly found in China, India, Sri Lanka, and Japan. Oolong, white, and green teas, as well as black and green, are produced using techniques that modify the chemicals in different ways (fermentation and processing conditions).

Flavonoids (catechins, TFs, and TRs), phenolic acids (CGA, CA, GA, and muramic acid), methylxanthines (caffeine), amino acids (theanine), carbohydrates, lipids, proteins, -carotene, volatile compounds fluoride, and traces of vitamins C, K, A, and folate are among the ingredients found in black tea . hydroxyl-4 flavanols, flavones, flavanols, anthocyanins, and phenolic acids (PA) are additional polyphenols found in tea. More than half of all amino acids, including the most unique amino acid, l-theanine (-flavanols), are also found in black tea. Black tea's flavor and aroma are produced in part by the degradation of L-theanine . Alanine and arginine, two additional amino acids, are responsible for the tea's bitterness.[39]

COMPONENTS	APIs
Catechins Thearubigins	(-)-EGCG
Methylxanthines	Caffeine
Theaflavins	Resulting from the oxidation of cathechins during processing of black tea.
Phenolic acids	CA Quinic acid GA
Amino acids	Theanine

Table - Key components of Black tea [39]

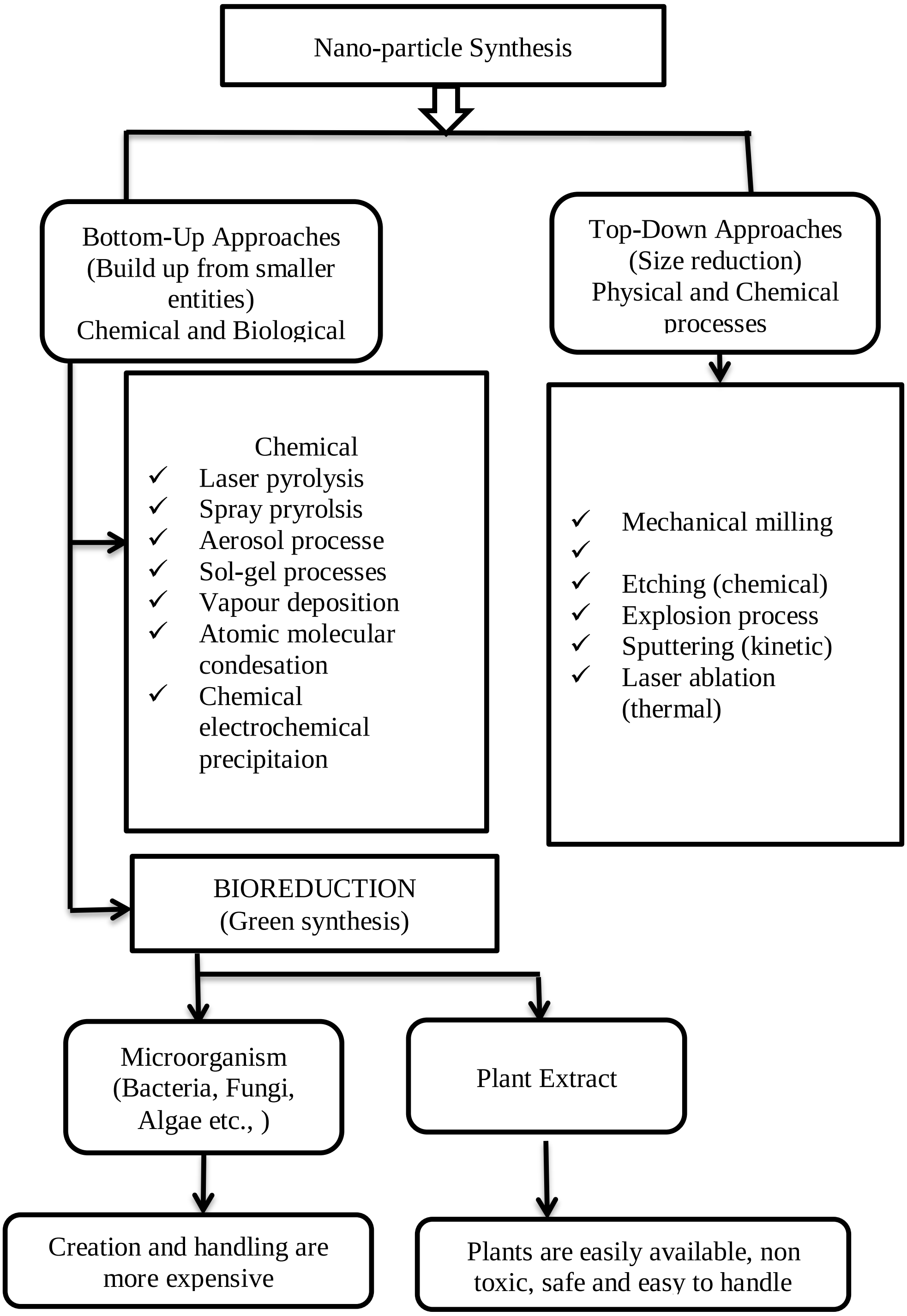
CHAPTER 5

5.1 SYNTHESIS OF NANOPARTICLE

Top-down and bottom-up strategies can both be used to synthesize nanoparticles. The top-down method entails the mechanical, chemical, or physical decomposition of bulk materials into smaller particles. The difficulty in obtaining uniform nanoparticle size and shape places a limit on this method. On the other hand, in the approach known as bottom-up, tiny particles are created from tiny components, like molecules or atoms, and gathered into larger structures. The dimension, shape, and makeup of the leading nanoparticles are highly controllable using this method.

The bottom-up method is applied in the case of silver nanoparticle synthesis mediated by black and green tea. Tea extracts are used in the procedure because they have reducing and stabilizing properties thanks to the polyphenols and flavonoids they contain. The silver ions are converted into nanoparticles by the polyphenols and flavonoids in the tea extracts. As electron donors, the reducing substances in the tea extracts reduce the metal ions to their elemental form. The stabilizing components in the tea extracts create a shield around the nanoparticles to stop them from aggregating or oxidizing.[40]

Figure 4 - Various approaches for fabrication of Metal Nanoparticles. [26]



5.2 OPTIMIZATION OF NANOPARTICLES SYNTHESIS PROCESS

1. **Ph**

   The extract's intrinsic pH was also altered to various pH levels ranging from 3.0 to 9.0. (i.e., 6.0). The color changed visibly after 12 hours. The results show that the ideal pH for tea extract is 6.0, which is its natural ph. It displays an appropriate amount of synthesis under the extract's natural pH circumstances. This shows that the biogenic method of making NPs is inexpensive, simple, and does not need particular temperature conditions.[41]

2. **Temperature**

   At several temperatures, such as 10°C, 37°C, and 55°C, the extract was incubated after being adjusted to its ideal pH value. The visible color changes after 12 hours. ambient room temperature (28.2° C) is the ideal temperature at which tea extract should be controlled. Some of the plant material's enzymes may have been released and activated during the first extraction procedure, which required boiling the material at a high temperature. Similar outcomes where smaller NPs were formed at higher temperatures were reported. The reduction and capping of these nanoparticles was hypothesized to be caused by enzymes that are activated at higher temperatures [41].

   This extract was able to produce NPs later on in the synthesis process at standard room temperature circumstances, demonstrating the viability and efficiency of this biological approach. The ability of biologically created metal nanoparticles to avoid agglomeration and stabilize the particles in the medium is an impressive characteristic. This data implies that the production and stabilization of Agnp in aqueous media may be a function that biological molecules may be able to carry out. It is well known that proteins can attach to NPs through their free amine groups, suggesting that surface-bound proteins may be able to stabilize the NP [41].

