2017-10-13

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING MANGO LEAF EXTRACT AND EVALUATION OF ITS ANTIBACTERIAL ACTIVITIES

AGEGNEHU, GASHAW

BAHIR DAR UNIVERSITY

COLLEGE OF SCIENCE

DEPARTMENT OF CHEMISTRY

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING MANGO LEAF EXTRACT AND EVALUATION OF ITS ANTIBACTERIAL ACTIVITIES

BY: AGEGNEHU GASHAW

BAHIR DAR, ETHIOPIA

AUGUEST 2017

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING MANGO LEAF EXTRACTS AND EVALUATION OF ITS ANTIBACTERIAL ACTIVITIES

A Thesis Submitted to the department of chemistry in partial fulfilment of the requirements for the degree of Master of Science in Chemistry.

BY: AGEGNEHU GASHAW

ADVISOR: MULUKEN AKLILU (PhD)

DEPARTMENT OF CHEMISTRY COLLEGE OF SCIENCE

BAHIR DAR UNIVERSITY

AUGUEST 2017

Thesis Approval Sheet

As members of the board of examiners for the MSc thesis open defense examination, we certify that we have read and evaluated the thesis prepared by *Agegnehu Gashaw* and examined the candidate. We recommended the thesis to be accepted as fulfilment for the requirements of the Degree of Master of Science in chemistry.

Board of Examiners

Advisor	Signature	Date
Dr. Muluken Aklilu	_____	_____
External examiner		
Dr. ----------	_____	_____
Internal examiner		
Dr. ----------	_____	_____
Chair man		
Dr. ----------	_____	_____

AUGUEST 2017

Bahir Dar, Ethiopia

Declaration

I hereby declare that the work is being presented in this thesis entitled **"**Green synthesis of Silver nanoparticles using Mango leaf extracts and evaluation of its antibacterial activities**",** that I submitted to the Department of Chemistry, Bahir Dar University in partial fulfilment of the Master Degree in chemistry is a record of bonafied and original research work carried out by me under the guidance and supervision of Dr. Muluken Aklilu, Department of chemistry, Bahir Dar University. To the best of my knowledge no part of this thesis has been submitted to any other university or institution for the award of any degree or diploma.

Name: Agegnehu Gashaw
Signature:
Place: Science Collage
Bahir Dar University
Date of Submission: August 2017

ACKNOWLEDGEMENTS

First and most I just want to thank my almighty God who gave me the strength and patience throughout the work.

I would like to express my deepest gratitude to my advisor Dr. Muluken Aklilu for his day to day follow up, valuable suggestions, advice, encouragement and ultimate help to the various stage of this thesis work.

I would like to thank the staff members of chemistry department of Bahir Dar University; Dr. Alemu Tesfaye, and Dr. Belete Asefa for their comment, advices and support.

I would like to thank Kiflom G/medhn for his valuable material support and sharing ideas.

My heart-felt word of thanks also goes to my friends Yalew Chekol, Melaku Tarkegne, Molla Zwedie, Yiktel Adege and G/selassie G/medhin for their friendly co-operation, support and advice in various ways.

I also thank the laboratory technicians of the Chemistry department; Mr. Kidane Teklay, for his material and technical support throughout my work. The same goes to Mr. Misganaw Liyew, laboratory technician of the Biology department for their technical support during the antibacterial test for the nanoparticle.

I would like to thank Department of Chemistry, Bahir Dar University for letting me use the laboratory with its chemicals, materials and instruments necessary for the work.

I also thank the Department of Material Science and Engineering of Bahir Dar University who gave the permission to use the UV-Vis spectrometer.

I would like to thank West Belessa Educational sector for giving me permission to doing my work.

I would like to thank Ministry of Education for giving me this sponsor and funding the require budget to accomplish my thesis.

Abstract

In the present investigation a fast, convenient and environment friendly method has been used for the synthesis of silver nanoparticles (AgNPs) by biologically reducing 30 ml of 4 mM $AgNO_3$ solution with extract of 2 ml of 5 % (m/v) mango leaf under optimum conditions (pH of 11). The formation of silver nanoparticles was indicated by the colour changes from colourless to light yellow then to yellow brown and finally reddish brown. Biosynthesized AgNPs were also characterized by UV-Visible and FT-IR spectroscopy. The reduction process was simple and convenient to handle and was monitored by UV-Visible spectroscopy that showed surface plasmon resonance (SPR) of the AgNPs at 418 nm. The presence of active flavanoids, phenolic groups, alkaloids, and carbohydrates which were in the biomass of the mango leaf extract before and after reduction was identified using qualitative screening methods (observing the colour changes) and FT-IR Spectroscopy. The FT-IR also showed that there were (3430, 2333, 2102, 1638 and 647) cm^{-1} newly formed peaks that is different from the spectrum of the pure mango leaves of (3484, 1627 and 703) cm^{-1}. These biologically synthesized AgNPs were tested for their antimicrobial activity against four human pathogens i.e. S. typhi, E. coil, S. aureus and S. pyrogens. They were found to have significant effect in controlling the growth of the human pathogens with a maximum zone of 22 mm in S. typhi and a minimum zone of 7 mm in S. pyrogens.

Keywords: AgNPs, Mango leaf, Green synthesis, UV-Vis, FT-IR, Antimicrobial activity

Table of Contents

page

LIST OF FIGURES

LIST OF TABLES

LIST OF SCHEME

LIST OF ABBREVIATIONS

NPs	Nanoparticle
NMs	Nanomaterials
SPR	surface Plasmon resonance
SERS	surface-enhanced Raman scattering
MEF	metal-enhanced fluorescence
AgNPs	silver nanoparticles
S. typhi	Salmonella typhi
E. coil	Escherichia coli
S. aureus	Staphylococcus aureus
S. pyrogens	Streptococci pyrogens

1. INTRODUCTION

1.1 Back ground of the study

Nanoscience refers to the study, manipulation and engineering of matter, particles and structures on the nanometre scale (one millionth of a millimetre, the scale of atoms and molecules). Important properties of materials, such as the electrical, optical, thermal and mechanical properties, are determined by the way molecules and atoms assemble on the nanoscale into larger structures. Moreover, in nanometre size structures these properties often different than on macro scale, because quantum mechanical effects become important **[1].**

The application of nanoscience is nanotechnology. Nanotechnology is a field of science which deals with production, manipulation and use of materials ranging in nanometres **[2]**. In recent days nanotechnology has induced great scientific advancement in the field of research and technology. The focus of nanoscience is now mainly shifting towards the assembly of individual NPs of high-order structures and nanomaterials **[3]**. Nanoparticles of different size and shapes can be synthesized in different material compositions and surface modifications **[4]**. The synthesized nanoparticles are found to exhibit size or shape dependent properties **[4,5]**. It has prospects in sensors**,** super capacitors, drug carriers, diodes, photonic and photovoltaic cells, or data storage media application **[6−8]**.While the analogies between nanoparticulate building blocks at the nanoscale and the atomic building blocks at the molecular scale appears quite appealing for high order structure **[9]**, yet it must be remembered that nanoparticles are rarely monodispersity and no two particles are ever identical unlike atoms. This intrinsic polydispersity of nanoparticles make self-assembly more complex which affects the overall characteristics derived from the size-dependent properties of individual nanoparticles (e.g., magnetic susceptibility, surface plasmon resonance, SPR) **[10]**. In order to synthesize highly ordered structures of nanoparticles with well-defined properties and functions, it is highly desirable to lower down the polydispersity of their nanoparticulate components to achieve maximum stability **[8,10]**. The ability to modify low-polydispersity particles is important for different applications, like catalysis where the catalytic activity of the nanoparticles is considered important **[11]**.

Metallic nanoparticles have different physical and chemical properties from bulk metals because of size difference of the metallic nanoparticles (e.g., lower melting points, higher specific surface areas, specific optical properties, mechanical strengths, and specific magnetizations), properties that might shows its attraction in various industrial applications

[10]. Gold, silver and copper are the most common types of element to form metallic nanoparticles. From these common types of Ag exhibits the highest efficiency of Plasmon excitation **[12]**. Moreover, optical excitation of Plasmon resonances in nanosized Ag particles is the most efficient mechanism by which light interacts with matter. A single Ag NPs interacts with light more efficiently than a particle of the same dimension composed of any known organic or inorganic chromospheres. The light-interaction cross-section for Ag can be about ten times that of the geometric cross-section, which indicates that the particles capture much more light than it's physically incident on them **[13]**. Silver is also the only material whose Plasmon resonance can be tuned to any wavelength thin the visible spectrum. The size, shape, and surface morphology of Ag NPs play a vital role in controlling their physical, chemical, optical, and electronic properties. The Nps that attract the attention of most researchers are produced from bulk silver and gold **[14]**.

Currently, silver nanotechnology, is becoming popular due to its extensive applications and distinctive properties (*e.g.* size and shape dependent several properties such as, optical, magnetic and electrical properties), which can be incorporated into biosensor materials, antimicrobial applications, composite fibres, cosmetic products, cryogenic superconducting materials, and electronic components **[3].** A silver nanoparticle, because of their anti-microbial property, is gaining popularity in medical applications **[15]**. In addition to this, silver is known to exhibit an oligodynamic effect because of its ability to exert bactericidal activity at minute concentrations **[16].** Studies have revealed their potential to kill the pathogens and thus they are used as disinfectants in Public Health Care from time immemorial even before the advent of penicillin **[15]**.

Silver nanoparticles synthesis by different methods using different reducing agents. The most common methods to synthesis sliver nanoparticles are chemicals, physicals and biological methods. Several physical and chemical methods are employed for synthesizing and stabilizing silver nanoparticles **[15]**. But most of the chemical methods used for the synthesis of nanoparticles involve the use of toxic, hazardous chemicals that create biological risks and these chemical processes are not eco-friendly. This enhances the growing need to develop environmentally friendly processes through green synthesis and other biological approaches. Green synthesis approaches mainly include mixed-valence polyoxometalates, biological, polysaccharides and irradiation method which has certain advantages over conventional methods that involve chemical agents which are found to be associated with environmental

toxicity **[16]**. Sometimes the synthesis of nanoparticles using various plants materials and their extracts can be beneficial over other biological synthesis processes which involve the very complex procedures of maintaining microbial cultures **[17].**

In the recent days, silver nanoparticles have been synthesized by biological methods from the naturally occurring sources and their products like green tea, Neem(Azadirachta indica), leguminous shrub(Sesbania drummondii), various leaf broth, natural rubber, starch, Aloe vera plant extract, lemongrass leaves extract, etc**[18]**. A lot of literature has been reported till to date on biological synthesis of silver nanoparticles using microorganisms including bacteria, fungi and plants; because of their antioxidant or reducing properties typically responsible for the reduction of metal compounds in their respective nanoparticles. Although; among the various biological methods of silver nanoparticle synthesis, microbe mediated synthesis is not very suitable for industrial feasibility because of requirements of highly aseptic conditions and their maintenance. So, the use of plant extracts for this purpose is potentially advantageous over microorganisms due to the ease of improvement, the less biohazard and elaborate process of maintaining cell cultures **[6].**

In the present scenario, there is a growing interest in biological reduction of metal ions into metal nanoparticles particularly in the field of biology and medicine because of their distinct particle size and shape dependent properties **[19]**. Bionanotechnology, a new approach, has emerged as the integration between biotechnology and nanotechnology for developing biosynthetic and environmental-friendly technology for the synthesis of nanomaterials.

It is one of the best plant forms for synthesis of nanoparticles as it is free from toxic chemicals as well as providing natural capping agents for the stabilization of silver nanoparticles. Now, plant mediated synthesis of metal nanoparticles is receiving lots of attention due to its simplicity, speedy synthesis of nanoparticles of attractive and diverse morphologies and elimination of detailed maintenance of cell cultures and eco-friendliness **[20].** The reason for selecting plant for biosynthesis is because they contain important reducing agents like Citric acid, Ascorbic acids, flavonoids, reductases etc., dehydrogenases and extracellular electron shuttlers that may play an important role in biosynthesis of metal nanoparticles **[21,22].** This is due to the reason that plants possess components that can act as reducing agent, capping agent, as a chelating agent and stabilizers for nanoparticles synthesis. The green reduction of metal (Ag, Zn, and Cu) nanoparticles using biomolecules present in plant extracts such as enzymes, proteins, amino acids, vitamins, polysaccharides has been

deeply studied in recent years **[23].** Mango is one of the plants growing in Ethiopia. Mango (*Mangifera Indica)* plant parts (pulp, peel, seed, bark, leaves, and flowers) being utilized domestically or industrially, the mango thus could be a cheap and readily available and composed of a variety of Phenolic acid, Phenolic esters, flavonoids, terpenoids, thiamine and mangiferin **[24]** and these may be responsible for reduction of metal ions and efficient stabilization of nanoparticles.

The advantage of using mango leaf for bio reduction of metal ions is that it is easily available throughout the year. The present study was synthesizing silver nanoparticles at bio reduction of $AgNO_3$ solution using aqueous mango leaf extracted.

1.2 Statement of the problem

These days different researches on synthesis of metallic nanoparticles have been reported in many areas of applications. The nanoparticles are mostly prepared using chemical and physical methods. However the qualities of the synthesized materials by physical methods are not as high as chemically synthesized materials **[4]**. Hence there arises a growing need to develop green synthesis routes for the nanoparticles. Still, using microorganisms as a green method of synthesis requires lots of maintenance, is complex, and involves very complex procedures of maintaining microbial cultures **[9,13]**. It is also slow and is not very suitable for industrial feasibility because of its requirements of highly aseptic conditions.

Different researchers have been used plant extracts to synthesis metallic nanoparticles. The results of their work showed us plant extracts are good sources for the synthesis of metal and metal oxide nanoparticles. These results motivated us to carry out further researches on the use of plant extracts for the preparation of nanoparticles. We know that there are many plants that are grown only in Ethiopia. Researches related with nanomaterials synthesis using plant mediated are new in Ethiopia. Therefore this study carried out synthesizing silver nanoparticles by using mango leaf extract.

On the other hand microbes are becoming resistant to the usual antimicrobials and so more consistent way of protecting or killing microbes (such as bacteria) is becoming a more focused area of study.

1.3 Significance of the study

This work is expected to have its own contribution on the green synthesis of the silver nanoparticles using locally available of green mango leaf extracts. These researches will

bring a technology transfer and also initiate researchers to explore Ethiopian indigenous and growing plants and use for the nanoparticle preparation. It is also hoped that, it will be used as one possible means of synthesizing silver nanoparticles of desired quality with low cost and convenient methods of preparation so that the toxicity or hazardous nature of the chemical methods of synthesizing silver nanoparticle can be avoided. It is believed that this work will have a positive impact on increasing the number of available research works dealing with silver nanoparticles synthesis using the indigenous plant and effective its antibacterial activity. Having easily available silver nanoparticles is also expected to make the protection and /or removal of bacterial microbes easy and effective. And this will definitely be one of the ways of keeping people healthy.

1.4 Objective of the study

1.4.1 General objective

The main purpose of this study was synthesizing the Silver nanoparticles using Mango leaf extract and evaluation of its antibacterial activity.

1.4.2 Specific objectives

To achieve the above general objective there were some specific tasks which needed to be performed.

- Testing the existence of different phytochemicals in the mango leaf extract using different standard solutions as well as FT-IR spectroscopy.
- Synthesizing the Silver nanoparticles from the mango leaf extract and $AgNO_3$.
- Characterizing the synthesised Silver nanoparticles using visual observation, UV-Vis and FT-IR spectrometer.
- Examining the anti-bacterial activity of the silver nanoparticles by measuring its zone of inhibition over different bacterial species.

2. REVIEW LITERATURE

2.1 Historical aspects and growth of Nanotechnology and Nanoparticles

The term “Nano” comes from the Greek word *dwarf* which generally elaborates the particle of size roughly in the range of 1 to 100 nanometres. The theoretical concept of nanotechnology was first begun with lecture delivered by Richard Feynman in 1959. He gave a lecture titled “There's Plenty of Room at the Bottom”, suggesting the possibility of manipulating things at atomic level. He speculated on the possibility and potential of Nano sized materials **[25].**

The term "Nanotechnology" was first defined by Norio Taniguchi of the Tokyo Science University in 1974 and Nanotechnology was shortened to “Nanotech”. However, the real burst of nanotechnology didn’t come until the early 1990s. In the early 1990s Huffman and Kraetschmer, discovered how to synthesize and purify large quantities of fullerenes (molecules composed entirely of carbon, in the form of a hollow sphere, ellipsoid, or tube. are similar in structure to graphite, which is composed of a sheet of linked hexagonal rings, but they contain pentagonal (or sometimes heptagonal) rings that prevent the sheet from being planar). Shortly after a meeting of the Materials Research Society in 1992, Dr. T. Ebbesen described to a spellbound audience his discovery and characterization of carbon nano tubes. Using the same or similar tools as those used by Huffman and Kraetschmer, hundreds of researchers further developed the field of nanotechnology. No one knows how many products on the market today contain nanoparticles or are manufactured with the help of nanotechnologies **[25]**.