3. **Precursor concentration**

   concentration 2, 4, 5, 8, and 10mM concentrations of AgNo3 solution were used to identify the ideal precursor concentration. The extract's color changed visibly after 12 hours. It was discovered that 5mM was the ideal concentration for tea extract. The Ag+ ion concentration in solution, the enzymes produced by the plant extract, and the pH of the solution all affect the crystallite morphologies of the biosynthesized NPs.[41]

It should be emphasized that the particle size is an essential component for optoelectronics and other uses of the nanomaterial, and that the crystallite structure is not the only significant factor determining the properties of metal nanoparticles . Therefore, metal ion concentration was changed to obtain the appropriate NP size.

The most significant dose of that produced an SPR band at 430 nm for NPs generated from tea extract was determined to be 5 mm other concentrations, such as 2 mM, 4 mM, and 8 mM, produced SPR bands at 370 nm and below, showing the absence of NPs, while 10 mM produced an SPR band at 450 nm, suggesting the development of larger-sized NPs.[41]

5.3 METHODOLOGY

Preparation of tea extract Black and Green

Two methods for the experiment

1. Oven dry
2. Microwave dry

Took 1:10 ratio of tea leaf and distilled water respectively.

25g of sample in 250mL of distilled water. Keep it on the hot plate for 10 mins at 60 degrees.

Filtered out using muslin cloth.

PREPARATION OF 1mM $AgNO_3$

Take 0.169g(molecular weight of $AgNO_3$) of $AgNO_3$ in 100mL of distilled water.

Figure 4- 1mM $AgNO_3$

MICROWAVE DRY FOR BLACK TEA

1. Keep the black tea in the microwave for 6 mins at medium temperature.
2. Take 10g of sample in 100mL distilled water.
3. Keep it at the hot plate for 10 min.
4. Filter out by using muslin cloth.
5. Repeat procedure 1 to 4 taking 5g of tea in 50mL of distilled water.

OVEN DRY FOR BLACK TEA

1. Keep the black tea in the oven for 3 hr.
2. Take 10g of sample in 100mL distilled water.
3. Keep it at the hot plate for 10 min.
4. Filter out by using muslin cloth.
5. Repeat procedure 1 to 4 taking 5g of tea in 50mL of distilled water.

Figure 5: Dried Black Tea

Figure 6: Black Tea Extract

MICROWAVE DRY FOR GREEN TEA

1. Keep the green tea in the microwave for 6 mins at medium temperature.
2. Take 10g of sample in 100mL distilled water.
3. Keep it at the hot plate for 10 min.
4. Filter out by using muslin cloth.
5. Repeat procedure 1 to 4 taking 5g of tea in 50mL of distilled water.

OVEN DRY FOR GREEN TEA

1. Keep the green tea in the oven for 3 hr.
2. Take 10g of sample in 100mL distilled water.
3. Keep it at the hot plate for 10 min.
4. Filter out by using muslin cloth.
5. Repeat procedure 1 to 4 taking 5g of tea in 50mL of distilled water.

Figure 7: Dried Green Tea

Figure 8: Green Tea Extract

PREPARATION OF SILVER NANOPARTICLE

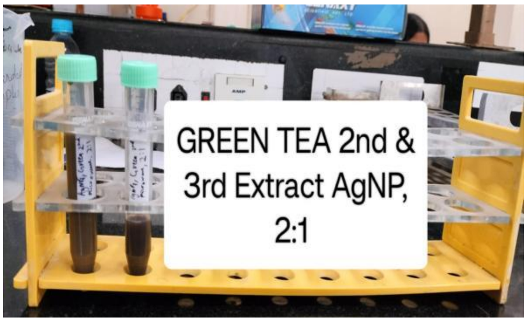
Concentration variation for the preparation of silver nanoparticles

1. 1:1 10mL extract and 10mL $AgNO_3$
2. 1.5:1 15mL extract and 10mL $AgNO_3$
3. 2:1 20mL extract and 10mL $AgNO_3$

From both black and green tea extracts

Figure 9: (a)Black Tea AgNP's (b) Green Tea AgNP's

(c)Black Tea AgNP's



(d) Black Tea 2nd and 3rd Extract AgNP's

(e) Green Tea 2nd and 3rd Extract

5.4 CHARACTERIZATION

Acoustic wave technique, contact angle measurements, electron microscopy, scanning probe microscopy, atomic force microscopy, x-ray diffraction, neutron diffraction, x-ray scattering, x-ray fluorescence spectrometry, and various spectroscopies are some of the methods that can be used to characterize nanomaterials. In numerous articles, the creation and characterization of various nanomaterials are covered, along with their morphological, structural, and mechanical properties.

The most crucial tool for morphological research in the biomedical field is microscopy

For the investigation of morphological properties at the micrometric size scale, optical microscopy, which is based on the transmission of light supported by various glasses and lenses, has traditionally been used extensively.

The last century has seen numerous advancements in our understanding of how electrons interact with matter, whether through diffraction or transmission.

They have supported the advancement of morphological research's resolution quality through the development of scanning and transmission electron microscopy (SEM and TEM).[42]

The electron microscope is a type of microscope that makes an image of the specimen using an electron beam. Compared to a light microscope, it can achieve much higher magnifications and has a higher resolving power, enabling it to see much smaller objects in greater detail.

A microscope that uses an accelerated electron beam as illumination is called an electron microscope.

Due to the fact that an electron's wavelength can be up to 100,000 times shorter than that of photons of visible light Compared to light microscopes, electron microscopes have a higher resolution and can see the details of smaller objects' structures.

When an electron beam interacts with a sample, signals are produced that reveal information about the sample's morphology, composition, and structure.

But the primary characterization is been done using the UV-visible spectroscopy.[42]

UV-visible spectroscopy

In many scientific fields, including bacterial culturing, drug identification, nucleic acid purity checks and quantitation, quality control in the beverage industry, and chemical research, ultraviolet-visible (UV-Vis) spectroscopy is a widely used technique. The operation of UV-Vis spectroscopy, data analysis procedures, the method's advantages and disadvantages, and some applications are covered .[43]

The number of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample is measured by the analytical technique known as UV-Vis spectroscopy. The sample composition has an impact on this property, potentially revealing what is in the sample and at what concentration. Because this spectroscopy method depends on the use of light, let's first consider the properties of light. The energy of light has a certain value that is inversely proportional to its wavelength. As a result, shorter light wavelengths carry more energy while longer ones carry less.

The promotion of electrons in a substance to a higher energy state, which we can observe as absorption, requires a specific amount of energy. In a substance, electrons in various bonding environments require different amounts of energy to move them to higher energy states.