Nanotechnology is a field of science which deals with design, production, manipulation and use of materials ranging in nanometres called nanomaterials **[26]**. It is the application of science to control matter at the molecular level **[25]**. It is also the study and application of small object which can be used across all fields such as chemistry, biology, physics, material science and engineering **[27]**, it has appealed many researchers from the above listed fields.

Generally, nanotechnology deals with structures sized between 1 to 100 nm, nanoparticles. It involves developing materials or devices within that size range **[25]**. Tremendous growth in nanotechnology has opened up novel fundamental and applied frontiers in materials science and engineering, such as nanobiotechnology, bionanotechnology, quantum dots, Surface-enhanced Raman Scattering (SERS), and applied microbiology **[27]**. The branch of

nanoscience and technology is truly multi-disciplinary and is an emerging technology with full of promises to have an impact on virtually every spectrum of civilization including communications, computing, textiles, cosmetics, sports, therapy, automotives, environmental monitoring, fuel cells and energy devices, water purification, food and beverage industry, etc **[25,28]**.

Nanoscience and nanotechnologies are revolutionizing our understanding of matter and are likely to have profound implications for all sectors of the economy, including; agriculture and food, energy production and efficiency, the automotive industry, cosmetics, medical appliances and drugs, household appliances, computers, and weapons. Nanotechnology has the capacity to improve our ability to prevent, detect, and remove environmental contaminants in air, water, and soil in a cost effective and environmentally friendly manner **[25]**.

The earliest, widespread description of nanotechnology referred to the particular technological goal of precisely manipulating atoms and molecules for fabrication of macro scale products. This definition reflects the fact that quantum mechanical effects are important at this quantum realm scale, and so the definition shifted from a particular technological goal to a research category inclusive of all types of research and technologies that deal with the special properties of matter which occur below the given size threshold. It is therefore common to see the plural form "nanotechnologies" as well as "nanoscale technologies" to refer to the broad range of research and applications whose common trait is size. Because of the variety of potential applications (including industrial and military), governments have invested billions of dollars in nanotechnology research.

Scientists currently debate the future implication of nanotechnology. Nanotechnology may be able to create many new materials and devices with a vast range of application, such as in nanomedicine, nanoelectronics, biomaterials energy production, and consumer products. On the other hand, nanotechnology raises many of the same issues as any new technology, including concerns about the toxicity and environmental impact of nanomaterials, and their potential effects on global economics, as well as speculation about various dooms day scenarios. These concerns have led to a debate among advocacy groups and governments on whether special regulation of nanotechnology is warranted **[29]**.

Nanotechnology will eventually provide us with the ability to design custom-made materials and products with new enhanced properties, new nanoelectronics components, new types of "**smart**" medicines and sensors, and even interfaces between electronics and biological systems **[30]**.These newborn scientific disciplines are situated at the interface between physics, chemistry, materials science, microelectronics, biochemistry, and biotechnology. Control of these disciplines therefore requires an academic and multidisciplinary scientific education. Nanoscience and nanotechnology are at the forefront of modern research. The fast growing economy in this area requires experts who have an outstanding knowledge of nanoscience in combination with the skills to apply this knowledge in new products. A multidisciplinary scientific education is crucial to provide industry and research institutes with top quality experts who have a generic background in the different sub disciplines such as electronics, physics, chemistry, material science, biotechnology, and at the same time be experts in one particular field **[25].**

2.2 Nanoparticles (Nps) / Nanomaterials (NMs)

Nanomaterials (NMs) are defined as materials with at least one external dimension lies in the size range of approximately 1-100 nanometres. While Nanoparticles (NPs) are solid particles with all three external dimensions at the nanoscale that can drastically modify physico-chemical properties compared to the bulk material **[16]**. Bulk materials possess relatively constant physical properties regardless of their size, but at the nanoscale this is often not the case. The nanoparticles possess unique physico-chemical, optical and biological properties which can be manipulated suitably for desired applications **[28].** They exhibit unique electronic, magnetic, optical, catalytic and medicinal properties as compared with the traditional and commercial bulk materials. This is due to its quantum size effect, large surface to volume ratio and their electronic energy states become discrete **[13,28].**

As particles are reduced from a micrometer to a nanometre size, the resultant properties can change dramatically. For example, electrical conductivity, hardness, active surface area, chemical reactivity and biological activity are all known to be altered **[31].** Because; as the material becomes smaller the percentage of atoms at the surface increases relative to the total number of atoms of the material bulk. This can lead to unexpected properties of nanoparticles which are partly due to the surface of the material dominating over the bulk properties. It is also known that with the decrease in the dimensions of the materials to the atomic level, their properties change **[32].**

All nanoparticles, regardless of their chemical constituents, have surface area to volume ratios that are extremely high. This causes nanoparticles physical properties to be dominated by the effect of the surface atoms and capping agents on the nanoparticles surface. A particle with a high surface area has a greater number of reaction sites than a particle with low surface area, and thus, results in higher chemical reactivity **[33].** High surface area to volume ratio is important for applications such as catalysis.

Nanoparticles, due to their extremely small size and large surface area, possess many interesting properties. Due to this they find novel applications in various areas of electronics, optoelectronic, magnetic, information storage, recording media, sensing devices, catalysis, chemistry, environment, energy, agriculture, medicine and drug delivery, communication technology, aircraft technology, heavy industry and consumer goods etc **[10,34].**

In recent years the interest in nanomaterials has increased dramatically due to their unique chemical and physical properties **[8,35].** Nanoparticles are of great interest; because they act as bridge gap between the atomic/molecular structure to the material in bulk as they exhibit completely new or improved properties based on specific characteristics such as size, shape, distribution, ionic strength, capping agent and morphology **[10].** And also on account of their potential applications which are strongly influenced by their size, morphology and structure **[22].**

Nanoparticles have been influencing material science considerably. It seems this dominance will continue in the future years, because of fundamental and technological importance with implementing incessant researches in this field. Nanoparticles are considered as building blocks for the next generation of technology with applications in many industrial sectors **[23]**.

2.3. Noble Metal nanoparticles

Metal nanoparticles have been the subject of focused research, due to their unique optical, electronic, mechanical and chemical properties that are significantly different from those of bulk materials. For this reason, metallic nanoparticles have found uses in many applications in different fields, such as catalysis, photonics, and electronics **[5,9].**

As the metal particles are reduced in size, bulk properties of the particles disappear to be substituted to that of quantum dot, following quantum mechanical rules. It can thus be easily understood that metal nanoparticles chemistry is different from that of the bulk materials

[24]. Many kinds of nanoparticles, including metal nanoparticles, oxide nanoparticles, semiconductor nanoparticles, and even composite nanoparticles, have been widely used in electrochemical sensors and biosensors.

Metallic nanoparticles are very interesting, because of the fact that these particles show size-dependent characteristics of the material. Especially in the nanometre scale, these size-dependent properties are observable. Metal nanoparticles with at least one dimension approximately 1-100 nm have received considerable attention in both scientific and technological areas due to their unique and unusual physico-chemical properties compared with that of bulk materials .This means the nanoparticles have change in colour when their size differs, where as bulk materials does not have change in colour in even if their size varies **[36].**

The term metal nanoparticle is used to describe nanosized metals with dimensions (length, width or thickness) within the size range 1-100 nm. The existence of metallic nanoparticles in solution was first recognized by Faraday in 1857 **[32]** and a quantitative explanation of their colour was given by Mie in 1908 **[37].**The colours of metal nanoparticles are varying because of the size of the particles. This colour formed by the resonant excitation of a collective oscillation of the conduction band electrons in the particles termed particle Plasmon.

2.3.1 Optical properties of metallic nanoparticles

Probably the optical property of nanoscale metallic particles is one of the most interesting topics in nano-related researches. Colloidal metallic nanoparticles possess optical absorption spectra with an absorption peak that looks similar to the absorption peak of colloidal semiconductor quantum dots. However, this absorption does not derive from transitions between quantized energy states. Instead, in metallic nanoparticles, collective modes of motion of the electron gas can be excited. They are referred to as localized surface Plasmon. The peak in the absorption spectrum is the resonance frequency for the generation of surface Plasmon. Therefore, the size dependence is not that significant comparing with semiconductor quantum dots **[38]**. The purpose of this section is to give an overall review on basic concepts in the optical properties of metallic nanoparticles, especially on noble metals, like gold and silver.

2.3.2. Surface Plasmon in Spherical Metallic Nanoparticles

The strong optical extinctions of conductive metal nanoparticles arise from an electrodynamics phenomenon known as surface Plasmon, which are generated by the

collective excitation of free electrons Surface Plasmon can be categorized into two types: localized Plasmon resonance, in which incident light is absorbed or scattered by the oscillating electric dipoles within a metal nanoparticle, and surface Plasmon polarities, which propagate along metal surfaces in a waveguide-like fashion until released at some distance from their point of origin. These two types are depicted in Figure 2.1 Localized Plasmon resonance is important for generating local field factors, which enhance linear and nonlinear optical effects near the metal surface. However, metal nanostructures often support both types of Plasmon simultaneously, surface Plasmon resonance (SPR) and surface-enhanced Raman scattering (SERS) **[37−39]** and it is difficult to decouple one from the other. Excitation of conduction electrons produces local electromagnetic fields near the metal surface.

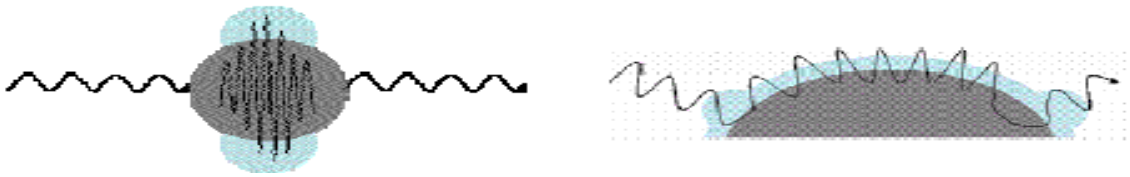


Figure 2.1 Incident light on Nano-structured metal surfaces can generate localized (standing) Plasmon resonances (left) as well as surface waves (right) **[39].**

2.3.3 Stabilization of Metal Nanoparticles against Agglomeration

Fine particles, particularly nanoscale particles, since they have large surface areas, often agglomerate to form either clusters or larger particles to minimize the total surface or interfacial energy of the system **[39,40].**When the particles are strongly stuck together, these hard agglomerates are called aggregates. Agglomeration of fine particles can occur at the synthesis stage, during drying and subsequent processing of the particles. Thus, it is very important to stabilize the particles against agglomeration at each step of particle production and powder processing. Agglomeration of fine particles is caused by the attractive Vander Waals force and/or the driving force that tends to minimize the total surface energy of the system **[41]**. Repulsive inter particle forces are required to prevent the agglomeration of these particles.

2.4. Silver nanoparticles (AgNPs)

The Ag+ ions are primary requirement for the synthesis of AgNPs which can be obtained from various water soluble salts of silver. However, the aqueous $AgNO_3$ solution with Ag+ ion concentration range between 0.1-10 mM (most commonly 1-5 mM) has been used by the majority of researchers **[23,26].**Silver nanoparticles are mostly smaller than 100 nm and

consist of about 15-20,000 silver **[42].**At the nano-scale, the silver particles exhibit deviating physico-chemical properties (like pH dependent partitioning to solid and dissolved particulate matters) and biological activities compared with the regular metal **[43].**This is due to the higher surface area per mass, allowing a larger amount of atoms to interact with their surroundings. Because of the properties of silver at the nanoscale, nanosilver is nowadays used in an increasing number of consumers and medical products. The optical properties of silver nanoparticles were used by glass founders as far back as in the time of the Roman Empire. That evidenced, so-called Lycurgus cup (4th century AD) now exposed in the British Museum. A detailed study of the composition of its bronze-mounted insets of stained glass, carried out in the late 20th century, revealed the presence of metal nanoparticles (with the average diameter of 40 nm) that consists of silver (70 %) and gold (30 %) alloy **[44]**.

The extremely small size of nanoparticles is quite significant, because of their large surface area relative to their volume. Particularly silver, have caught the attention of scientists because of its widespread application in the development of new technologies such as electronics, material sciences and medicine **[12].** New applications of nanoparticles and nanomaterials are coming up rapidly. Nanocrystalline silver particles have found tremendous applications in the field of high sensitivity bio molecular detection, diagnostics **[23],** Catalysis, antimicrobials, therapeutics, and microelectronics **[4].**

2.4.1 Silver nanoparticle application

The nanoparticles used so far, the silver nanoparticles considered as the most promising as they contain remarkable antimicrobial properties due to their large surface area to volume ratio, which is of interest for researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains **[45]**. In nanotechnology, silver nanoparticles are the most promising one. It is observed that silver nanoparticles were doing not affect living cells, so not able to provoke microbial resistance. It is believed that silver nanoparticles can attach to the cell wall and disturb cell-wall permeability and cellular respiration **[46]**. A number of nanoparticles based therapeutics has been approved clinically for vaccines, infections and renal diseases. One of the applications of silver nanoparticles in drug discovery, drug delivery and new drug therapies has declared war on dreadful diseases and they use on body natural transport pathway and natural mechanism of uptake of drug by the diseased cell **[47].**

Silver is well-known since ancient time due to its medicinal value and for its preservative properties. Silver nanoparticles have many applications (Figure 2.2); spectrally selective

coatings for solar energy absorption and intercalation material for electrical batteries, as catalysts in chemical reactions, for bio-labelling etc **[48]**. Silver containing particles also used in textile fabrics, as food additives, and in package and plastics to eliminate microorganisms. Because of such a wide range of applications, various methods concerning the fabrication of silver nanoparticles, as well as various silver-based compounds containing metallic silver (Ag^0) have been developed **[49]**. Silver nanoparticles used in environmental-friendly antimicrobial nanopaint, antimicrobial nature of silver ions plays a prominent role in food packaging systems **[49,50]**. Silver nanoparticles have antibacterial properties mediated by silver ions, it used as preservative in food and various food related products **[51]**.Silver nanoparticles are reported to show better wound healing capacity, better cosmetic appearance and scar less healing when tested using an animal model **[52].**

Figure 2. 2 The silver nanoparticles applications **[52].**

Silver nanoparticles are not only being used in various technologies but also incorporated in to a wide group of consumer products that gets benefits from their unique properties (optical, conductive, and antibacterial properties).

(i) Diagnostic Applications**:** Silver nanoparticles can be used in numerous assays and biosensors where the silver nanoparticle materials can play a role as biological markers for quantitative detection.

(ii) Antibacterial Applications**:** Their antibacterial property can be incorporated in apparel, wound dressings, footwear, paints appliances, cosmetics, and plastics.

(iii) Conductive Applications**:** Silver nanoparticles are also used in conductive inks and integrated into composites to increase thermal and electrical conductivity.

(iv).Optical Applications**:** Silver nanoparticles can be used to efficiently harvest light and for enhanced optical spectroscopes including metal-enhanced fluorescence (MEF) and surface-enhanced Raman scattering (SERS).

Silver nanoparticles have been found to possess both anti-bacterial and anti-inflammatory properties that can promote faster wound healing. Because of these advantageous properties, silver nanoparticles have been integrated into commercially available wound dressings, pharmaceutical preparations, and medical implant coatings **[53].**

2.5. Synthesis methods of sliver nanoparticle

The synthesis of nanomaterials is of current interest due to their wide variety of applications in fields such as electronics, photonics, catalysis and medicine the synthesis approach significantly impacts the properties of such nanoparticles and these properties in turn have a significant impact on their biomedical applications**.** Basically there are two main approaches for nanoparticle synthesis i.e. the Top down approach and the Bottom up approach.

In the Top down approach, production of nanosilver involves mechanical grinding of a bulk piece of the material into nanocrystalline particles. Bulk material is broken down into particles at nanoscale with various lithographic techniques. Physical techniques such as lithography, laser ablation, sputtering deposition, pulsed electrochemical etching and vapour deposition are among the most commonly used top-down methods**.**

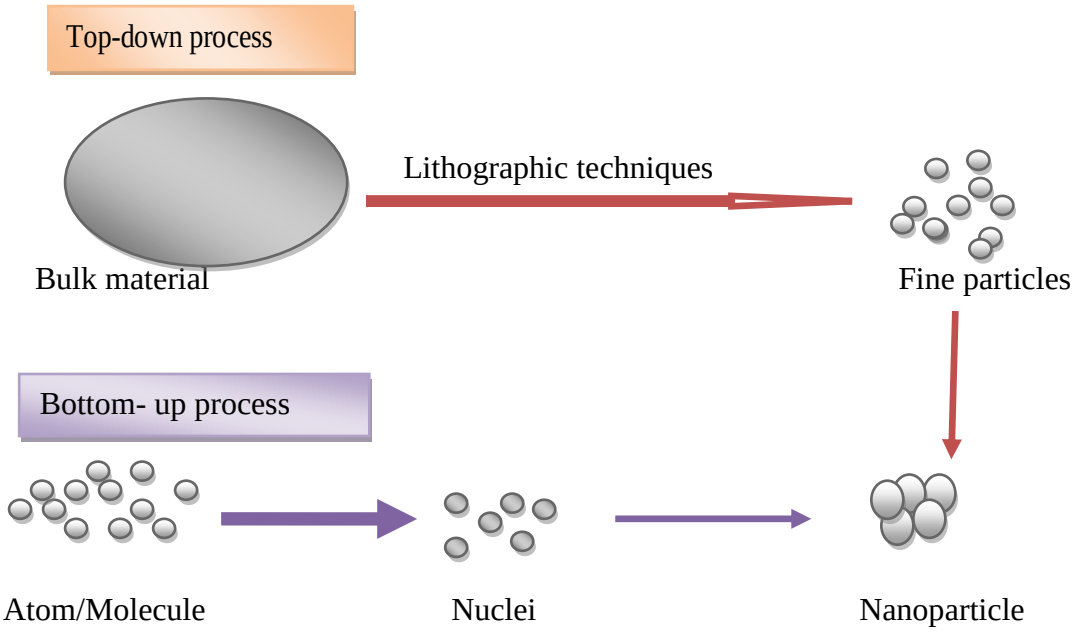


Figure 2.3 The top-down approach versus the bottom-up approach **[45].**

The Bottom up approach basically involves chemical and biological methods to synthesize nanoparticles. Atoms self-assemble to new nuclei which grow into a particle of nanoscale. It involves the synthesis of material, atom by atom, molecule by molecule, or cluster by cluster **[15].**This process requires controlled growth/condensation of solute molecules formed during a chemical reaction. Desired shape and size of the nanoparticles can be achieved by the controlled condensation of nanoparticles. Biosynthesis of nanoparticles is either scale down or bottom up approach where the main reaction occurring is reduction/oxidation. The microbial enzymes or the phyto-chemicals with antioxidant or reducing properties are usually responsible for reduction of metal compounds into their respective nanoparticles **[15].** Bottom-up approaches include; sol-gel processing, chemical vapour deposition, plasma or flame spraying synthesis, laser pyrolysis, micro emulsion and the green synthesis.

There are different methods used to synthesize sliver nanoparticles from different sources, such methods are:

2.5.1 Physical synthesis method

Arrays of conventional methods have been employed in synthesis of nanoparticles. They can be categorized mainly under physical and chemical methods. Some of the physical methods known for the synthesis of nanoparticles include radiolysis, microwave, ultrasonication, laser ablation and electrochemical methods **[15]**. Most important physical approaches include evaporation-condensation and laser ablation. The absence of solvent contamination in the prepared thin films and the uniformity of nanoparticle distribution are the advantage of physical approaches in comparison with chemical methods **[54]**. However the quality of the material is not as high as chemically synthesized materials. It consumes a great amount of energy while raising the environmental temperature around the source material, and requires a great deal of time to achieve thermal stability. Metal nanoparticles can be synthesized by evaporation–condensation mechanism, which could be carried out using a tube furnace at atmospheric pressure **[55]**.However, generating silver nanoparticles using a tube furnace has many drawbacks. Tube furnace besides occupying a large space, also consumes a great deal of energy simultaneously raising the environmental temperature around the source material. It is also very time consuming. A typical tube furnace needs power consumption of more than several kilowatts and a preheating time of several tens of minutes to attain a stable operating temperature **[56]**.

Silver nanoparticles also could be synthesized by laser ablation of metallic bulk materials in solution **[57,58**].The ablation efficiency and the characteristics of produced nanosilver particles depend upon many factors such as the wavelength of the laser impinging the metallic target, the duration of the laser pulses (in the femto-, pico- and nanosecond regime), the laser fluence, the ablation time duration and the effective liquid medium, with or without the presence of surfactants. Also the generation of silver nanoparticles (Ag NPs) using a tube furnace has several drawbacks, because a tube furnace Occupies a large space, consumes a great deal of energy while raising the environmental temperature around the source material, and requires a lot of time to achieve thermal stability.

2.5.2 Chemical synthesis method

It is seen that physical methods have limited success and therefore chemical methods for the synthesis of inorganic nanoparticles are widely used **[15]**. Chemical reduction method is widely used to synthesize silver nanoparticles because of its readiness to generate silver nanoparticles under gentle conditions and its ability to synthesize silver nanoparticles on a large scale **[59]**.Nanosilver can be synthesized chemically by several processes such as, reduction or oxidation of metal ions, inert gas condensation, or by the sol gel methods **[15]**. The chemical synthesis process of the silver nanoparticles in solution has the following three main components: (i) metal precursors, (ii) reducing agents (iii) and stabilizing/capping agents. It is also shown that the size and the shape of synthesized AgNPs are strongly dependent on these stages. Furthermore, for the synthesis of small sized monodispered silver nanoparticles in a solution, it is highly essential that all nuclei are formed at the same time. In this case, all the nuclei are very much likely to have exactly same or similar size. It will also have same subsequent growth. The initial nucleation followed by the subsequent growth of initial nuclei can be controlled by regulating the reaction parameters such as reaction temperature, pH, precursors, reduction agents (i.e. NaBH4, ethylene glycol, glucose) and stabilizing agents (i.e. PVA, PVP, sodium oleate) **[3,19,49].**

In general, different reducing agents such as sodium citrate, ascorbate, sodium boron hydride (NaBH4), elemental hydrogen, polyol process, Tollens reagent, N, N-dimethyl formamide (DMF), and poly (ethylene glycol)-block copolymers are used for reduction of silver ions (Ag+) in aqueous or non-aqueous solutions. The aforementioned reducing agents reduce silver ions (Ag+) and lead to the formation of metallic silver (Ag^0), which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to formation of

metallic colloidal silver particles **[36,60,61]**. However chemical synthesis methods are more negative side compare to biological synthesis method, some of chemicals are toxic such as NaBH4, citrate, ascorbate and harsh reaction parameters like high temperatures in the polyol method **[61]**.

2.5.3. Drawback of Conventional methods

The drawback of the physical technique is that; resultant nanoparticles have defective surface formation, low production rate, high cost of manufacturing and large energy requirement, it raises the environmental temperature around the source material, and requires a great deal of time to achieve thermal stability. Almost all of the possible chemical methods employ toxic chemicals and energy intensive routes which make these choices eco-hazardous and preclude their applications in biology, medicine and clinical applications. They are not safe, where flammable or corrosive reducing agents such as; titanium tetrachloride and hydrazine have been used. This method also requires strong and weak chemical reducing agents as well as capping agents like sodium borohydride, sodium citrate and alcohols. These agents are generally highly toxic, flammable, cannot be easily disposed of. Some wet chemical methods employ the toxic organic reactant such as ethylene glycol, while certain methods use additional reducing agent such as sodium**.** They also involve using of toxic chemicals and it's dangerous by products, concentrated reducing agents, high level of radiation, and contamination from precursor chemicals. Hence it is a fact that reproducibility and stability of the nanoparticles with controlled size are very difficult to achieve by popular chemical reduction methods which involve expensive reagent, hazardous reaction condition and need longer time as well as tedious process to isolate nanoparticles which is alarming threat in every aspect of flora, fauna and human health **[16,31].** Hence green syntheses of nanoparticles are now commonly preferred using microorganisms and plant extracts. Development of clean, biocompatible, nontoxic, eco-friendly, cheap and sustainable methods for nanoparticles, in general and AgNPs in specific, synthesis deserves merit. As a result the interest in this field has shifted toward 'green' chemistry and bio-processor approach **[62]**, Green synthesis is considered as the most ideal synthesis route nowadays.

Green plant and microorganisms have a remarkable ability to form nanosilver. Biological synthesis using plant materials and microorganisms is proven to be eco-friendly and economical. These days, a large number of microorganisms and plant extracts are used for nanosilver synthesis **[15].** Plant Extracts obtained from bio-organisms may act as reducing

and capping agents in silver nanoparticles synthesis. The reduction of Ag+ ions by means of biomolecules found in these bio-organisms extracts such as amino acids, enzymes/proteins, polysaccharides, and vitamins **[44]** is environmentally benign, yet shown to be chemically complex. An extensive study of literature reports successful silver nanoparticles synthesis using bio-organic compounds.

Biosynthesis of nanoparticles is advantageous over physical and chemical methods as it has a low cost (cost effective) and does not affect environment, in addition to that it doesn't use high pressure, energy, temperature and toxic chemicals to synthesis silver nanoparticles **[63].**

2.5.4. Green synthesis of Metal Nanoparticles

Recent studies have shown that green based methods using microorganisms and plants to synthesize nanoparticles are safe, inexpensive, and an environment-friendly alternative **[64].** Both microorganisms and plants have long demonstrated the ability to absorb and accumulate inorganic metallic ions from their surrounding environment. These attractive properties make many biological entities efficient biological factories capable of significantly reducing environmental pollution and reclaiming metals from industrial waste. Importantly, the ability of a biological entity to use its inherent biochemical processes to transform inorganic metallic ions into metal nanoparticles has led to a relatively new and largely unexplored field of research **[15,64].** The advantage of using plants over other eco-friendly biologically based systems such bacteria and fungi, is that it avoids the use of specific, well-conditioned culture preparation and isolation techniques that tend to be expensive and elaborate. Conversely, biosynthesis of nanoparticles using plants or plant based extracts tends to be safe, have relatively short production times, and have a lower cultivation cost compared to other biological systems **[23,64].**Furthermore, plant based biosynthesis is a relatively straight forward process that can be easily scaled up for large-scale production of nanoparticles. Recently, the biological synthesis of nanoparticles using plants and plant extracts appears be to an attractive alternative to conventional chemical synthesis and the more complex culturing and isolation techniques needed for many microorganisms. Moreover, combinations of molecules found in plant extracts perform as both reducing and stabilizing (capping) agents during nanoparticle synthesis **[15,37,44,64]**. These biological molecules are chemically complex, but have the advantage of being environment-friendly. Many biological approaches for intracellular and extracellular nanoparticles synthesis have been reported till date using microorganisms including plants, fungi and bacteria. Plants have been providing a better platform for synthesis of nanoparticles; they are free from toxic chemicals and

providing a natural capping agent. A number of plant extract mediated synthesis of AgNPs have been reported in the literature. For instance the use of Red Apple (*Malus domestica),* *Myristica fragrans* (nutmeg), *Portulaca oleracea*(*Piper betle* leaves), *Adansonia digitata* leaf extract, *Adansonia digitata* leaf extract, *Aeglemarmelos, Murraya koenigii, Cardiospermum halicacabum* L. leaf extract e.t.c**, [65]**.

2.5.4.1 Microbial Routes for Nanoparticle Synthesis

Many studies have shown that microorganisms, both unicellular and multicellular have the ability to synthesize inorganic materials. The biological synthesis can be considered a bottom-up approach where nanoparticle formation occurs due to the reduction/oxidation of metallic ions via biomolecules such as enzymes, sugars, and proteins secreted by the microorganism **[64,65].** However, a complete understanding of nanoparticle synthesis mechanism occurring in microorganisms is yet to be fully developed. Bacteria are known to synthesise metallic nanoparticles by either intracellular or extracellular mechanisms this is because each type of microorganism tends to behave and interact differently with particular metallic ions. The interaction and biochemical processing activities of a specific microorganism and the influence of environmental factors such as pH and temperature ultimately determines the formation of nanoparticles with a particular size and morphology **[64].**

In nature, bacteria are frequently exposed to diverse and sometimes extreme environmental situations. Survival in these harsh conditions ultimately depends on their ability to resist the effects of environmental stresses. Natural defence mechanisms exist in bacteria to deal with a variety of stresses such as toxicity arising from high concentrations of metallic ions in the environment. Biological strategies for dealing with high concentrations of metallic ions include changes in metal ion concentration via red-ox state changes, efflux systems, intracellular precipitation, and accumulation of metals, and extracellular formation of complexes **[65].** Size, shape, and composition of a nanoparticle can be significantly influenced by pH and temperature **[44].** For example, particle size is an important factor since novel and unique physicochemical properties are more pronounced at smaller sizes. Therefore, there is a need to optimize synthesis parameters during nanoparticle formation to enhance the overall particle properties. In particular, selecting the appropriate culture media for a specific bacteria and the particular metallic salt is important since these two parameters form the basis of nanoparticle synthesis and can influence particle yield **[65].** Studies by **[64]**

using bacterium Rhodopseudomonas capsulata have shown that particle size and morphology can be influenced by both metallic salt concentration and medium pH.

2.5.4.2 Green Synthesis of Silver Nanoparticles via Plants

It has long been known that plants have the potential to hyper-accumulate and biologically reduce metallic ions **[64,65].** Because of these interesting properties, plants have been considered a more environment-friendly route for biologically synthesizing metallic nanoparticles and for detoxification applications **[64].** Plant extracts containing bioactive alkaloids, Phenolic acids, polyphenols, proteins, sugars, and terpenoids are believed to have an important role in first reducing the metallic ions and then stabilizing **[64 – 66].** The variation in composition and concentration of these active biomolecules between different plants and their subsequent interaction with aqueous metal ions is believed to be the main contributing factors to the diversity of nanoparticle sizes and shapes produced. Importantly, the synthesis of nanoparticles from reducing metal salts via plants is a relatively straight forward room temperature process.

The synthesis of nanoparticles using plants or parts of plants can prove advantageous over other biological processes by eliminating the elaborate processes of maintaining microbial cultures, valuable alternative for the large-scale production of metal nanoparticles, can overcome the time consuming process of employing microbes, provides a single step technique for the biosynthesis process, used to produce more stable, to produce NPs of various sizes and shapes, faster rate of production and less biohazardness **[64]**.

In this study, researcher was used a simple, effective, low cost and environmentally safe method for synthesis of silver nanoparticles using extracted mango leaf mixed with silver nitrate solution. Therefore, this work was supported by different researchers to show green methods; typically plant mediated is important for this study. Some of the researchers studied that sliver nanoparticles were syntheses from different green plants. The different parts of plant such as stem, root, fruit, seed, callus, peel, leaves and flower are used to synthesis of metallic nanoparticles in various shapes and sizes by biological approaches. For instance, plant extracts from alfalfa, the broths of lemongrass, geranium leaves etc. have served as green reducing agents in silver nanoparticles synthesis **[32].**

Table2.1 Plants using synthesizing nanoparticles **[32]**.

Plant	Size of the particle (nm)
Eucalyptus hybrid (Safeda)	50-150
Azadirachta indica	5-100
Emblica officinalis	10-20
Aloe vera	15±4.2

2.6. Mango plant (*Mangifera indica*)

Species: *Mangifera indica L.*

Family: Anacardiaceae

Genus: Mangifera

Figure 2.4 Description of Mango plant (photograph).

2.6.1 Historical and botanical aspects of mango

Mango (Mangifera indica L.) fruit belongs to the family of Anacardiaceae in the order of Sapindales and is grown in many parts of the world, particularly in tropical countries. According **[67]** to mango fruit occupies the 2nd position as a tropical crop, behind only bananas in terms of production and average used. Mango leaves are alternately arranged, lanceolate (long and narrow) shaped, 6 to 16 inches in length and leathery in texture. The leaves are pinkish, amber, or pale green-colour when young and become dark green at maturity. In Ethiopia mango is produced mainly in-west and east of Oromia, SNNPR, Benishangul and Amhara **[67]**.

It has been well documented that mango fruits are an important source of micronutrients, vitamins and other phytochemicals. Moreover, mango fruits provide energy, dietary fibre, carbohydrates, proteins, fats and Phenolic compounds **[67]**, which are vital to normal human growth, development and health. Each part of a mango tree, such as its leaves, flowers, bark,

fruit, pulp, peel and seeds contains essential nutrients that can be utilized. The bioactive compounds in mango leaves are Phenolic compounds, mangiferin, kaempferol, quercetin, anthocyanins, mono- and diterpenes, fiber, antioxidant minerals **[68]**.

There is vast literature on chemical analysis of the aroma of several mango cultivars around the world with a wide range of compounds having been identified as esters, lactones, mono- and sesquiterpenes. Monoterpene hydrocarbons such as *cis*-ocimene, α-pinene, β- pinene, myrcene and limonene have been reported as key contributors to the aroma of the fresh mango fruits and leaves, depending on the variety **[69].** The aroma is one of the significant and decisive parameters of quality in the selection of a product. Aroma compounds are present in raw foods in free volatile form and also as non-volatile precursors such as substituted cystein sulfoxides, thioglycosides, glycosides, carotenoids and cinnamic acid derivatives **[70]**.