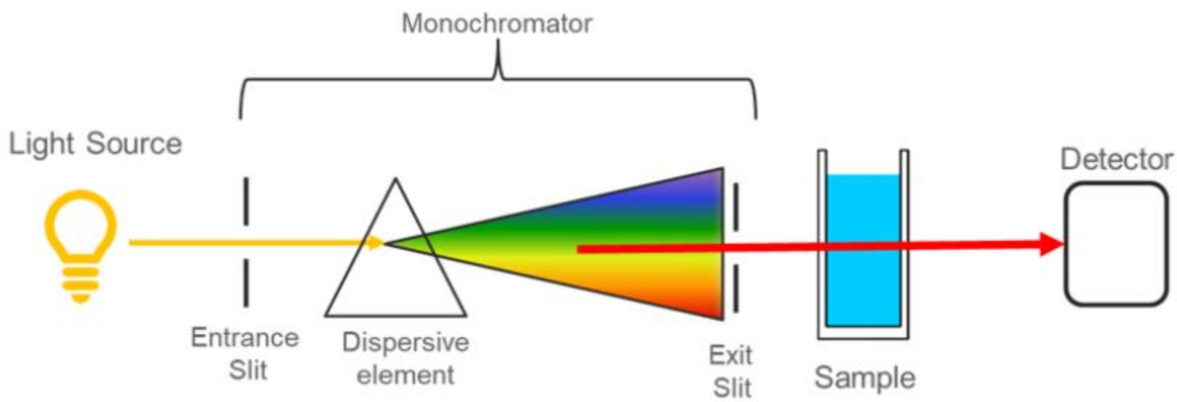
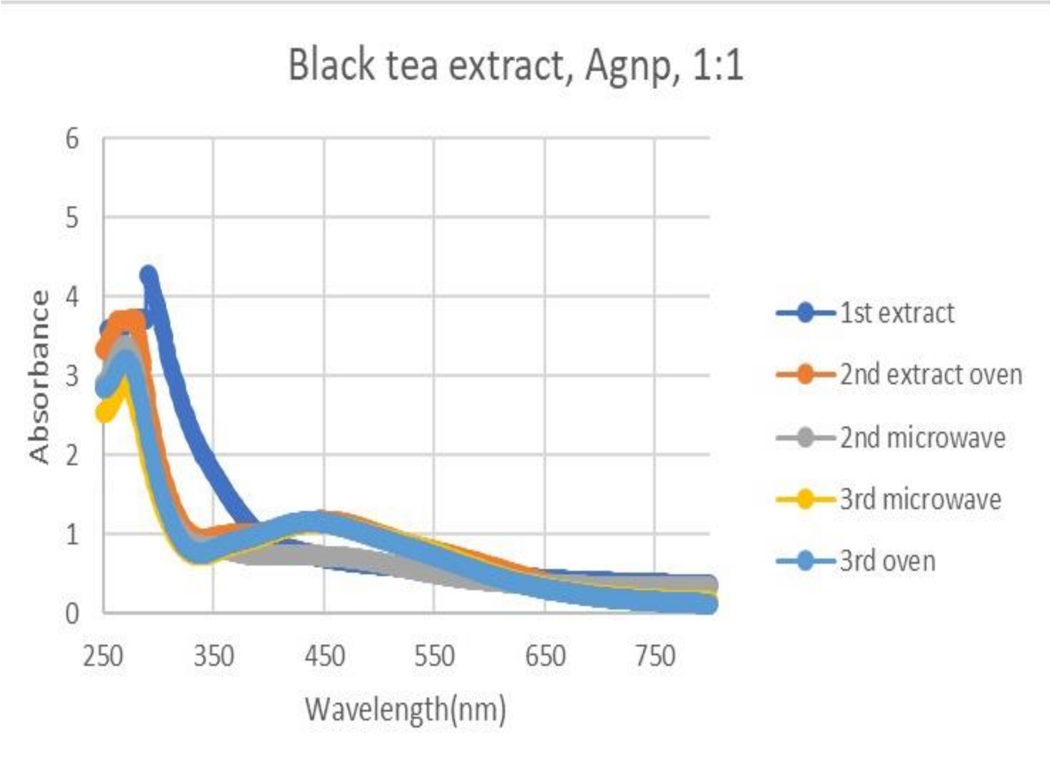


Figure 10 working of UV-Vis spectroscopy [30]

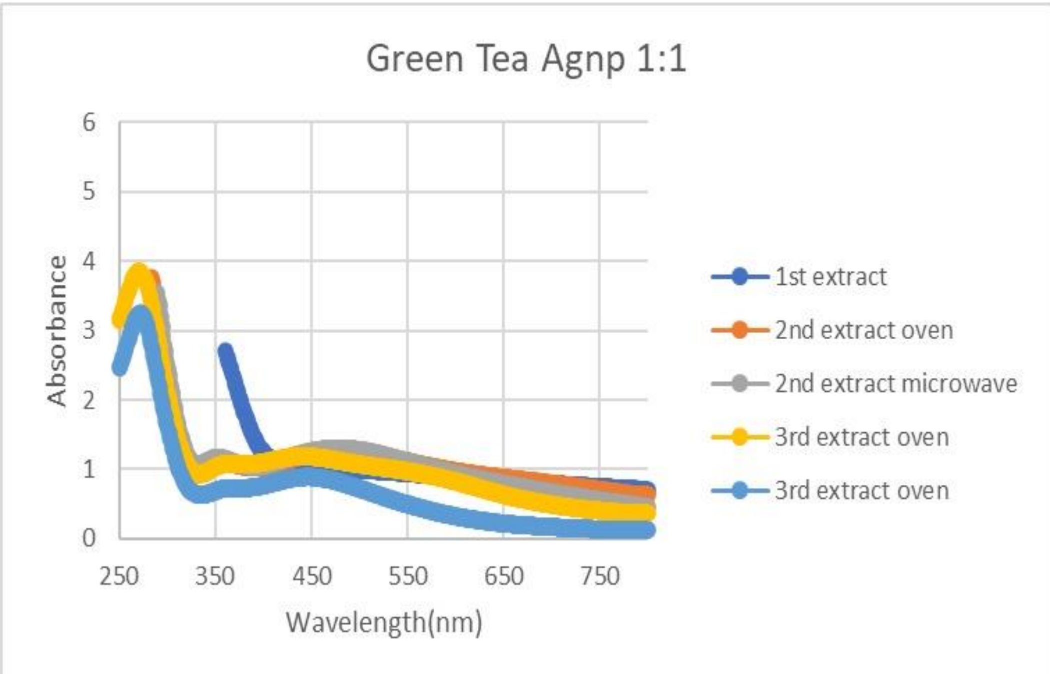
This explains why different substances absorb light at different wavelengths. Humans can see a range of visible light, from about 380 nanometers, which we perceive as violet, to 780 nanometers, which we perceive as red.

UV light has shorter wavelengths than other types of visible light up to about 100 nm. Since light can be described by its wavelength, UV-Vis spectroscopy, which locates the precise wavelengths that correspond to maximum absorbance, can be used to analyze or identify various substances (see the Applications of UV-Vis spectroscopy section).[43]

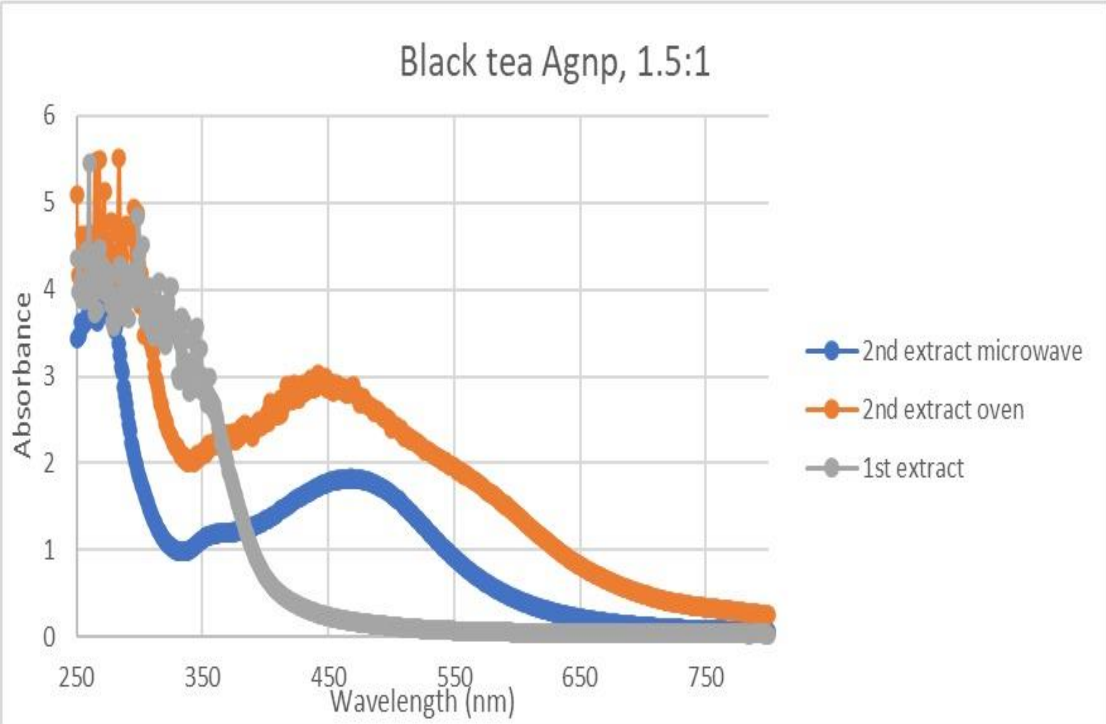
Diluted nanoparticles at the conc. Of 300µm nanoparticle + 2700µm distilled water.

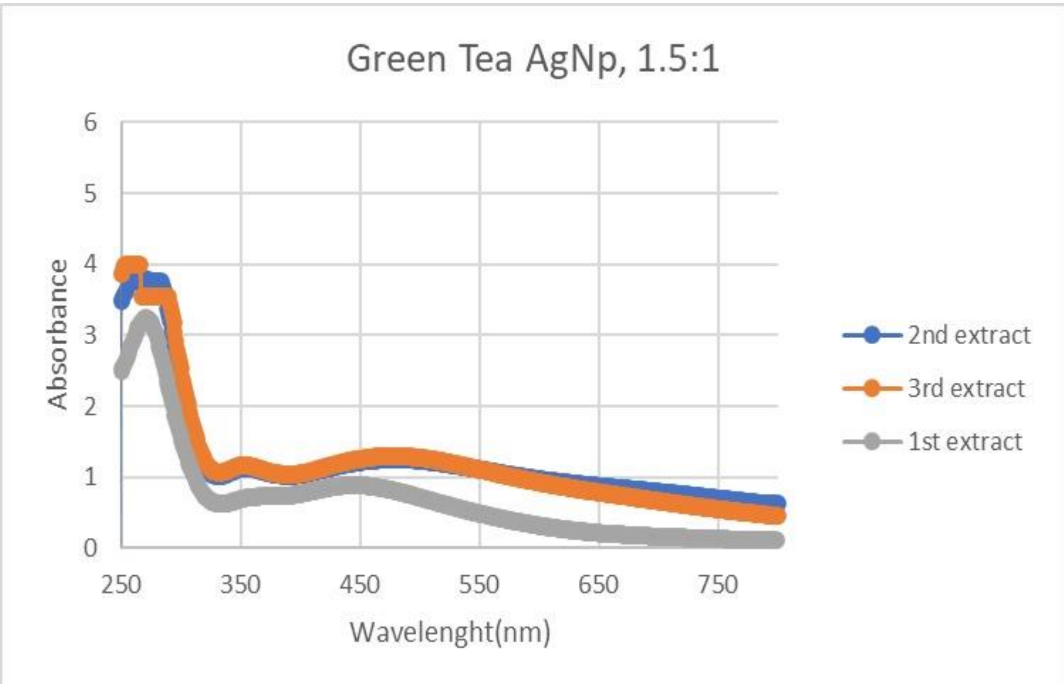


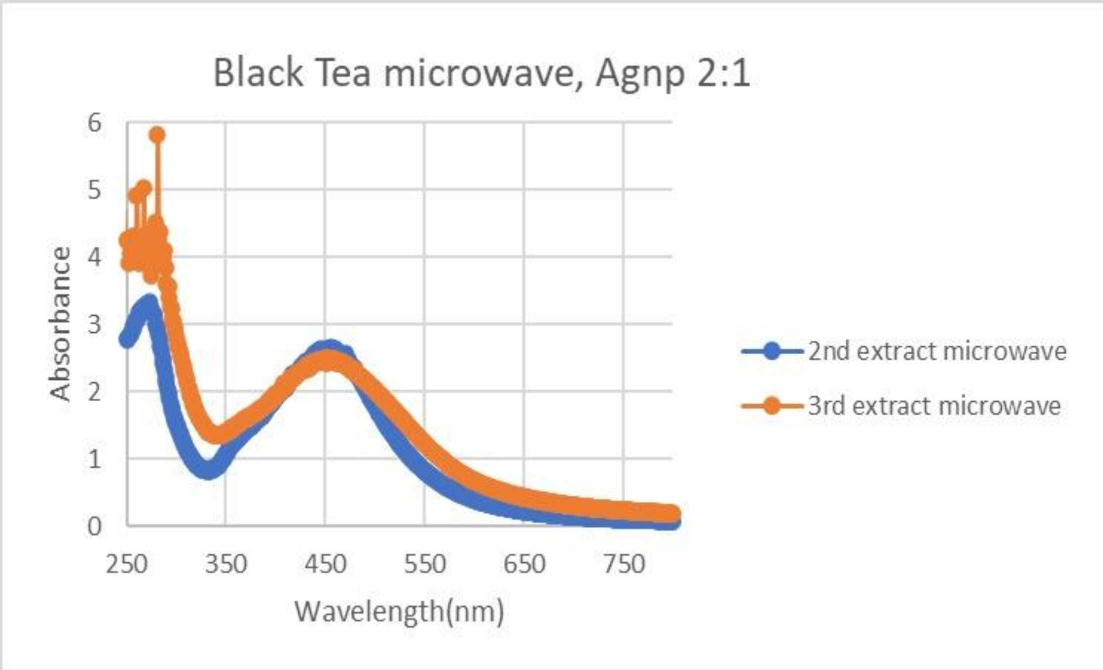
Black tea extract, Agnp, 1:1
Absorbance
0
1
2
3
4
5
6
250
350
450
550
650
750
Wavelength(nm)
1st extract
2nd extract oven
2nd microwave
3rd microwave
3rd oven