2.6.2 Mango phytochemicals and their Potential Significance to Human Health

In the past few years, there has been increasing interest in the study of mango phytochemicals compounds such as alkaloid, saponins, terpenoids, flavonides, phenolics e.t.c from mango fruits, peels, seeds, leaves, flowers, and stem bark due to their antioxidative and health-promoting properties that make consumption of mangoes and derived products a healthy habit. Bioactive compounds found in the mangos, among other plants and herbs have been shown to have possible health benefits with antioxidative, anticarcinogenic, antiatherosclerotic, antimutagenic, and angiogenesis inhibitor activities **[67]**.Interestingly, many herbs, fruits, and vegetables are known to contain large amounts of Phenolic antioxidants other than the well-known vitamins C, E, and carotenoids. Mango fruit and leaves are an excellent source of dietary antioxidants, such as ascorbic acid, carotenoids, and especially Phenolic compounds **[70]**. They are also an excellent source of dietary fiber, provitamin A and vitamin C.

Antioxidant nutrients and phyto nutrients inhibit the oxidation of living cells by free radicals by protecting the lipids of the cell membranes through free radical scavenging, blocking the initiators of free radical attack, neutralizing or converting free radicals into less active, stable products thus breaking the chain reaction and assisting in salvaging oxidized antioxidants enabling them to continue to be of benefit **[68]**. Polyphenols have the ability to scavenge free radicals via hydrogen donation or electron donation **[69]**. The mango is a potential source of Polyphenolic compounds with high antioxidative activity that help protect the body against

damage linked to oxidative stress. The quantities and characteristics of different mango phenolics differ in the different plant parts besides being affected by the geographic locations of the plants. Mangiferin, which is mainly concentrated in the leaves of the mango tree, is a unique polyphenols to the mango with high pharmaceutical activity, a potential which has been exploited in medicine and food supplements. Being a very popular plant, especially within the tropics and owing to its uniqueness of all parts (pulp, peel, seed, bark, leaves, and flowers) being utilized domestically or industrially, the mango thus could be a cheap and readily available supplier of dietary polyphenols with great antioxidative potential that will help reduce degenerative diseases such as cancer, atherosclerosis, diabetes, and obesity. Mango leaves proved as antimicrobial, antioxidants, for diabetes and prevent cancer **[69]**. Mango leaves containing organic compounds tarakserol-3 beta and ethyl acetate extract synergism with insulin activates GLUT4 and stimulates the synthesis of glycogen, so it can reduce the symptoms of hyper glycaemia. Mango leaves can also be used to treat diarrhoea, fever, insomnia, hypertension, and can also be used to lower high blood pressure **[70].**

The main classes of polyphenols are defined according to the nature of their carbon skeleton and they are: Phenolic acids, flavonoids, stilbenes, and lignans **[71].** Polyphenolic composition of mango leaves Galloyl, hydroxybenzoyl esters, and epicatechin have been identified in mango leaves. Mangiferin was the major component of the leaves. Some studies have reported that mango leaves are a rich source of polyphenols **[72]**, Phenolic acid and flavonols **[73]**, flavonol and xanthone, mangiferin **[74].** A number of Phenolic compounds have been reported in mango (Mangifera indica) leeaves including ellagic acid, mangiferin, mangiferin gallate, isomangiferin, isomangiferin gallate, quercetin, kaempferol, rhamnetin and their related conjugates **[23]**.

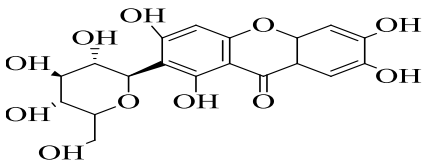


Figure 2.5 Basic structure of mangiferin **[23]**.

Phenolic compounds confer unique taste, flavour, and health-promoting properties found in vegetables and fruits. The term “Phenolic acids”, in general, designates phenols that do possess one carboxylic acid functional group, moreover the reason for including Phenolic

acids in the family of plant poly phenols lies in the fact that they are bio-precursors of poly phenols and, more importantly, they are metabolites of poly phenols.

The reduction activity of Phenolic and flavonoid compounds depends on the number of free hydroxyl groups in the molecular structure, which would be strengthened by steric hindrance **[67].**

Naturally occurring Phenolic acids contain two distinctive carbon frameworks the hydroxycinnamic and hydroxybenzoic structures. Mango plant one of Ethiopia indigenous plants cultivated in Ethiopia, "Bahir Dar University" was the one selected. It is known for its highly anti-oxidant property. It was expected to have higher amounts of the phytochemicals explained above. It was then believed that it is a favourable mango plant leaves type to reduce the silver ions of the salt solution to silver metal for the synthesis of the AgNPs.

2.7. Studies Related to Phytochemical Constituents of Mango leaf

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients **[64]**.They protect plants from disease and damage and contribute to the plant's colour, aroma and flavour. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as Phytochemicals. Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 Phytochemicals have been catalogued and are classified by protective function, physical characteristics and chemical characteristics dietary. Phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices. Mango leaves posses biomolecules such as Phenolic acids, flavonoids, and alkaloids which could be used as reducing, stabilizing, chelating and capping agents to react with silver ions and as scaffolds to direct the formation of AgNPs in solution **[67].**

2.7.1 Mechanism of silver nanoparticles formation

The synthesis of AgNPs by biological entities is due to the presence of large number of organic chemical like carbohydrate, fat, proteins, enzymes & coenzymes, phenols flavanoids, terpenoids, alkaloids, gum, etc capable of donating electron for the reduction of Ag+ ions to Ag^0. The active ingredient responsible for reduction of Ag+ ions varies depending upon organism/extract used. For nano-transformation of AgNPs, electrons are supposed to be derived from dehydrogenation of acids (ascorbic acid) and alcohols (catechol) in

hydrophytes, keto to enol conversions (cyperaquinone, dietchequinone, remirin) in mesophytes or both mechanisms in xerophytes plants **[75]**. A schematic diagram showing the silver ion reduction, agglomeration and stabilization to form a particle of nano size is shown in (Figure 2.6).

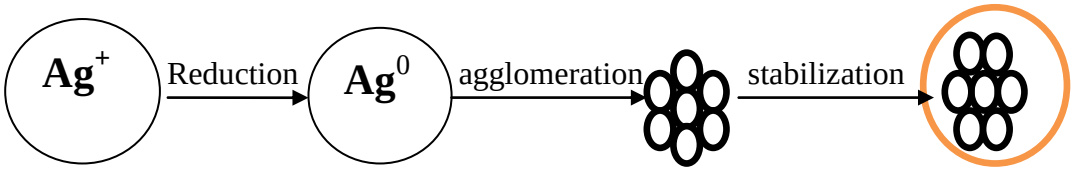


Figure 2.6 The Synthesis mechanism of AgNPs **[67].**

Many researchers were reported Vegetables have contained ascorbic acid **[67]**. Ascorbic acid was selected as the reductant of choice, because of its ability to precipitate metallic silver in acidic solution according to:

$Ag\ (aq)^{+} + e^{-} \longrightarrow Ag^{0}(s)$.....1

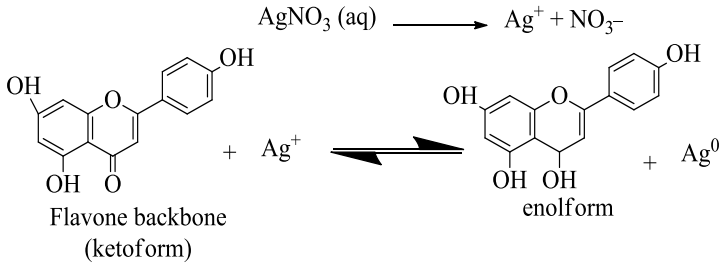
$2Ag\ (aq)^{+} + C_6H_8O6\ (aq) \longrightarrow 2Ag(s) + 2H^{+} + C_6H_6O_6$.....2

Scheme 2.1: Possible reaction mechanism for the formation of AgNPs using ascorbic acid **[75].**

The important aspect of this process is the increase in the acidity of the reaction mixture during the reduction, due to the release of protons.

In this study silver nanoparticles was prepared using extracted mango leaf with silver nitrate solution in Green synthesis method. The mango leaves posses biomolecules such as Phenolic acids, flavonoids, and alkaloids which could be used as reducing, stabilizing, chelating and capping agents to react with silver ions and as scaffolds to direct the formation of AgNPs in solution. Although the exact mechanism for the synthesis of nanoparticles using plant extracts has not been devised yet, it was suggested that different polyol components are responsible for the synthesis of the nanoparticles. The FT-IR spectrum again confirmed the formation of AgNPs nanoparticles in the presence of plant extracts. The peaks of the spectrum have indicated the secretion of some water-soluble organic components (phytochemicals) from the plants, which might have contributed in the formation of AgNPs via similar mechanism. This gives strong evidence for the involvement of polyphenols in the rapid biosynthesis and for the stability of metallic nanoparticles in the aqueous medium. This definitely reveals that flavonoids, poly phenols and other phytochemicals found in plant extracts are the ones responsible for the synthesis of nanoparticles like silver by firstly donating electrons to the metallic cation and stabilizing the newly formed nanoparticles. Since mango leaves which is composed of phytochemicals like Phenolic acids, flavonoids,

and alkaloids, it was expected to be helpful in synthesizing the AgNPs by donating electrons to the silver (I) cation of the silver salt solution.



Scheme 2.2: Possible reaction mechanisms for the formation of Ag NPs using plant extract **[76].**

2.8 Factors Affecting AgNPs Synthesis

The major physical and chemical parameters that affect the synthesis of AgNPs are reaction temperatures, metal ion concentration, extract contents, pH of the reaction mixture, duration of reaction and agitation. Parameters like metal ion concentration, extract composition and reaction period largely affect the size, shape and morphology of the AgNPs **[76].** Most of the authors have reported suitability of basic medium for AgNPs synthesis due to better stability of the synthesized nanoparticles in basic medium **[73].** Some other advantages reported under basic pH are rapid growth rate **[77]** good yield and mono dispersity **[78]** and enhanced reduction process. Small and uniform sized nanoparticles were synthesized by increasing pH of the reaction mixture **[78,79]**. The nearly spherical AgNPs were converted to spherical AgNPs by altering pH **[72]**, However, very high pH (pH > 11) was associated with the drawback of formation of agglomerated and unstable AgNPs **[80].**

The Reaction conditions like time of stirring and reaction temperature are important parameters. Temperatures up to 100˚C were used by many researchers for AgNPs synthesis using bio-polymers and plant extracts, whereas the use of mesophilic microorganism restricted the reaction temperature to 40˚C. At higher temperatures the mesophilic microorganism dies due to the inactivation of their vital enzymes. The temperature increase (30˚C −90˚C) resulted in increased rate of AgNPs synthesis **[76]** and also promoted the synthesis of smaller size AgNPs **[78].** On the whole, most of workers have synthesized AgNPs at room temperature (25˚C to 37˚C) range **[73−75].**

2.9 Anti-Microbial Activity

The AgNPs have been found to exhibit promising anti-microbial activity. Researchers have used several novel techniques to confirm and quantify the anti-microbial activity of AgNPs.

2.9.1 Disc/Well Diffusion Methods

The disc diffusion method, a most commonly used technique to access the antimicrobial activity of a liquid, has been employed by many researchers to confirm antimicrobial action of the AgNPs solution **[73]**. In this method, uniform sized disc of adsorbent material are dipped in the increasing concentration of AgNPs and placed over surface of the targeted microbe inoculated on the nutrient medium plates. An inhibition zone formation around the disc reflects antimicrobial action of the nanomaterials **[72]** and well diffusion **[29]**. In the Well diffusion method instead of using discs, small disc shaped pits are created on the agar plate for filling the test solution. In both the techniques, the microbe inoculated plates are incubated under standard condition for the formation of clear inhibition zone. The inhibition zone diameter around the disc or well, directly relates the effects of AgNPs on the chosen microbe.

2.10 The antibacterial activity of the AgNPs

The AgNPs are well known for their excellent antibacterial ability and superior physical properties and are widely used in a growing number of applications ranging from home disinfectants and medical devices to water purificants **[47]**. AgNPs can be exploited in medicine and pharmacy for dental materials, burn treatments, coating stainless steel materials, and sunscreen lotions **[37]**. AgNPs act as an antibacterial, antiviral, and antifungal agent when incorporated in coatings, nanofiber, first aid bandages, plastics soap, and textiles, in self-cleaning fabrics, and as conductive filler **[23]**.

The AgNPs have been an effective biocide against broad-spectrum bacteria including both Gram-negative and Gram-positive bacteria. The plant synthesis of AgNPs have potent antibacterial action against gram positive bacteria, *Lactobacillus fermentum, Streptomyces* sp, *Bacillus cereus, Brevibacterium casei, S. aureus, B. licheniromis* **[75,76]**, and gram negative bacteria, *E. Coli, Entrobacteria,* and *Ureibacillus thermo sphaerius* **[69,73]**.

The antibacterial action of AgNPs on gram positive and gram negative bacterial strains is not the same but competes one over the other. There are contradictory reports regarding antibacterial action against gram positive and gram negative bacteria. According to some

researchers the gram negative bacteria are reported to be more sensitive to AgNPs compared to gram positive bacteria **[82]** whereas reverse results were observed by other researchers **[76].** The reported differential sensitivity of both the bacterial species could be attributed to the difference in structural characteristics of the bacterial species as well as shape and size of AgNPs, bacterial inoculums size, exposure time and nutrient medium used during analysis of antibacterial action. The anti-bacterial action of AgNPs is quite complex and not well studied. Mechanisms of antimicrobial effects of AgNPs are still not fully understood, but several studies have revealed that AgNPs may attach to the negatively charged bacterial cell wall and rupture it, which leads to denaturation of protein and finally cell death. The cell death due is also related to uncoupling of oxidative phosphorylation, induction of free radical formation, interference with respiratory chain at cytochrome level, interaction with protein thiol groups and membrane bound enzymes. Additionally, interaction with phosphorus and sulphur containing compounds such as DNA and protein **[43,53,54]**. Its mechanism is only tentatively explained.

The antimicrobial action of AgNPs can be categorized in two types: the inhibitory action and bactericidal action. In the former strategy bacterial cells are not killed but their division is prevented whereas in the later bacterial cells will die due to the action of AgNPs **[58]**. The antibacterial action mechanism of AgNPs is summarized in (Figure 2.7)**.** The graphical presentation shown in Figure 2.7 is the result of bacterial growth loaded with Ag NPs synthesized from different green sources.

The mechanism behind the bactericidal action of Ag NPs was illustrated by release of Ag+ ions, which serves as reservoirs for anti-microbial action **[83]**. The Ag+ cations produced interacts with the negative charge on the cell wall and affects the membrane permeability. The nano-silver cations which have greater affinity towards sulphur and phosphorus containing compounds present in the outer membrane, respiratory enzymes, proteins and DNA, penetrate through the cell wall and plasma membrane by destabilizing them and cause protein denaturation by dissipating proton motive force, respiratory inhibition, intracellular ATP depletion and DNA damage. The above stated mechanism is in agreement with the reports of many authors **[76,77,81,83]**.

It is also assumed that the high affinity of metallic nanoparticles towards sulfur and phosphorus is the key element of the antimicrobial effect. Due to the abundance of sulfur-containing proteins on the bacterial cell membrane, metallic nanoparticles (such as AgNPs)

can react with sulfur-containing amino acids inside or outside the cell membrane, which in turn affects bacterial cell viability. The general understanding is that metallic nanoparticle of typically less than 20 nm diameters get attached to sulfur-containing proteins of bacterial cell membranes leading to greater permeability of the membrane, which causes the death of the bacteria.

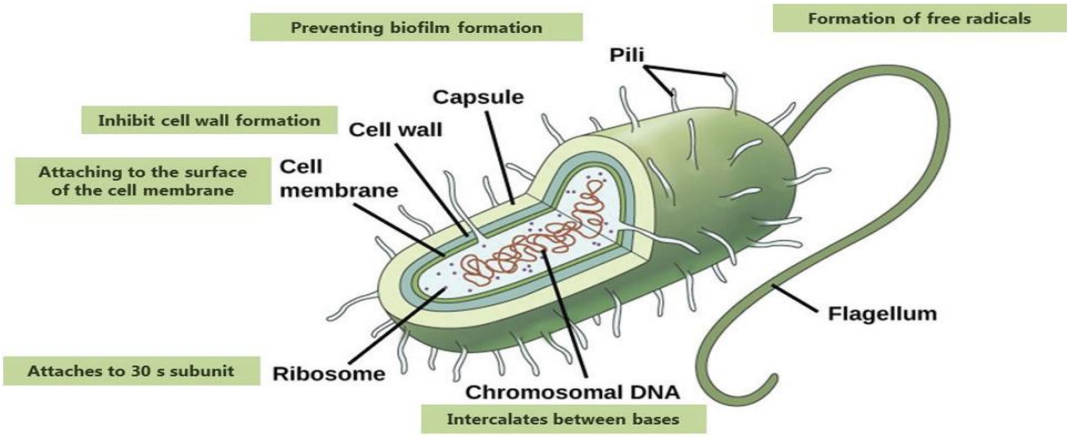


Figure 2.7 Mechanism of action of Silver Nanoparticles anti microbial activity **[83].**

Therefore it was clear that green synthesized AgNPs has been used as an antibacterial agent. And the mango leaves synthesized AgNPs was expected to be effective in killing bacteria. In this work antibacterial activity of AgNPs was examined by disc diffusion method of for two bacterial strains. This was quantitatively done by measuring the clear zone of inhibition. Generally, strains are thought to present a major public health problem **[81]**. The antibacterial activity of the AgNPs was examined against the two both Gram-positive and Gram-negative bacteria.