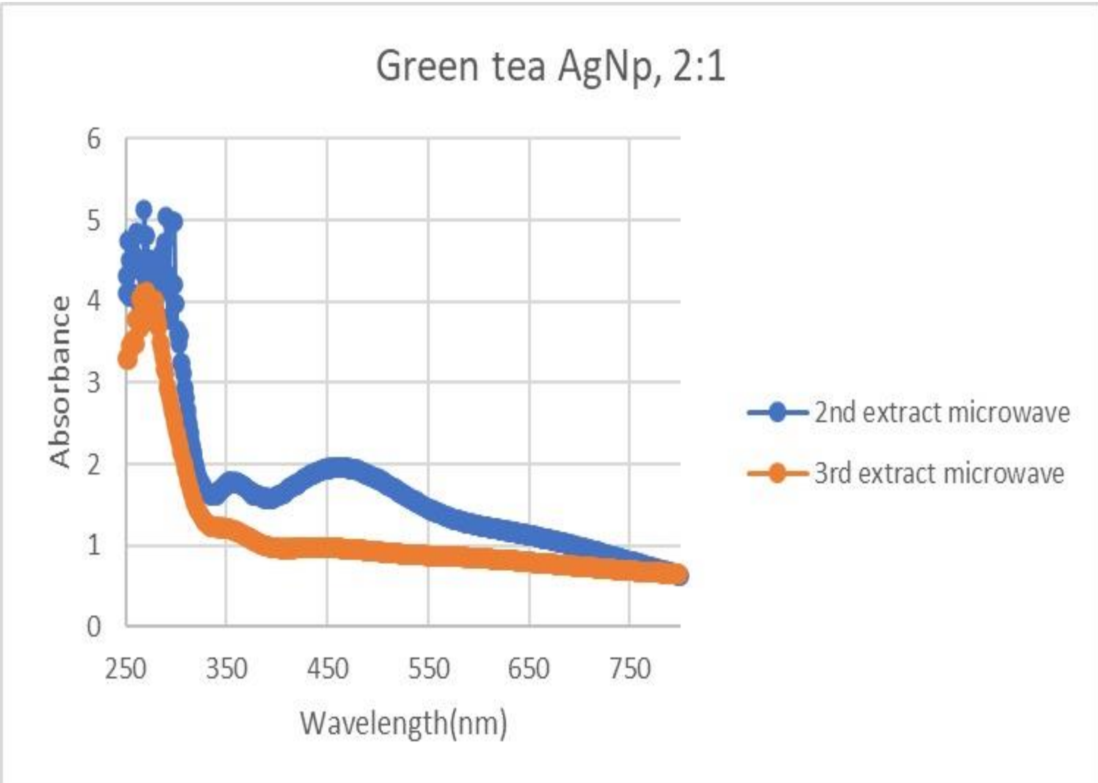


Green Tea Agnp 1:1
Absorbance
0
1
2
3
4
5
6
250
350
450
550
650
750
Wavelength(nm)
1st extract
2nd extract oven
2nd extract microwave
3rd extract oven
3rd extract oven


Black tea Agnp, 1.5:1
Absorbance
Wavelength (nm)
0
1
2
3
4
5
6
250
350
450
550
650
750
2nd extract microwave
2nd extract oven
1st extract


Green Tea AgNp, 1.5:1
Absorbance
Wavelenght(nm)
0
1
2
3
4
5
6
250
350
450
550
650
750
2nd extract
3rd extract
1st extract


Black Tea microwave, Agnp 2:1
Absorbance
0
1
2
3
4
5
6
250
350
450
550
650
750
Wavelength(nm)
2nd extract microwave
3rd extract microwave


Green tea AgNp, 2:1
Absorbance
0
1
2
3
4
5
6
250
350
450
550
650
750
Wavelength(nm)
2nd extract microwave
3rd extract microwave

CHAPTER 6

6.1 APPLICATIONS OF SILVER NANOPARTICLE

The physicochemical characteristics of silver nanoparticles, such as their high melting point temperature, magnetic, electrical and thermal conductivity, light absorption, and high heat transfer, have drawn a lot of attention. Silver nanoparticles have been employed as an antibacterial agent because of their high surface-to-volume ratio and simple interaction with other particles to increase their antimicrobial efficacy. When compared to other metallic nanoparticles, silver nanoparticles have a high degree of reactivity.

Due to their unique qualities and small size, silver nanoparticles find significant use in heat transfer systems, sensors, high strength materials, catalysts, antimicrobial materials, sensors, high strength materials, sensors, high strength materials, high strength materials, etc. We talked about regulating the growth temperature during the synthesis of Cu nanoparticles from a single precursor. The development process is greatly aided by the selective absorption of oleyl amine.[43]

Due to the fact that some microorganisms are resistant to more traditional antimicrobial treatments, water pollution by germs poses a serious risk to human health. Water treatment facilities have used silver nanoparticles as a disinfectant.

Effective antibacterial activity is provided by silver nanoparticles stabilized on carbon, polymers, sepiolite, and polyurethane foam. Silver nanoparticles have been used to treat B. subtilis because of their high affinity for surface-active bacterial groups (Ruparelia et al. 2008). Due to their high surface-to-volume ratio, continuously renewable surface, and fluctuating microelectrode potential values, nanoparticles are also frequently used as catalysts. Silver nanoparticles that are stable have good catalytic qualities.[44]

Due to its poor performance and significant nitrogen oxide emission, the use of biodiesel in car engines is restricted. For diesel engines using soy-based biodiesel, nano silver particles were employed as a fuel additive (B10). The electrochemical approach was used to create silver nanoparticles, which ranged in size from 40 to 50 nm. When compared to other formulations, nano silver particles demonstrate improved engine performance and decreased nitrogen oxide emission.[45]

Metal nanoparticles' antimicrobial effects have drawn a lot of interest from a medical and technological perspective. In applications like water treatment, food processing, and the defense of medical equipment, metallic Cu particles were used as an anti-infective agent to replace Ag and other noble metal composites.[46]

Outstanding anti-bacterial activity against two Gram-negative and Gram-positive strains is demonstrated by silver nanoparticles that have been functionalized with glutamine and created using an injection pump-assisted reaction environment and in situ redux condensation. The glucosamine functionalized silver nanoparticles are spherical in shape with a hybrid morphology, according to the dynamic and structural light scattering.

Due to its unique qualities, such as biocompatibility, high stability against aggregation, and hydrophilic nature, metallic nanoparticles and their composites offer flexible interface activity in biological and biosensing applications. Despite the fact that metallic silver's bulk microstructures and nanostructures have different uses, functionalized silver nanoparticles exhibit cutting edge characteristics like high thermal stability, electrochemical viability for biomolecule (C-reactive protein, CRP) detection, and increased crystallinity.[47]

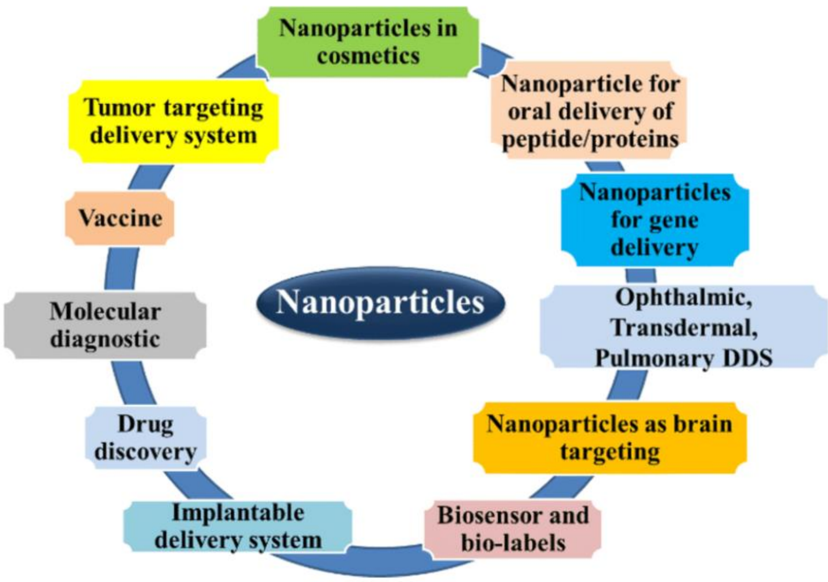


Figure 11- showing the applications of nanoparticles[47]

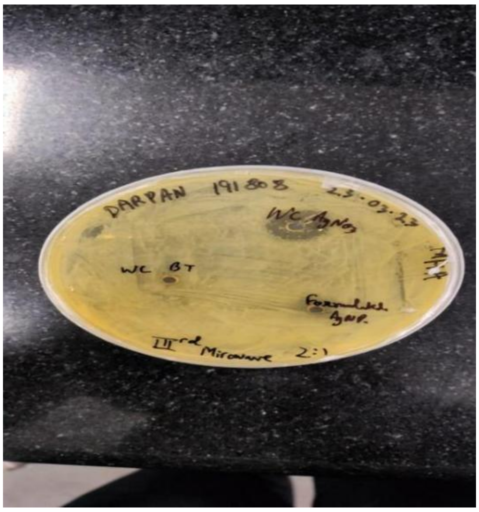
After understanding the various application of silver nanoparticles, we as a team decided to test the Antibacterial activity against the coli strain DH5- alpha, using MHA media.

MHA Media Preparation using autoclave

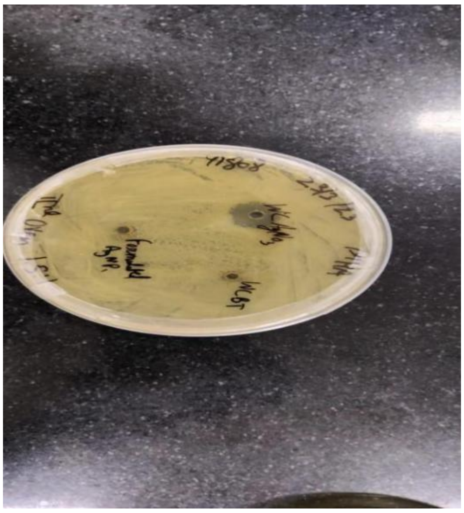
1. MHA powder should be weighed out in accordance with the manufacturer's instructions before being added to a sterile, clean flask.
2. The MHA powder should be thoroughly mixed into the flask before the appropriate volume of distilled water is added.
3. put the flask containing the MHA solution into an autoclave and run a sterilization cycle in accordance with the manufacturer's instructions.
4. Once the MHA media has been sterilized, let it cool to room temperature before transferring it as needed into sterile petri dishes or tubes.

Antibacterial activity of silver nanoparticles synthesized by black and green tea leaves on E. coli DH5-alpha:

1. Use black and green tea leaves in accordance with the established protocol to create the silver nanoparticles.
2. Make an E. coli DH5-alpha suspension in sterile saline
3. A small amount of bacterial strain was dispersed on the plate and wells were formed.
4. Spend a few minutes letting the nanoparticles spread out onto the disc or into the agar.
5. Apply the E. coli DH5-alpha suspension as an inoculum to the agar plate's or the disc's surface using sterile techniques.
6. Incubate the plate at 37°C for 24 hours.
7. Observe the zone of inhibition around the disc or on the agar plate to determine the antibacterial activity of the synthesized nanoparticles.

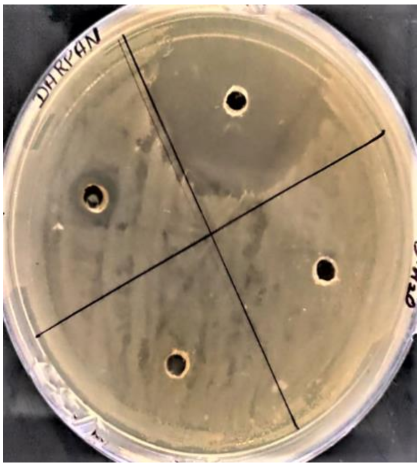


(a)



(b)

(c)



(d)

Figure 12: MHA plate

CHAPTER 7

7.1 FUTURE PERSPECTIVE AND CHALLENGES

Due to their excellent physicochemical properties, high electrical conductivity, biocompatibility, surface activity, etc., silver-based nanoparticles are promising for a variety of applications. While many synthesis techniques have been addressed in this study, the necessity to concentrate on straightforward and efficient metallic NP synthesis remains. Controlling the form, size, and morphology of the nanoparticles is vital for creating and optimizing silver nanoparticles with particular properties suitable for product development. Reaction factors such as temperature, pressure, duration, pH, etc., must be adjusted.

AgNP's are beneficial for creating innovative formulations for plant protection, such as Agnp-based nano pesticides, nano herbicides, and nano fertilizers. Numerous studies have also demonstrated that AgNP's can be effective against fungus and insect pests of agricultural plants. These formulations are more cost-effective and can lessen the toxicity problem brought on by excessive pesticide application. Potential applications for Agnp-based biosensors include the control of pests and the identification of illnesses that lead to food spoilage. [48]

7.2 CONCLUSION

One of the most significant and beneficial metal nanoparticles are silver. For several processes, such as catalytic reduction, they are commonly used as catalysts. In this review, eco-friendly approaches to the synthesis of silver nanoparticles have been outlined because the formation of oxides makes it challenging to produce and stabilize metallic nanoparticles

It is preferable to obtain nanoparticles from green sources because they act as both the stabilizing and reducing moieties. Depending on the circumstances, the produced nanoparticles exhibit a variety of plasmonic peaks. The cited applications show that silver nanoparticles have numerous uses. Silver is a noble metal, but nanoparticles are poisonous.

Top-down (physical approach) and bottom-up (chemical and biological method) methods can be used to synthesize nanoparticles, respectively. The physical approach of making nanoparticles calls for pricey machinery, extremely high temperatures, and vacuum systems. Although biological techniques are also utilized to create nanoparticles, they are ineffective for producing nanoparticles due to a lack of understanding and experience.[49]

Chemical methods are used to create nanoparticles because they are low-cost, easy to use, very flexible, easy to find equipment for, don't require vacuum systems, are environmentally benign, and produce high yields in ambient settings. According to a thorough analysis of chemical processes, silver nanoparticles can be synthesized using electrochemistry, and there are numerous techniques for doing so when employing chemical reduction. However, the manufacture is expensive due to the usage of an inert gas atmosphere to avoid oxidation and many poisonous and expensive chemicals as reducing agents. In this instance, a straightforward and inexpensive electrochemical technique is chosen to produce silver nanoparticles more quickly.[50]

The main benefit of this approach is that it eliminates the need for inert gas atmosphere, hazardous chemicals, high pressure, and energy while allowing control over the form, size, and morphology of silver nanoparticles.

The influence of pH, among other factors, has yet to be optimized for silver nanoparticle formation. Some accounts mention synthesis at high pH. Therefore, it is important to evaluate silver nanoparticle behavior throughout a broad pH range. The mechanism of green synthesis has been characterized using a variety of methods.

This field needs attention because polymeric stabilized solutions for silver nanoparticles have not been extensively studied. The cytotoxicity of silver nanoparticles has been well characterized, but more research into their use in electrodes and electronic applications is possible. It is hoped that these environmentally friendly nanoparticles will improve the conductivity of various electrodes.[51]

Due to their numerous special qualities, NPs have prospective uses in a variety of industries, including pest control, environmental management, electronics, etc. Additionally, Silver-based nanoparticles affect the biotic and abiotic elements of the environment in both favorable and unfavorable ways.

Silver nanoparticles, however, may be hazardous to the environment and other creatures when present in higher concentrations.