3. MATERIALS, CHEMICALS AND METHODS

3.1 Chemicals and Reagents

All the chemicals used were analytical grade in this experiment. Silver nitrate (98 %, Blulux Laboratories (P) Ltd), Ferric chloride (99 %), Hydrochloric acid (35.4 %), Sulphuric acid (98 %) (All are products of Loba Chemie Pvt. Ltd, India), Ethanol (99.5 %, UNI-CHEM Chemical Reagents), Methanol, Gentamicin, Chloroamphenicol, KBr (Uvasol, Germany), Agar Hilten Muller (HiMEDIA, India), Chloroform (99.9 %, Fisher Scientific UK Limited, UK), Sodium Hydroxide (98 %), Ammonia solution (25 %) (Both of them are products of Blulux Laboratories (P) Ltd), Meyer's reagent, Benedict's solution, Magnesium Chloride (98 %), Iodine solution (80 %), Potassium Iodide (99 %) (All of them are produced by Abron Chemicals, India).

3.2 Instruments and Apparatus

The necessary apparatus and instruments used for this study were UV-Vis spectrometer (Agilent technologies, Cary 60UV-Vis), FT-IR (Perkin Elmer), electronic beam balance, digital PH meter (Adwa), store chamber (Lec refrigerator plc, England), and others were used for different purposes.

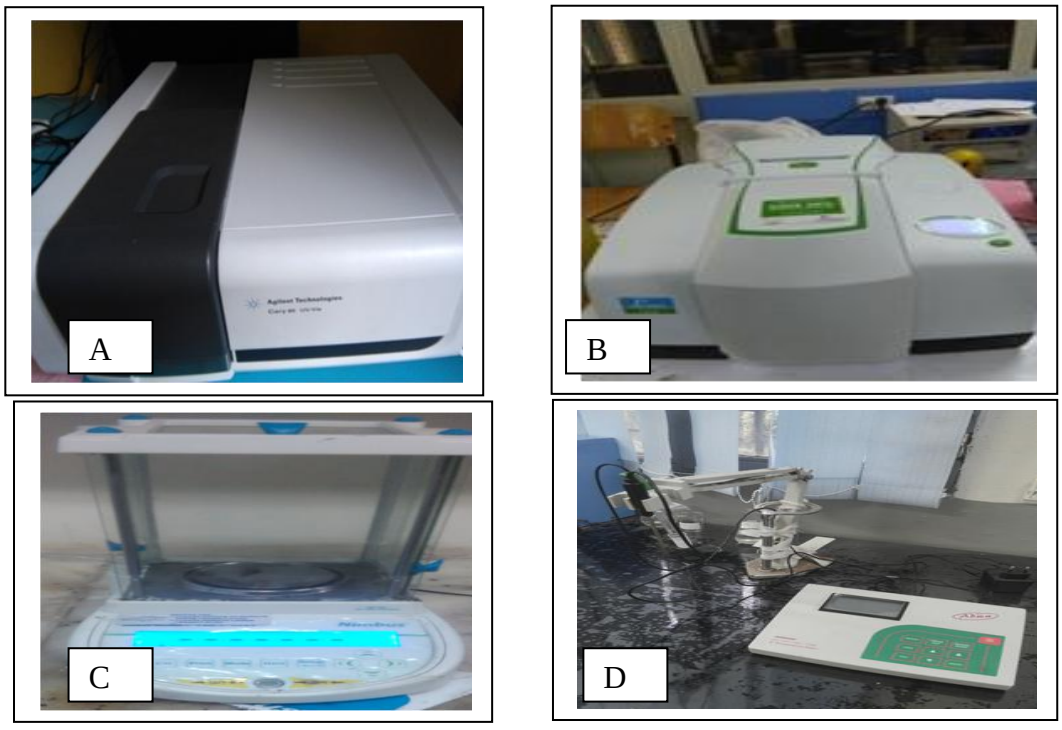


Figure.3.1 Some of the instruments used during the experiment. (A) UV-Vis spectrometer (B) FT-IR spectrometer (C) Electronic beam balance (D) Digital PH meter.

3.3 Experimental Procedures

Typically, a plant extract mediated bio-reduction involves mixing the aqueous extract with an aqueous solution of the appropriate metal salt. A silver nanoparticle was prepared by using aqueous mango leaves mixed with silver nitrate solution. The synthesis of nanoparticle was carried out at room as well as other recommended temperatures and was completed within few minutes. But results after hours of reactions have also been used to analyze the effect of reaction time on the formation and stability of the AgNPs. All the glassware were washed with distilled water and dried in air. De-ionized and distilled water were used in all of the experimental works.

3.3.1 Preparation of Mango leaves Extract by heating methods

Ethiopia medicinal plant typically, Green mango tree was selected from peda campus, Bahir Dar University, on the basis of cost-effectiveness and ease of availability. Fresh and healthy mango leaves were collected locally and rinsed thoroughly first with tap water followed by distilled water to remove all the dust and unwanted visible particles, cut into small pieces and dried at room temperature. The aqueous leaves extract was prepared by placing 5 g of washed and dried fine cut leaves in 250 ml glass beaker along with 100 ml of De-ionized water. The mixture was then heated for 15 minutes at 60°c until the colour of the aqueous solution changes from watery to pale Green. Then the extract was cooled to room temperature and filtered twice through Whatman No.1 filter paper to remove particulate matter and to get clear solution. The filtrate was collected and then stored in refrigerator at 4°C in 250 ml Erlenmeyer flask in order to be used for further experiments. In each and every steps of the experiment, sterility conditions were maintained for the effectiveness and accuracy in results without contamination.

The following diagram shows the steps of preparing the mango leaves extract samples.

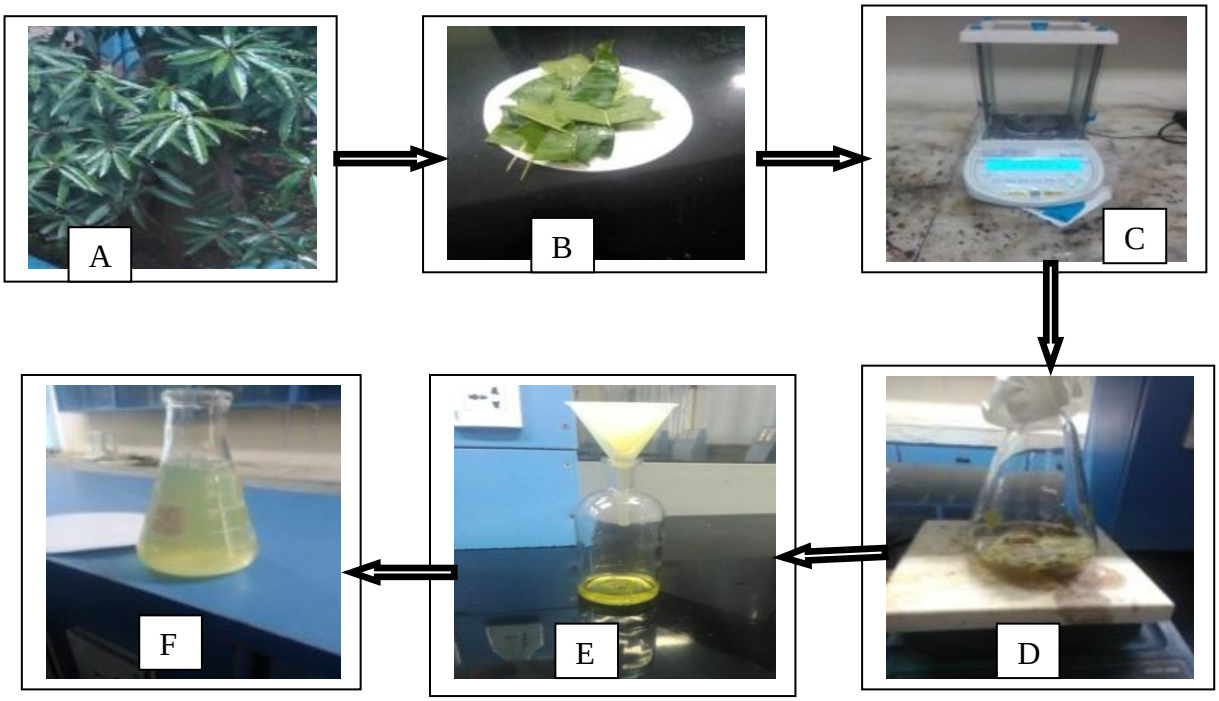


Figure.3. 2 The general procedure of preparation of mango leaves aqueous extract. (A) fresh mango leaf was collected (B) washed several times and cut in to small peace (C) fine cut dried in air and weighted by electronic beam balance (D) 5 g of mango leaf was mixed with 100 ml de-ionized water and heated for 15 minute at 60 °c (E) filter the cooled solution (F) clear extracted solution was collected.

3.3.2 Phytochemical screening-Qualitative analysis

The researcher was used chemical tests for the screening and identification of bioactive chemical constituents like alkaloids, carbohydrates, glycosides, saponins, terpenoids, Phenolic compounds, proteins, amino acids, flavonoids, and tannins, in the medicinal plants were used as reducing and capping agent, to cheek whether these components are found in mango leaves by using standard procedure **[67]**.

3.3.2.1. Test for Alkaloids: (Wagner's test: Iodine- Potassium iodide solution)

1.2 gm of Iodine and 2 gm of H_2SO_4 was mixed and then diluted to 100 ml. 10 ml of the alcoholic extract was acidified by adding 1.5 % (v / v) of HCl and a few drops of Wagner's reagent. Formation of yellow or brown precipitate was to confirm the presence of alkaloids.

3.3.2.2. Test for Glycosides

A small amount of alcoholic extract was dissolved in 1 ml of water. Aqueous NaOH solution was dissolved in 1 ml of water and added to the extract. Formation of reddish brown colour was taken as an indicator for the presence of glycosides.

3.3.2.3. Test for Tannins: (Ferric chloride test)

1.0 ml extract was stirred with 1.0 ml ferric chloride; the occurrence of a greenish black precipitate for the presence of tannins was checked.

3.3.2.4. Test for Flavonoids

0.2 ml extract was added to 2 ml 10 % (m / v) $FeCl_3$ solution and the mixture was shaked. A woolly brownish precipitate for the presence flavonoid was checked.

3.3.2.5. Test for Saponins

0.2 ml extract was mixed with 5.0 ml distilled water, shaked for 20 minutes and the persistence of foams for the presence of saponins was checked.

3.3.2.6 Test for Steroids (Salkowski test)

2 ml of chloroform and 1 ml of concentrated H_2SO_4 acid were added carefully along the sides of the test tubes. The mixture was then examined to reveal a red colour in the chloroform layer which confirms the presence of steroids.

3.3.2.7. Test for Phenols

The mango leaves extract was treated with 3−4 drops of ferric chloride solution. It was then left to form bluish black colour that indicates the presence of phenols.

3.3.2.8. Test for Carbohydrates: (Benedict test and Iodine test)

A few drops of Benedict solution was added in to the plant extract and it was checked for its formation of brick read colour which confirms the presence of glucose and a few drops of Iodine was added in other extract where a dark blue colour was expected to confirm the presence of starch.

3.4. Preparation of Silver Nitrate stock Solutions.

6 mM $AgNO_3$ was prepared i.e., 1.02 g of $AgNO_3$ was taken and dissolved in 1000 ml de-ionized water. From this stock solution, different concentration of precursors, (0.25, 0.5, 1, 2, 3, 4 and 4.5) mM were prepared and used for the synthesis of silver nanoparticles by applying dilution law.

3.4.1 Synthesis of Silver Nanoparticles

For synthesizing of silver nanoparticles, first silver nitrate and mango leaf extract stock solutions were prepared. In a typical biosynthesis of silver nanoparticles, 2 ml of 5 % (5 g / 100 ml) of the filtrate from the plant extract was added with stirring to 30 ml of 4 mM $AgNO_3$ in 250 ml flask for gave a light yellow solution mixture at room temperature. The sample of the mixtures was taken at intervals 1-2 hour. I was observed that the colour of the solution mixture of silver nitrate and mango leaf extract changed from light yellow colour to yellow brown colour at room temperature after 1 hour of reaction time and then after 2 hour of reaction time, the colour of mixture was changed from yellow brown colour to reddish brown colour. It was allowed to cool down. Afterwards, the mixture was treated with 0.1 M Sodium hydroxide drop by drop for a specific pH of 11. Finally the nanoparticle formed was characterized using the visual observation and the UV-Vis spectra. At last, it was examined for its antibacterial activity.

The following diagram shows the steps of preparing the Silver Nanoparticles.

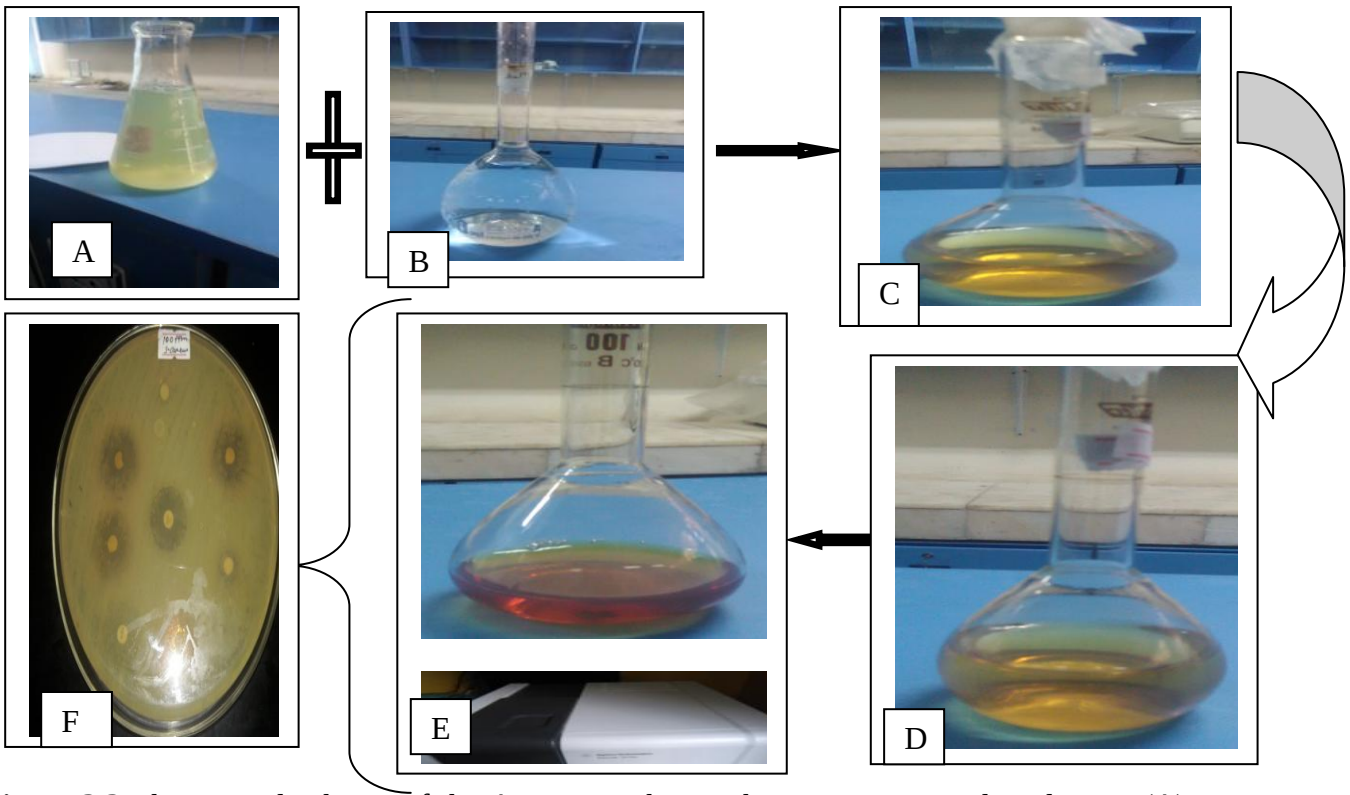


Figure.3.3 The general scheme of the AgNPs synthesis, characterization and application(A) extract of mango (B) silver nitrate (C) light yellow of AgNPs after 15 minute (D) yellowish brown of AgNPs after 1 hour (E) reddish brown of AgNPs after 2 hour (F) antibacterial activity of reddish brown of AgNPs.

3.5. Characterization of Silver Nanoparticles

The synthesized AgNPs was characterized by visual observation, UV–Visible absorption spectroscopy and Fourier Transform Infrared spectroscopy analysis.