To lessen the toxicity of metal oxide nanoparticles, various research on surface modification, including novel low-toxicity chemicals, size, dissolution factor, and selection of an appropriate exposure route have to be examined.[52]

REFRENCES

[1] S. Link et al., “Laser-Induced shape changes of colloidal gold nanorods using femtosecond and nanosecond laser pulses,” *J. Phys. Chem. B*, vol. 104, no. 26, pp. 6152-6163, 2000 [doi:10.1021/jp000679t].

[2] N. V. Tarasenko et al., “Synthesis of nanosized particles during laser ablation of gold in water,” *Appl. Surf. Sci.*, vol. 252, no. 13, pp. 4439-4444, 2006 [doi:10.1016/j.apsusc.2005.07.150].

[3] M. Kawasaki and N. Nishimura, “1064-nm laser fragmentation of thin au and Ag flakes in acetone for highly productive pathway to stable metal nanoparticles,” *Appl. Surf. Sci.*, vol. 253, no. 4, pp. 2208-2216, 2006 [doi:10.1016/j.apsusc.2006.04.024].

[4] T. Tsuji et al., “Preparation of silver nanoparticles by laser ablation in solution: Influence of laser wavelength on particle size,” *Appl. Surf. Sci.*, vol. 202, no. 1-2, pp. 80-85, 2002 [doi:10.1016/S0169-4332(02)00936-4].

[5] T. Tsuji et al., “Preparation of nanosized particles of silver with femtosecond laser ablation in water,” *Appl. Surf. Sci.*, vol. 206, no. 1-4, pp. 314-320, 2003 [doi:10.1016/S0169-4332(02)01230-8].

[6] D.-C. Tien et al., “Discovery of ionic silver in silver nanoparticle suspension fabricated by arc discharge method,” *J. Alloys Compd.*, vol. 463, no. 1-2, pp. 408-411, 2008 [doi:10.1016/j.jallcom.2007.09.048].

[7] J. Siegel et al., “Progressive approach for metal nanoparticle synthesis,” *Mater. Lett.*, vol. 89, pp. 47-50, 2012 [doi:10.1016/j.matlet.2012.08.048].

[8] B. Wiley et al., “Shape-controlled synthesis of metal nanostructures: The case of silver,” *Chemistry*, vol. 11, no. 2, pp. 454-463, 2005 [doi:10.1002/chem.200400927].

[9] D. D. Evanoff and G. Chemanol, “Size-controlled synthesis of nanoparticles. 2. measurement of extinction, scattering, and absorption cross sections,” *J. Phys. Chem. B*, vol. 108, no. 37, pp. 13957-13962, 2004 [doi:10.1021/jp0475640].

[10] G. Merge et al., “Redox Catalysis on ‘naked’ silver nanoparticles,” *J. Phys. Chem. C*, vol. 111, no. 33, pp. 12220-12226, 2007 [doi:10.1021/jp074257w].

[11] M. M. Oliveira et al., “Influence of synthetic parameters on the size, structure, and stability of do decanethiol-stabilized silver nanoparticles,” *J. Colloid Interface Sci.*, vol. 292, no. 2, pp. 429-435, 2005 [doi:10.1016/j.jcis.2005.05.068].

[12] M. Brust and C. J. Kiely, “Some recent advances in nanostructure preparation from gold and silver particles: A short topical review,” *Colloids Surf Phys. Eng. Aspects*, vol. 202, no. 2-3, pp. 175-186, 2002 [doi:10.1016/S0927-7757(01)01087-1].

[13] D. Kim et al., “Synthesis of silver nanoparticles using the polyol process and the influence of precursor injection,” *Nanotechnology*, vol. 17, no. 16, p. 4019-4024, 2006 [doi:10.1088/0957-4484/17/16/004].

[14] Y. Zhang et al., “Facile preparation and characterization of highly antimicrobial colloid Ag or Au nanoparticles,” *J. Colloid Interface Sci.*, vol. 325, no. 2, pp. 371-376, 2008 [doi:10.1016/j.jcis.2008.05.063].

[15] M. Chen et al., “Silver nanoparticles capped by oleyl amine: Formation, growth, and self-organization,” *Langmuir*, vol. 23, no. 10, pp. 5296-5304, 2007 [doi:10.1021/la700553d].

[16] A. Troupis et al., “Synthesis of metal nanoparticles by using polyoxometalates as photocatalysts and stabilizers,” *Agnew. Chem. Int. Ed. Engl.*, vol. 41, no. 11, pp. 1911-1914, 2002 [doi:10.1002/1521-3773(20020603)41:11<1911::AID-ANIE1911>3.0.CO;2-0].

[17] E. E. Agnew et al., “Bioactive compounds from medicinal plants: Focus on Piper species,” *S. Afr. J. Bot.*, vol. 112, pp. 54-69, 2017. doi:10.1016/j.sajb.2017.05.007.

[18] S. Mishra et al., “Potential of biosynthesized silver nanoparticles using *Stenotrophomonas* sp. BHU-S7 (MTCC 5978) for management of soil-borne and foliar phytopathogens,” *Sci. Rep.*, vol. 7, p. 45154, 2017. doi:10.1038/srep45154.

[19] Y. K. Mohanta et al., “Allah AH, Al Qarawi AA, Dhananjay Yadav TKM,” *Molecules*, vol. 23, pp. 1-18, 2018 Silver nanoparticles synthesized using wild mushroom show potential antimicrobial activities against food borne pathogens. doi:10.1007/s11164-016-2481-3.

[20] P. Nikolopoulos-Stamati et al., "Chemical pesticides and human health: The urgent need for a new concept in agriculture," *Front. Public Health*, vol. 4, pp. 148, 2016. doi:10.3389/fpubh.2016.00148.

[21] K. Nishiwaki et al., "Structure–activity relationships and docking studies of hydroxychavicol and its analogs as xanthine oxidase inhibitors," *Chem. Pharm. Bull. (Tokyo)*, vol. 66, no. 7, pp. 741-747, 2018. doi:10.1248/cpb.c18-00197.

[22] S. M. Ofuda, "Antifungal activity of silver and copper nanoparticles on two plant pathogens, *Alternaria alternate* and *Botrytis cinerea*," *Res. J. Microbial.*, vol. 9, no. 1, pp. 34-42, 2014. doi:10.3923/jm.2014.34.42.

[23] S. Panda et al., "Antithyroid and hepatoprotective properties of high-resolution liquid chromatography–Mass spectroscopy-standardized Piper beetle leaf extract in rats and analysis of its main bioactive constituents," *Pogo. Mag.*, vol. 14, no. 59, pp. 658-664, 2018. doi:10.4103/pm.pm_450_18.

[24] V. Parashar et al., "Parthenium leaf extract mediated synthesis of silver nanoparticles: A novel approach towards weed utilization," *Dig. J. Nanometer. Biostructures*, vol. 4, pp. 45-50, 2009. Google Scholar.

[25] S. Parveen et al., "Preparation, characterization and antifungal activity of iron oxide nanoparticles," *Microbe. Pathos.*, vol. 115, pp. 287-292, 2018. doi:10.1016/j.micpath.2017.12.068.

[26] S. Pawar et al., "Biochemical profiling of antifungal activity of betel leaf (*Piper beetle* L.) extract and its significance in traditional medicine," *J. Adv. Res Biotechnology.*, vol. 2, pp. 1-4, 2017. doi:10.15226/2475-4714/2/1/00116.