3.5.1Visual Observation:

The process begins by mixing a sample of plant extract with a metal salt solution. Biochemical reduction of the salts starts immediately and the formation of nanoparticles is indicated by a change in the colour of the reaction mixture. During synthesis, there is an initial activation period when process metal ions are converted from their mono or divalent oxidation states to zero-valent states and nucleation of the reduced metal atoms takes place **[81]**. This is immediately followed by a period of growth when smaller neighbouring particles amalgamate to form larger nanoparticles that are thermodynamically more stable while further biological reduction of metal ions takes place.

The formation of AgNPs is initially judged based on the colour change of the solution, using the naked eye. The characteristic absorbance is observed in the range of 380~450 nm (reddish brown colour), in which predominantly spherical AgNPs are synthesized **[65]**. Formation of silver nanoparticles was monitored visually. During synthesis of the silver nanoparticles, every colour change was noticed carefully.

3.5.2 UV-Vis Spectroscopy

The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 2 hours after diluting a small aliquot of the sample into distilled water. The optical property of biosynthesized silver nanoparticles samples were measured at room temperature to monitor the completion of bio-reduction of Ag^{+} in aqueous solution by UV-Vis spectrophotometer (Cary 60UV-Vis) operated at a resolution of 1 nm between 300 and 800 nm range. UV-Vis spectra were recorded and analyzed.

3.5.3. Fourier transforms infrared spectroscopy (FT-IR)

FT-IR spectroscopy can be used to investigate surface chemistry and identify surface residues such as functional groups like carbonyls and hydroxyls moieties that attach to the surface during nanoparticle synthesis.

FT-IR spectroscopy was used; a drop of the mango leaf extract was mixed with the KBr powder so as to form a paste. Afterwards the paste was taken in to the FT-IR and got scanned at a resolution of 8 nm in a wave number range of 400 to 4500 cm^{-1}. The same was done for

the AgNPs formed using KBr pellet method. FT-IR spectra provide information regarding the functional groups of the reducing agents that are involved in the reduction of Ag+ to AgNPs.

3.6. Antimicrobial susceptibility testing of the Silver Nanoparticles

There are different methods of antimicrobial testing of the silver nanoparticles. The following three methods have been shown to consistently provide reproducible and repeatable results when followed correctly (Clinical and Laboratory Standards Institute (CLSI) i.e. disk diffusion, broth dilution and agar dilution. Among the three known antimicrobial susceptibility testing methods Disk diffusion method was used in this study.

3.6.1 Source of microorganism

The bacterial strains were used obtained from Bahir Dar University of Science College: Microbiology Laboratory, Ethiopia. The name of the bacteria and the kind of food were selected to examine this test. Table 3.1 shows types of bacteria and the materials that were used for growing.

Table3. 1 Types of bacteria and their food for growing

Name of bacteria	Type of bacteria	Materials(food)	antibiotics
Streptococci pyrogens(S.pyrogens)	Gramme positive(G^{+ve})	Mueller Hinton Agar (HiMEDIA, India)	Gentamicin(CN)
Staphylococcus aureus (S. aureus)			
			Chloroamphenicol(C)
Salmonella typhi (S. typhi)	Gramme negative(G^{-ve})		
Escherichia coli (E. Coli)			

3.6.2 Antimicrobial activity study

For examined the antimicrobial activity of silver nanoparticles, disc diffusion method was performed. In order to examined antimicrobial activities, researcher was selected two different gram positive and negative bacteria .These bacteria were Staphylococcus aurous (+ve), Streptococci pyrogens (+ve), Salmonella typhi (-ve) and Escherichia coli (-ve). Then researcher was examined the effect of silver nanoparticles on bacteria growth compared with known antibiotics like Gentamicin (CN, 10 μg), Chloroamphenicol (C, 10 μg).

3.6.3 Preparation of Mueller Hinton Agar Plate (nutrient media)

Mueller-Hinton agar was prepared from a commercially available dehydrated base according to the manufacturer's instructions 19 g of Mueller-Hinton agar powder was weighed using a

clean electronic weighing balance and dissolved in 500 ml of sterile distilled water using 1000 ml conical flask. The mixture was stirred with a sterilized glass rod and covered with a cotton wool, over which an aluminium foil was tightly wrapped and then autoclaved for 20 min at 121°c. Soon after autoclaving, the agar was allowed to cool to 40°C in order to maintain the media in a molten stage. Petri dishes were dried in lower humidity by keeping them in a laminar flow hood. The freshly prepared and cooled Muller-Hinton agar was dispensed in to sterile flat-bottomed Petri dishes uniformly without it forming any bubbles and allowing it to solidify at room temperature.

3.6.3.1 Antimicrobial activity assay by disc diffusion method

The antimicrobial activity of silver nanoparticles by using disc diffusion method was testing. For growing the four selective bacteria, first the nutrient media materials, the Petri dish was free from micro organism bacteria using safety hood oven dry for sterilized by moist heat at 121°c with 1.5 atm pressure in an autoclave for 30 min and then covered by aluminium foil. After sterilization, the two negative and two positive bacteria were poured for each individual Petri dish plates was allowed to solidify. The media was used to make cultural growth of each of the bacteria. Using a sterile cotton swab, bacterial cultures were swabbed on the surface of sterile disc plates. Four sections on the base of the Petri dish, the four different samples water (negative control), Mango leaf extract, silver nitrate solution and solution containing AgNPs of each type separately were placed at the corners of the disc plates. Two drugs, Gentamicin (CN), and Chloroamphenicol (C) wear marked and labelled placed at the centres of the disc plates, used as a positive controller. Using sterile forceps (flamed with alcohol and cooled) one filter paper disc was removed and dips into the first test sample. Then, the sample and the drag were drained on the side of the container and placed firmly onto the appropriate section of the seeded agar plate. Finally the forceps was washed to be free from the sample. This procedure was repeated for the remaining samples and the two drugs. Remember to rinse and sterilize the forceps between each sample and to open the plate for the minimum possible time and placed on the surface of disc plates inoculated with a microbial culture. The plates were incubated at 37°C for 24 hours. The plates were examined (without opening), measured and recorded the size of any zones of inhibition (clear zone) around the filter paper discs by using ruler its diameter in millimetre. Then, the maximum zone of inhibition were observed and measured for analysis against each type of test microorganism and drugs **[20].**

4. RESULT AND DISCUSSIONS

4.1. Phytochemical test Analysis of the Extracted mango leaf

The mango leaf has been extracted and for it to be used as a reducing and capping agent, it was necessary to check whether the phytochemicals, such as flavonoids, polyphenols, carbohydrate etc, do naturally exist in the extract or not. The results of qualitative phytochemicals analysis of the mango leaf extract are shown below in Table 4.1 and Figure. 4.1.

Therefore the Mango leaf extract is composed of phytochemicals which are capable of reducing the Ag^{+} by donating electrons, capping and stabilizing the formed nanoparticles **[73]**. For instance, the Polyphenolic compounds are very important plant constituents because of the scavenging ability of their –OH groups **[57]**. The antioxidant property of Polyphenolic compounds is mainly due to its red-ox property which allows them to act as reducing agents.

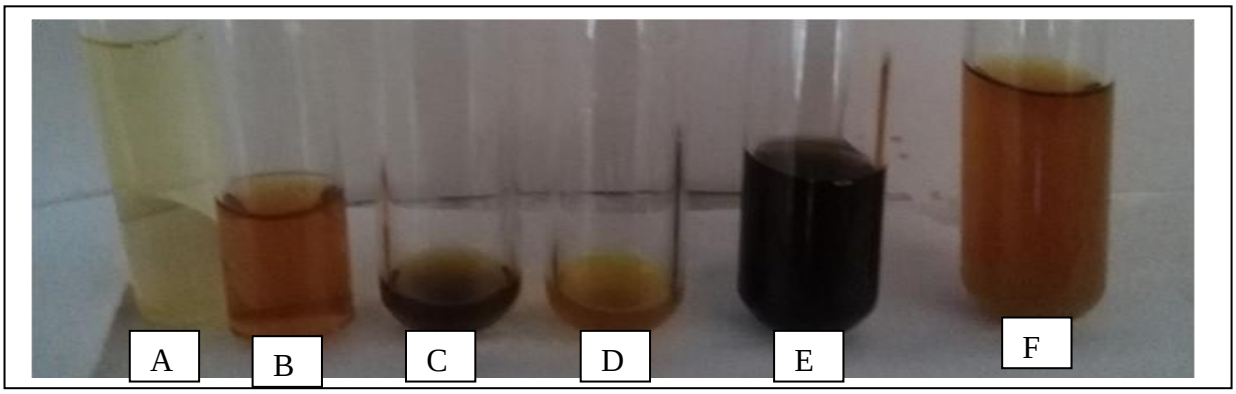


Figure.4.1 The colours observed when the extract is tested for (A) Alkaloids (B) Glycosides (C) Tannis (D) Flavonoids (E) Poly phenols and (F) Carbohydrate.

The colour changes are the results of formation of different complexes as a result of oxidation and reduction reactions. For instance the yellow colour for alkaloids indicates that the nitrogen or oxygen atoms of the amide groups of the alkaloids participation in a reaction. In most of the Ferric chloride tests, the iron (III) ion forms complexes having different colours **[76]**. These different colours assures the presence of Protein, Alkaloides, Flavonoids, Phenolic compounds and Tannins in extracted mango leaf.

Table4. 1 The qualitative analysis of phytochemicals in the Mango leaf extract.

No	Phytochemicals	Chemical test	Result	Colours observed	Sign(+)presence Sign(++) highly presence
1	Alkaloid	Wagner's test	+	Yellow	
2	Glycoside	Alkaline reagent test	+	Reddish brown	
3	Tannin	Ferric Chloride test	+	greenish black	
4	Flavonoid	Ferric Chloride test	+ +	Woolly brown	
5	Phenols	Ferric Chloride test	+ +	Bluish Black	
6	Carbohydrate (glucose)	Benedict test	+	Brick red	

4.2 Characterization of the synthesis of sliver nanoparticle

The formation of AgNPs nanoparticles was confirmed primarily based on change in colours of the reaction mixture, and then used UV-Visible spectrum and FT-IR spectrum which were frequently used to characterize and synthesized the metal nanoparticles.

4.2 .1 visual observation

The formation of AgNPs was primary well known by the colour change. The colour changes arise due to excitation of surface plasmon resonance in the metal nanoparticles indicating the formation of AgNPs. It was primarily confirmed based on change in colour of the reaction mixture as soon as the mixture was made. The change was observed from the colour less in to light yellow for the first 1 hour and after 2 hours duration, the colour was changed to from light yellow to yellowish brown and finally converted to reddish brown. Then after no further colour change had been observed. This indicates that silver salt present in the reaction mixture has been reduced completely **[63]**. In contrast, the colour of the mango leaf extract (pale green) and the $AgNO_3$ (white) solution remained unchanged. Figure 4.2 shows that the colour changes while adding mango leaf extract into sliver nitrate solution. This change in colour was due to the excitation of free electrons in nanoparticle which gives the SPR absorption band wave metal NPs in resonance with light wave.

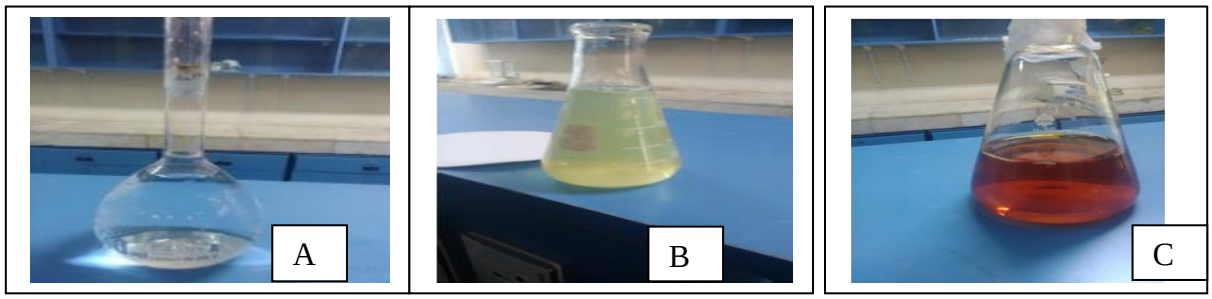


Figure.4. 2 The colour changes observed during the formation of AgNPs; (A) precursor (B) the Mango extract and (C) the nanoparticle.

In this experiment further pointed out, as shown in Figure 4.3, the change difference in the intensity of the colour as pH increases (1, 3,5, 7, 9, 11,and 13) at constant concentration of the reducing agent (mango leaf extract) and precursor. This is because as the pH increases, the rate of formation of the nanoparticles increases. Typically, at pH 1 and 3 these colours were not primarily confirmed with synthesizing AgNPs and the result is in good agreement with previous reported work **[73],** broad SPR bands at low pH, which indicated the formation of large size nanoparticles.

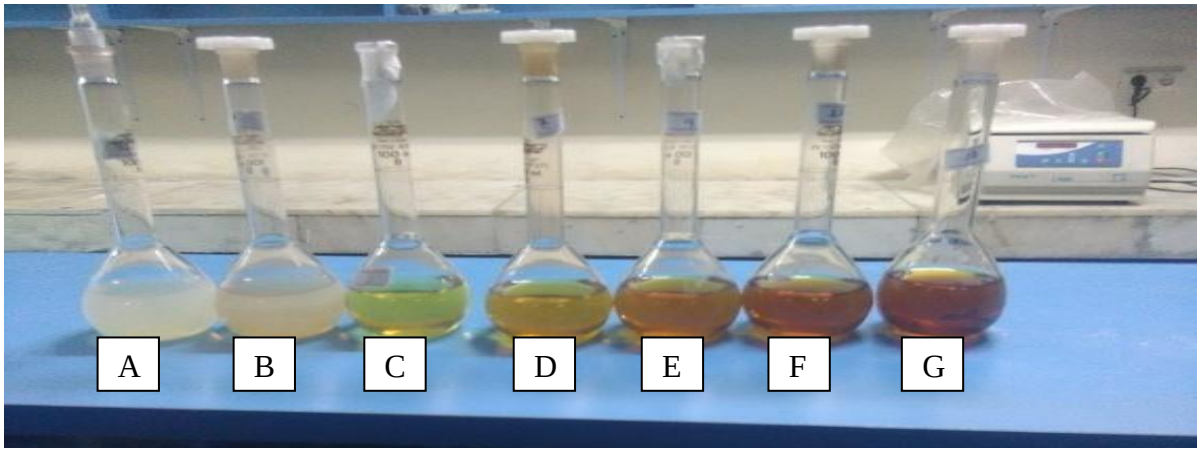


Figure.4. 3 The colour changes observed during the formation of AgNPs by changing pH at (A) 1 (B) 3 (C) 5 (D) 7 (E) 9 (F) 11 (G) 13.

4.2.2 UV-Visible spectra result analysis

The metal AgNPs have free electrons, which are responsible for the SPR absorption band. Such metal called plasma contains equal numbers of positive ions which are fixed in position and conduction electrons which are free and highly mobile. Under the irradiation of an electromagnetic wave, the free electrons are driven by the electric field to oscillate coherently. These collective oscillations of the free electrons are called Plasmon. These plasmons can interact, under certain conditions, with visible light in a phenomenon called SPR. The position, the shape and intensity of the SPR strongly depend on various factors including the size, shape and monodispersity of the NPs, as well as the composition of the surrounding media and interactions between stabilizing ligands and the NPs. Therefore the effect of different factors on the size, shape and monodispersity of the nanoparticles should be observed to design a suitable formulation for production of NPs. Silver metal nanoparticles exhibit the absorption of visible electromagnetic waves by the collective oscillation of conduction electrons at the surface. This is known as the surface Plasmon

resonance effect. The interest in this effect is the possibility of using it as a tracer for the presence of metal nanoparticles with a simple UV-Visible spectrometer **[65]**.

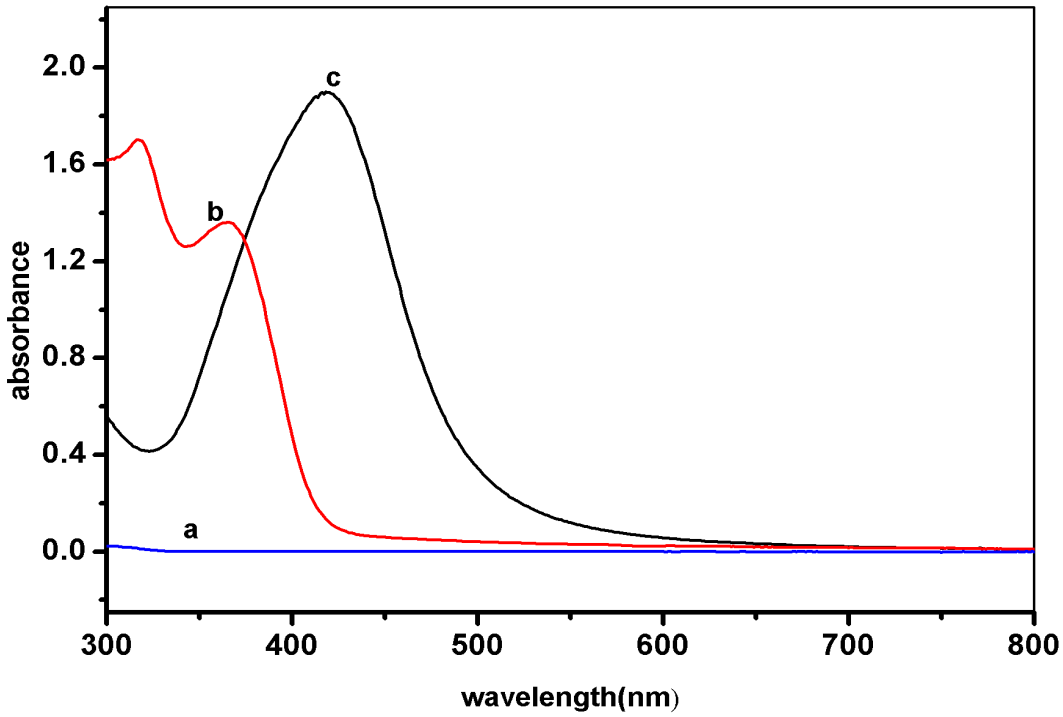


Figure.4.4. UV–Visible spectra of (a) precursor (b) Mango leaf extract and (c) AgNPs.

The Figure 4.4 shows the maximum wave length of mango leaf is 366 nm and the AgNPs is 418 nm. The above graph indicates that the absorbance and wave length of the silver nitrate and mango leaf extract graphs were completely different from the synthesized of AgNPs. From several literatures it was reported that the AgNPs usually show SPR peak centred at 420 nm **[62,65,77,79]**. In the synthesis of AgNPs mediated by *Coleus aromaticus* leaf extract, maximum production of nanoparticles depicted by sharp peak was observed at 425 nm in alkaline pH of 9.2 **[73]**.

The UV-Vis spectra result revealed a strong absorbance at 418 nm suggesting the formation of AgNPs (Figure.4.4), which is very closes to most results published at different journals. This Ag NPs result definitely agrees with the range of λ_{max} values, 400 nm – 450 nm, at different previous works using plants other than mango leaf **[63,65,69,81]**. The peak obtained in this work was very sharp and more intense for the green synthesis of AgNPs by using aqueous mango leaf extract. This is expected to be the effect of the smaller size of the nanoparticles synthesized which make the AgNPs to be more applicable. Therefore the colour changes and the above UV-Vis values confirm the formation of the AgNPs. This shows,

during the formation of the AgNPs, there is a red shift in wavelength which can be considered as an indicator of a newly formed shape and size of particles with different SPR **[60].**

During the formation or synthesized of the AgNPs, parameters were optimized so as to select the best conditions used during the work.

4.3. Optimization of Different Parameters of Silver Nanoparticles result analysis

Researchers have reported that four factors pH, temperature, reaction time and the ratio of plant extracts to silver nitrate affect the synthesis of AgNPs based on plant extracts **[74, 76]**. The first parameters to be optimized were the concentrations of both mango leaf extract and silver nitrate solutions. The second parameter was pH optimized in both acidic and basic media. Then the stability of AgNPs was studied.

4.3.1 Effect of silver nitrate concentration on the synthesis of Ag NPs

Figure 4.5 represents with different concentration of silver nitrate mixed with constant concentration of mango leaf extract. The result showed that at different concentration different of maximum wave length and the peak intensity were obtained. In this work 379 nm and 0.6218 at 0.25 mM, 407.98 nm and 1.0289 at 0.5 mM, 421.01 nm and 1.326 at 1 mM, 426.98 nm and 1.3934 at 2 mM, 426.98 nm and 1.6565 at 3 mM, 418 nm and 1.9 at 4 mM, and 418 nm and 2.549 at 4.5 mM of maximum peak wave length and absorbance intensity were obtained respectively. As seen from Figure 4.5 the concentration of silver nitrate increase, the intensity of the peak also would increase. Comparing the peaks obtained, it was shown that 4 mM$AgNO_3$ was the best concentration of the precursor to be used for the synthesis of the AgNPs with an intense peak and more blue shift, as compared to the (0.25, 0.5, 1, 2, 3, and 4.5) mM, as can be seen at Figure.4.5. This result definitely agrees with the concentration of $AgNO_3$ used for the synthesis of AgNPs using aqueous leaf extract of *Coleus aromaticus*. For the 0.25 mM and 0.5 mM the peaks became broader due to the small in number of the silver ions and excesses number of reducing agent so that low formation of AgNPs and form particles of larger size **[47].** But for the 4.5 mM dark green colour was observed that confirms the peak was agglomeration. This shows that when the concentration of the silver ions in the solution was increased, it has exceeded the amount of reducing agent phytochemicals of the mango leaf extract. This Higher concentration of silver nitrate suggests the formation of larger nanoparticles **[83].**The large size and aggregation of nanoparticles was occurred due to the occurrence of compete between silver ions and functional groups of 2 ml

extract of mango leaf. This investigation concludes that the optimum silver nitrate concentration 4 mM suitable for nanoparticles synthesis because the peak is more intense and sharp of the nanoparticles synthesis and size reduction was started quickly and then used for next experiment.

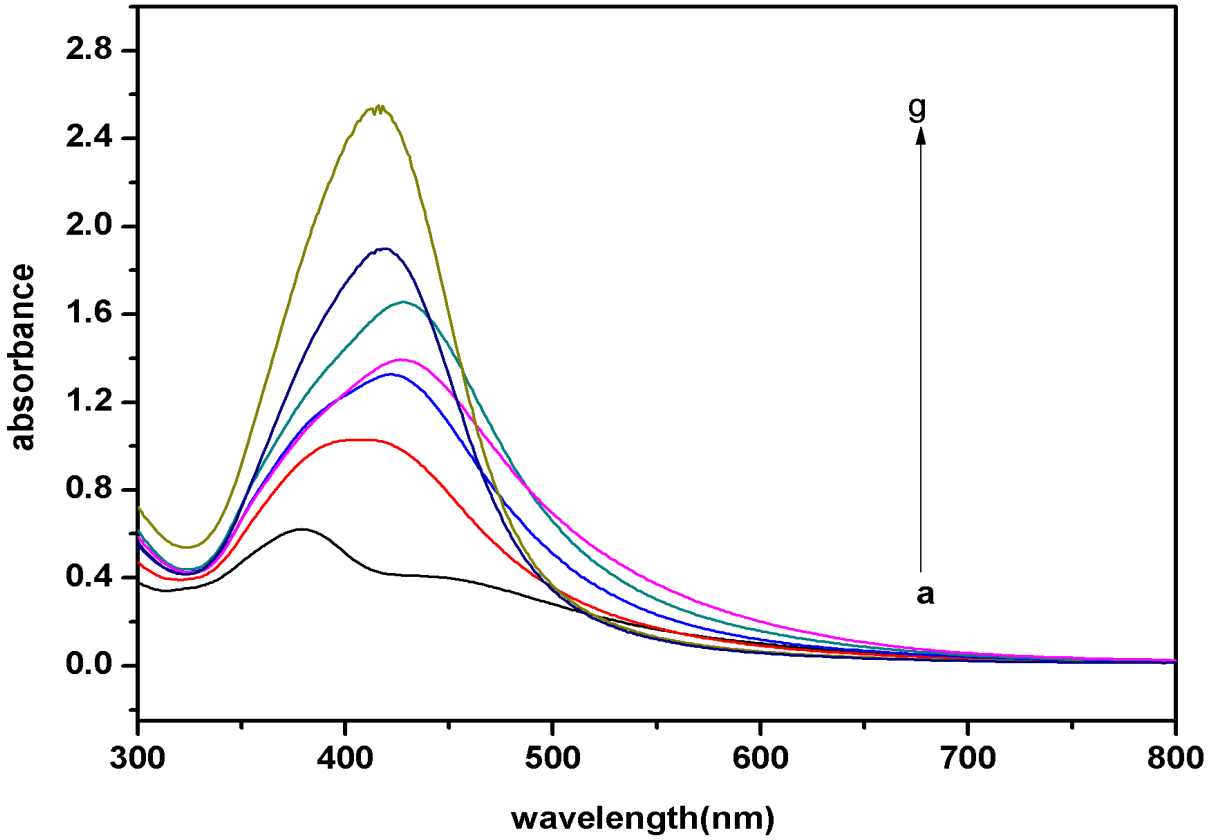


Figure.4.5.The UV-Vis spectra of AgNPs formed from 2 ml extract of 5 % (m/v) mango leaf and 30 ml of (a, b, c, d, e, f, g), (0.25, 0.5, 1, 2, 3, 4, 4.5 mM) respectively.

4.3.2. Effects of concentrations of mango leaf on synthesis of AgNPs

The results were obtained from UV-Visible spectroscopy analysis of the sample. In Figure 4.6 shows that the absorption spectra of the silver nanoparticles were obtained using different concentrations of mango leaf extract mixed with the same concentration of sliver nitrate. Figure.4.6 shows that; the concentration of Mango leaf extracts which gave a broader peak were 6 and 7 % (m / v) relative to the 4 % and 5 %. Because, it is clear that the extract peak is increase and the AgNPs peak is decrease. This reveals that the Mango leaf extract is dominating the solution and the nanoparticle formed has become more unstable. The sharpness peak of mango leaf extract increase beyond 7 % (m / v) indicates that the size of

the nanoparticle decreases more as the concentration of the mango leaf extract is increased which agrees with the concept that; progressive increase in the characteristic peak with increase in concentration of biological extracts with the same amount of salt ions is a clear indicator of nanoparticle formation **[65]**. Despite of all these facts, 5 % (m / v) mango leaf extract was taken as the best concentration of the extract for its smooth peak with good intensity. Because increasing the concentration above 5 % (m / v) was noticed giving the extract peaks and the mixture was suspected to be dominated by the extract. Therefore increasing the concentration above this value might ultimately giving the nanoparticle unstable and show a blue shift closer to the λ_{max} of the Mango leaf extract.

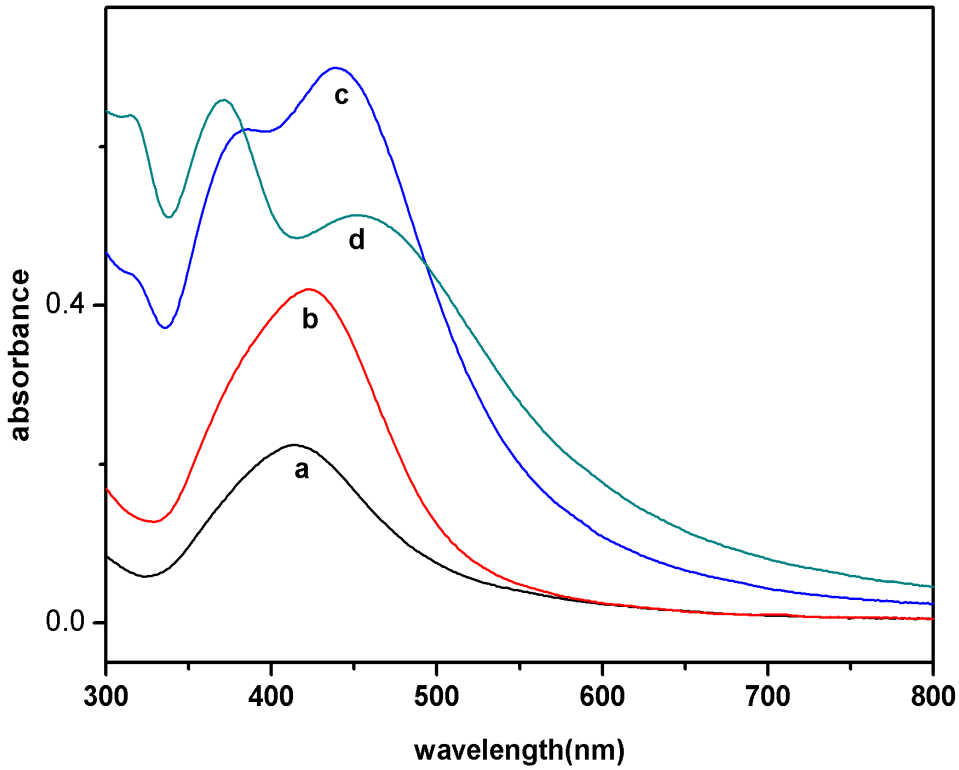


Figure.4. 6 UV-Visible spectra for the AgNPs formed from 30 ml of 4 mM $Ag(NO_3)$ and (a, b, c, d), (4, 5, 6, 7, % (m / v)) mango leaf extract respectively.

4.3.3 Effect of pH on synthesis of AgNPs

By changing the pH, the charges of the biomolecules in extracts are altered **[77]**. Because the Ag ion is a cation, the charge of the biomolecules also affects the synthesis of AgNPs. The pH of the reaction medium plays a role in the nanoparticle synthesis by inducing the reactivity of leaf extract with silver ions. The pH of the precursor, the extract, and the nanoparticle before addition of NaOH solution were 5.7, 6.2 and 5.8 respectively. The pH values play important role in the formation of small size nanoparticles as shown in Figure4.7.

(a−e). The nanoparticle solutions of different pH values (5, 7, 9, 11, and 13) were analyzed based on intensity of their peak intensity and red shift of their respective λ_{max} values. At pH of 5 lower intensity and very broad peak was revealed compared with others pH spectra. The effect of pH on the size and amount of AgNPs synthesized were observed. As the pH increases the maximum absorption intensity also increases due to the reduction of silver cation and formation of sharp peaks of small size nanoparticles were revealed. There is a clear indication that the reaction is favoured under alkaline medium with formation of a narrow peak that tends towards maximum absorbance of silver nanoparticle. The Best result was obtained for the Ag NPs solution with a pH value of 11, as it is revealed at Figure. 4.7 (d). This shows that a more basic media is vey suitable for the AgNPs synthesis. The surface plasmon absorbance of silver nanoparticles was obtained in the range of pH 9−11. The maximum intensities of the AgNP are at pH 13, however the solution was unstable that visually confirmed that the colour was deep black and the peak is full of noise. Researchers **[72,76−78,80]** reported that pH 9-11 is an ideal condition for the preparation of AgNPs plants using as stabilizing agents. This work result is agreed with previous researcher, which is the maximum wavelength of AgNPs was 418nm at pH 11.

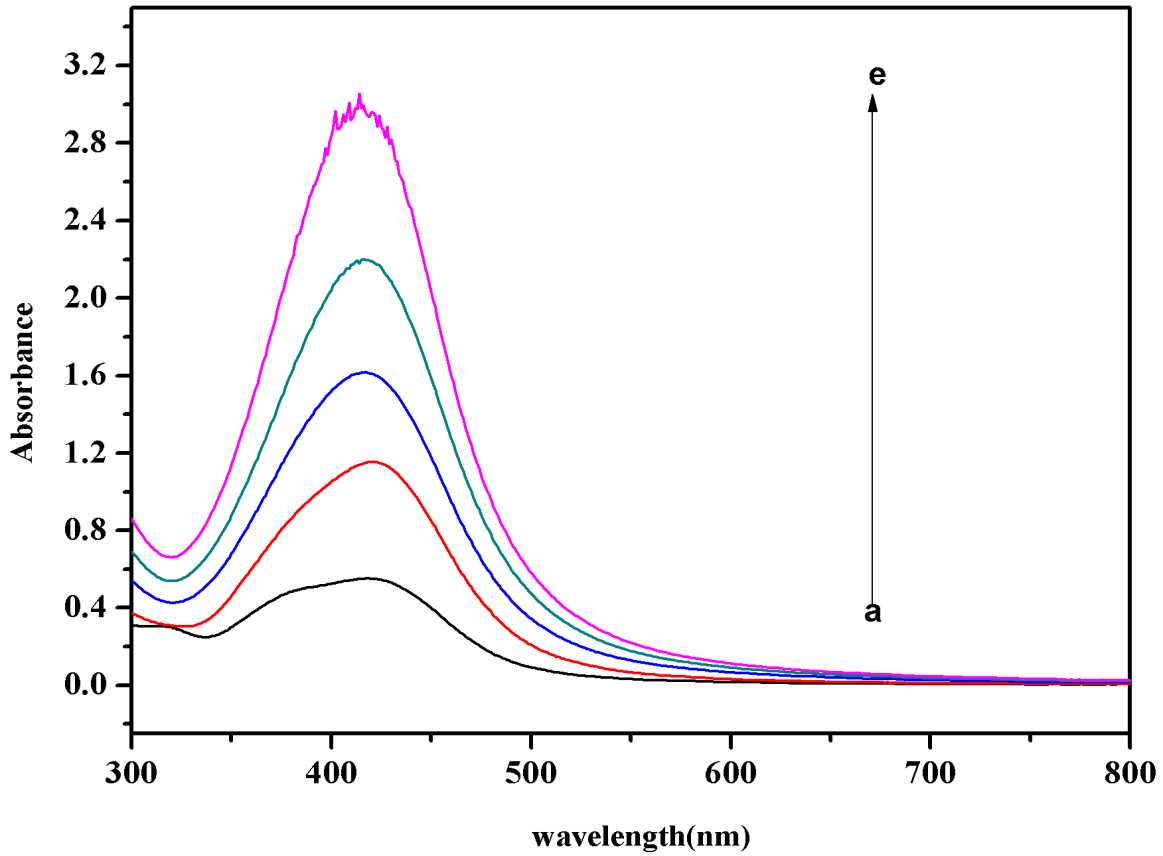


Figure.4. 7 The UV-Vis spectra of AgNPs formed from 2 ml of 5 % (m/v) mango leave extract and 30 ml of 4 mM at a pH of (a)5 (b)7 (c)9 (d)11 (e)13**.**

4.3.4. Effect of reaction time on synthesis of sliver nanoparticle

In this study, the formation of AgNPs was monitored by measuring UV-Vis spectra at different time intervals; (Figure4.8.a – d) as the time increased, the intensity of this absorbance increased, and indicating increases in the amount of AgNPs produced from the mixture. There seems to be more stabilizing effect indicated by gradual increase in the intensity of the nanoparticles with increase in reaction time. Moreover, there was observed increasing absorbance intensity from one day to six day followed by narrowing of the peaks which suggests particle size reduction **[76].** The intensity of the peaks continued to increase from with slight variation in the absorption maxima. In this study the maximum stability of AgNPs was observed at (Figure4.8.d). However, an optimum duration is required, as silver nanoparticle agglomeration after the optimum duration results in larger particle sizes **[81]**. After a week and continued beyond, in (Figure4.8.e) shows a lowering absorbance intensity in the Plasmon absorption maximum consequence of the unstable and bandwidth increase of the nanoparticles which may result due to desorption of the biomolecules, thereby reducing the concentration of the silver nanoparticles with increase the size of formed **[37]**. This is expected to be the result of small increase in size of the particles and rate of aggregation due to the increase in nucleation and collusion of the nanoparticles formed **[83].**

In other words, there could be aggregation which leads to reduction in total number of nanoparticles over the growth time. This observation is in accordance with earlier reports by other studies, and gives rise to aggregation, coalescence and Ostwald ripening **[35,70]**.