[27] P. Phum at et al., "Effects of *Piper beetle* fractionated extracts on inhibition of *Streptococcus mutans* and *Streptococcus intermedius*," *Drug Disco. Ther.*, vol. 12, no. 3, pp. 133-141, 2018. doi:10.5582/ddt.2018.01021.

[28] R. Prasad et al., "Nanotechnology in sustainable agriculture: Recent developments, challenges, and perspectives," *Front. Microbial.*, vol. 8, pp. 1014, 2017. doi:10.3389/fmicb.2017.01014.

[29] K. F. Princy and A. Gopinath, "Optimization of physicochemical parameters in the bio

fabrication of gold nanoparticles using marine macroalgae *Padina tetrachromatic* and its catalytic efficacy in the degradation of organic dyes," *J. Nanostructure Chem.*, vol. 8, no. 3, pp. 333-342, 2018. doi:10.1007/s40097-018-0277-2.

[30] J. B. Penury et al., "*Piper beetle*-mediated green synthesis of biocompatible gold nanoparticles," *Int. Nano Lett.*, vol. 2, no. 1, p. 18, 2012. doi:10.1186/2228-5326-2-18.

[31] M. Rafique et al., "Novel and facile synthesis of silver nanoparticles using *Albizia proceri* leaf extract for dye degradation and antibacterial applications," *Mater. Sci. Eng. C Mater. Biol. Appl.*, vol. 99, pp. 1313-1324, 2019. doi:10.1016/j.msec.2019.02.059.

[32] S. Rajaram Mohan et al., "Genetic architecture of resistance to *Alternaria brassica* in *Arabidopsis thaliana*: QTL mapping reveals two major resistance-conferring loci," *Front. Plant Sci.*, vol. 8, pp. 260, 2017. doi:10.3389/fpls.2017.00260.

[33] P. Rajasekhar Reddy and P. U. Rani, "Biosynthesis and characterization of Pd and Pt nanoparticles using *Piper beetle* L. plant in a photoreduction method," *J. Clusty. Sci.*, vol. 25, no. 5, pp. 1377-1388, 2014. doi:10.1007/s10876-014-0715-3.

[34] K. Ramachandran et al., "A facile green synthesis of silver nanoparticles using *Piper beetle* biomass and its catalytic activity toward sensitive and selective nitrite detection," *J. Ind. Eng. Chem.*, vol. 35, pp. 29-35, 2016. doi:10.1016/j.jiec.2015.10.033.

[35] D. Restani et al., "Nanotechnology-based recent approaches for sensing and remediation of pesticides," *J. Environ. Manage.*, vol. 206, pp. 749-762, 2018. doi:10.1016/j.jenvman.2017.11.037.

[36] M. Samet et al., "Effect of compost tea containing phosphonyls on potato plant growth and protection against *Fusarium solan* infection," *Environ. Sci. Pollute. Res. Int.*, vol. 25, no. 19, pp. 18921-18937, 2018. doi:10.1007/s11356-018-1960-z.

[37] M. Sathishkumar et al., "*Cinnamon zeylanicum* bark extract and powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity," *Colloids Surf. B Bio interfaces*, vol. 73, no. 2, pp. 332-338, 2009. doi:10.1016/j.colsurfb.2009.06.005.

[38] N. N. Swazi et al., "Antioxidant and cytoprotective activities of *Piper beetle*, *Areca catechu*, *Unchair gambit* and betel quid with and without calcium hydroxide," *BMC Complement. Altern. Med.*, vol. 13, p. 351, 2013. doi:10.1186/1472-6882-13-351.

[39] S. K. Shah and D. N. Jhade, “Evaluation of antifertility potential of *Piper beetle* (petiole) on female Wistar rats ‘rising approaches of herbal contraception’,” *Beachem. Biopsy’s. Rep.*, vol. 15, pp. 97-102, 2018. doi:10.1016/j.bbrep.2018.08.001.

[40] S. Shah et al., “Biofilm inhibition and anti-quorum sensing activity of photosynthesized silver nanoparticles against the nosocomial pathogen *Pseudomonas aeruginosa*,” *Biofouling*, vol. 35, no. 1, pp. 34-49, 2019. doi:10.1080/08927014.2018.1563686.

[41] A. Shahzad et al., “Size-controlled production of silver nanoparticles by *Aspergillus fumigatus* BTCB10: Likely antibacterial and cytotoxic effects,” *J. Nanometer.*, vol. 2019, pp. 1-14, 2019. doi:10.1155/2019/5168698.

[42] D. Singh et al., “Hydroxychavicol, a key ingredient of *Piper beetle* induces bacterial cell death by DNA damage and inhibition of cell division,” *Free Radic. Biol. Med.*, vol. 120, pp. 62-71, 2018a. doi:10.1016/j.freeradbiomed.2018.03.021.

[43] T. P. Singh et al., “*In vitro* study on antimicrobial, antioxidant, FT-IR and GC – MS / MS analysis of *Piper beetle* L.”. leaves extracts, *J. Food Meas. Charact.*, vol. 13, pp. 1-10, 2018b. doi:10.1007/s11694-018-9960-8.

[44] S. K. Srikar et al., “Green synthesis of silver nanoparticles: A review,” *Green Sustain. Chem.*, vol. 06, no. 1, pp. 34-56, 2016. doi:10.4236/gsc.2016.61004.

[45] S.”. et al., “Synergistic antifungal effects of thyme essential oil and *Lactobacillus plantarum* cell-free supernatant against *Penicillium* spp. and in situ effects,” *J. Food Process. Preserve.*, vol. 42, no. 1, pp. 1-8, 2018. doi:10.1111/jfpp.13400.

[46] B. Sumi Maria et al., “Synthesis of silver nanoparticles using medicinal *Ziziphus pylorus* bark extract,” *Appl. NanoSQUID.*, vol. 5, no. 6, pp. 755-762, 2015. doi:10.1007/s13204-014-0372-8.

[47] X. Sun et al., “Effects of silver nanoparticles on microbial community structure in activated sludge,” *Sci. Total Environ.*, vol. 443, pp. 828-835, 2013. doi:10.1016/j.scitotenv.2012.11.019.

[48] A. Verma and M. S. Mehata, “Controllable synthesis of silver nanoparticles using neem leaves and their antimicrobial activity,” *J. Radiat. Res. Appl. Sci.*, vol. 9, no. 1, pp. 109-115, 2016. doi:10.1016/j.jrras.2015.11.001

[49] A. Verma et al., “Green nanotechnology: Advancement in Phyto formulation research,” *Medicines (Basel)*, vol. 6, no. 1, p. 39, 2019. doi:10.3390/medicines6010039.

[50] B. Xue et al., “Biosynthesis of silver nanoparticles by the fungus *Erythroderma fulva* and its antifungal activity against genera of *Candida*, *Aspergillus* and *Fusarium*,” *Int. J. Nanomedicine*, vol. 11, p. 1899-1906, 2016. doi:10.2147/IJN.S98339.

[51] M. Amin et al., “Green synthesis of silver nanoparticles through reduction with *Solanum Artocarpus* L. berry extract: Characterization, antimicrobial and urease inhibitory activities against helicobacter pylori,” *Int. J. Mol. Sci.*, vol. 13, no. 8, pp. 9923-9941, 2012. doi:10.3390/ijms13089923.

[52] K. Anandalakshmi et al., “Characterization of silver nanoparticles by green synthesis method using *Pedalium murex* leaf extract and their antibacterial activity,” *Appl. NanoSQUID.*, vol. 6, no. 3, pp. 399-408, 2016. doi:10.1007/s13204-015-0449-z.