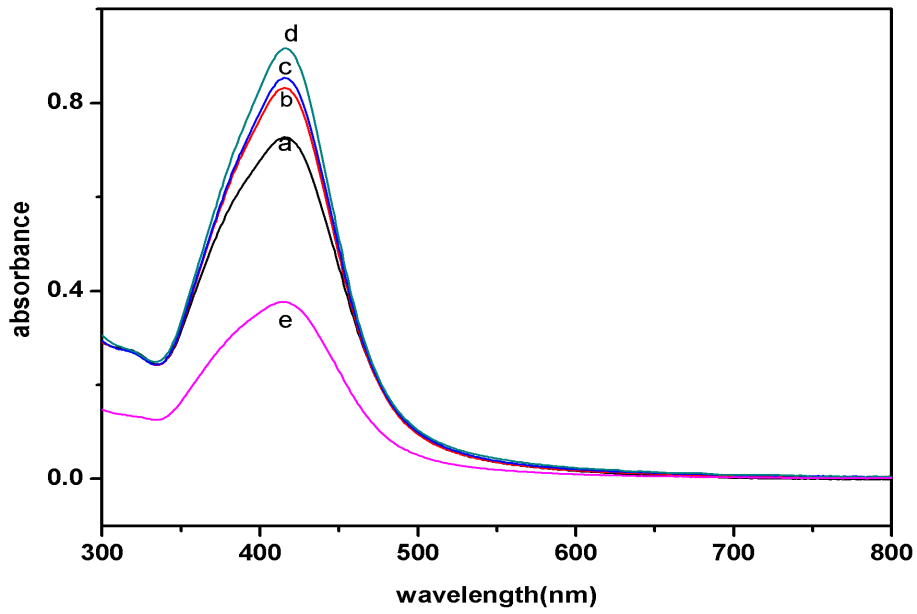


Figure.4.8.The UV-Vis spectra of Ag Nps. (a), after one day (b), after two days (c), after three days (d), after six days (e), after a week.

4.4. The FT-IR spectra analysis

FT-IR spectrum was analysed for identification of different biomolecules adsorbed on the surface of nanoparticles, and also to find out their role in reduction and stabilizing the nanoparticles **[77]**. The dual role of the plant extract, as a reducing as well as capping agent, and presence of some functional groups in both the mango leaf extract and AgNPs were investigated by FT-IR spectra analysis **[81]**. FT-IR analysis was used for the characterization of the pure mango leaf extract (Figure.4.9) and the resulting silver nanoparticle (Figure.4.10). The Absorbance bands in the region of 4000−400 cm^{-1}, the FT-IR spectrum of mango leaf extract strong bands at 3484 cm^{-1}, 1627 cm^{-1}, and 703 cm^{-1}, Figure 4.9. A strong, broad band peak at 3484 cm^{-1} in mango leave can be attributed hydrogen bonded O-H groups of alcohols, phenols and also to the presence of amines N-H of amide. This agrees with the conclusion that the mango leaf extract was composed of poly phenols, flavonides, alkaloids and other similar phytochemicals containing O-H and N-H bonds. The medium sharp peak at 1627 cm^{-1} in mango leaf extract could be attributed to C=C stretching, vibrations about C=O amide, and conjugated C=O of the proteins that are responsible for capping and stabilizing of AgNPs. The strong peaks observed in the range of 703 cm^{-1} has been assigned to phenol groups, C-N stretching vibrations of aliphatic and aromatic amines. From the analysis of the FT-IR spectrum in Figure 4.9, the major peaks of mango plant extract were observed completely peak shifted in silver nanoparticles. The shifting in these bands is clearly indicating that the coordination of carboxylic acids in the protein of mango leaf extract with Ag NPs play a major role on dispersing, stabilizing and capping of AgNPs **[81]**.

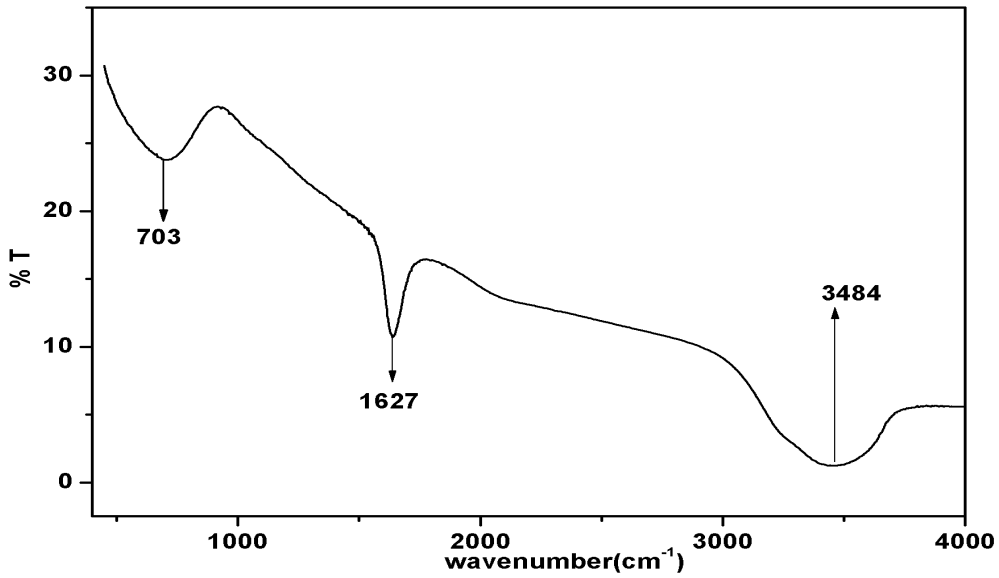


Figure.4.9 FT-IR spectra analyses of mango leave extract.

The FT-IR spectrum of synthesized silver nanoparticles by the mango leave extract, figure 4.10 shows strong bands at 3430, 2362, 2102, 1638, 1285, and 647 cm^{-1}. A broad and strong band b/n (3230− 3642) cm^{-1} is due to bounded hydroxyl (-OH) or amine (-NH) groups of mango leaves extract, that corresponds to O-H groups, H bonded alcohols and phenols.

The medium intensity bands at 2362 cm^{-1} and at 2102 cm^{-1} in the FT- IR spectrum of the AgNPs indicate the presence of alkynes groups in the material bound to the Ag NPs (Figure 4.10). Studies by **[16,23,65,77]** have also identified numerous organic extracts in the samples and proposed that these groups could serve as organic reducing or capping agents. A medium sharp peak at 1638 cm^{-1} corresponds to secondary amine due to carbonyl stretch and –N-H stretch vibrations in the amide linkages of the proteins. The spectral bands (1450−1650) cm-1 show presence of proteins which are responsible for the reduction of metal ions or affinity for metal nanoparticles **[65].** The bands (1250-1450) cm-1 suggest the presence of flavanones or terpenoids adsorbed on the surface [**65]**, which confirmed that in AgNPs at 1285 cm^{-1}. The functional groups mainly OH and –C=C– are derived from heterocyclic compounds or alkanols e.g. alkaloid, flavones and tannins present in mango leaf extract and are the capping ligands of the nanoparticles **[27]**. The nanoparticles bond showed strong peak at 647 cm^{-1} suggests the presence of Vander Waals forces of interaction between oxygen groups in alkanols structures in mango leaf extract on the surface of AgNPs **[28].**

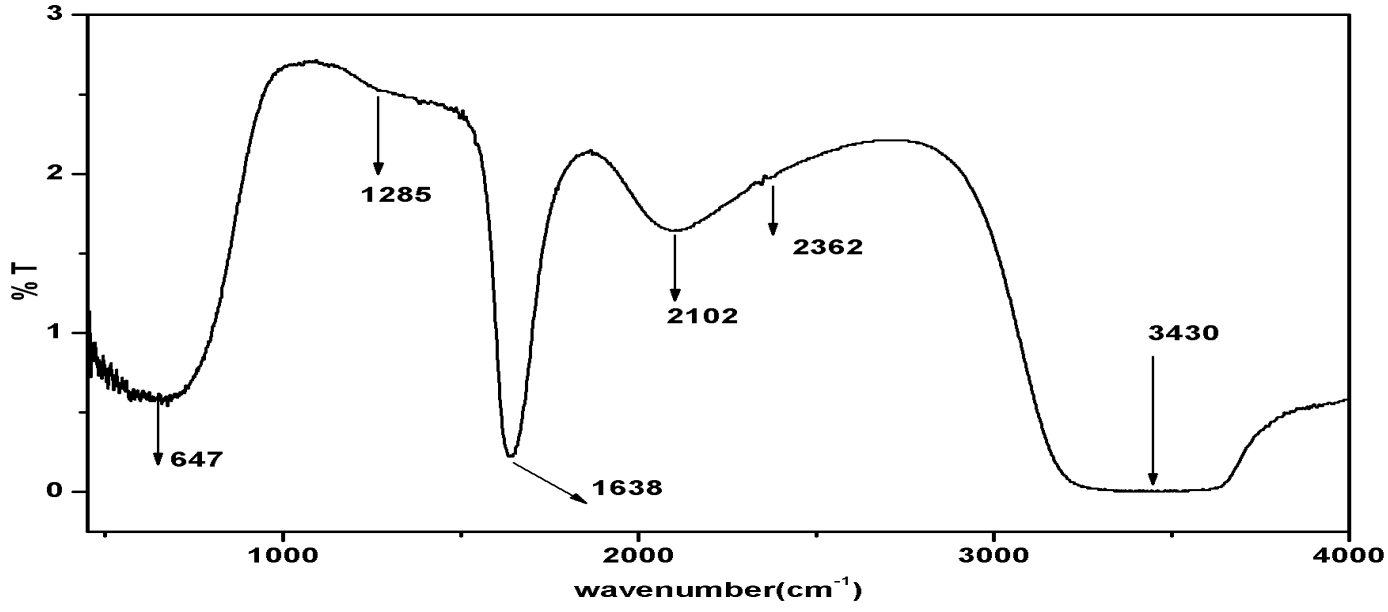


Figure.4.10 FT-IR spectra analysis of silver nanoparticles.

From the analysis of the FT-IR spectrum, conclude that carboxyl group were found adsorbed on the particles surface, hence that confirms the presence of biomolecules like terpenoids, flavonoids which acts as a capping agent for the synthesized nanoparticles. These also throw some light on the dual role of biological molecule in reducing metal ions and capping. Capping of nanoparticles by protein stabilizes silver nanoparticles and prevents agglomeration in the medium. FT-IR analysis confirmed that mango leaves extract can perform dual functions of reduction of (Ag+) to (Ago) and also stabilization of silver nanoparticles. FTIR spectrum of AgNPs suggested that AgNPs were surrounded by different organic molecules such as terpenoids, alcohols, ketones, aldehydes and carboxylic acids.

4.5 Antibacterial Activity Study of Silver Nanoparticles (AgNPs)

Comparison zone of inhibition using disc diffusion method Plant synthesized silver nanoparticles against standard drug resistance gram positive and gram negative bacteria. This activity depends on the strain sensitivity and on the contact surface, where in the silver can inhibit the respiratory chain enzyme systems of some bacteria and alter their DNA synthesis. Antimicrobial activity of silver nanoparticles was evaluated based on the diameters of clear inhibition zone surrounding the paper disks. The diameter of the zone of inhibition around the antibiotic disc with silver and without silver nanoparticles against the test strains is shown in (Figure 4.11). If there is no inhibition zone, it is assumed that there is no antimicrobial activity. Both (Figure4.11) and (Table 4.2) were shows that representative disk diffusion plates with different bacteria after 24hrs incubation. The silver nanoparticles synthesized showed inhibition zone against all the studied bacteria. The diameter of inhibition zone of *Salmonella typhi* is larger than that of *Escherichia coli* from the two negative and staphylococcus-aureus is greater than *Streptococci pyrogens* from the two positive bacteria. Plant synthesized silver nanoparticles showed excellent antibacterial activity against; Maximum zone of inhibition was revealed at 22 mm in *Salmonella typhi* and minimum of 7 mm in *Streptococci pyrogens,* (Table 4.2).

Table4. 2 The antibacterial activity of Ag Nps synthesized using Mango leaf extract

Name of the bacteria	**Mango leaf extract**	**zone of inhibition in (mm)**		
		Ag NPs	**Reference drugs (antibiotics)**	
			Gentamicin(CN)	**Chloroamphenicol(C)**
Streptococci pyrogens (S.pyrogens) Gramme positive	0	7	17	13
Staphylococcus aureus (S. aureus) Gramme positive	1	13	25	23
Salmonella typhi (S. typhi) Gramme negative	3	22	30	27
Escherichia coli (E. Coli) Gramme negative	2	15	22	18

The results revealed that the bacterial sensitivity to nanoparticles was found to vary depending on the microbial species. It was also possible to observe from the Figure 4.11 that the AgNPs was more sensitive to the bacterial as compared to the Mango leaf extract and the precursor, $AgNO_3$. This is expected to be due the reason that the size of the silver particles is smaller than the other two substances' particles which catalyzes the antibacterial activity. Gram positive bacteria have a large peptidoglycan structure **[72].** Peptidoglycan (cell wall) provides bacterial shape and rigidity. The cell wall consists of alternating units of N-acetyl glucosamine and N-acetylmuramic acid. The polysaccharide chains are cross-linked by a peptide bridge. It is a primary target of antimicrobial therapy because it is specific to prokaryotes, these accounts for the differential staining with Gram stain. Some Gram positive bacteria are also capable of forming spores under stressful environmental conditions such as when there is limited availability of carbon and nitrogen Spores therefore, allow bacteria to survive exposure to extreme conditions and can lead to re-infection **[76]**. Gram negative bacteria have a small peptidoglycan layer but have an additional membrane, the outer cytoplasm membrane **[67]**. This creates an additional permeability barrier and results in the need for transport mechanisms across this membrane. When we compared positive and negative bacteria from the (Table4.2), the negative bacteria have more bacterial effect than positive bacteria. This indicating *Salmonella typhi* and *Escherichia coli* are more susceptible to silver nanoparticles solution than *staphylococcus-aureus* and *streptococci-phyrogens*. This is because gram-positive bacteria were much more difficult to kill, due to the quite different

cell wall structure as compared to gram-negative bacteria. This work result is agreed with others previous reported **[69,73].** Based on these results, it can be concluded that these synthesized AgNPs had significant antibacterial action on both of the gram classes of bacteria. Because of the large surface area of the nanoparticles, it could be tightly adsorbed on the surface of the bacterial cells so as to disrupt the membrane, which would lead to the leakage of intracellular components, thus killing the bacterial cells. It was reported that the size of the inhibition zone increased significantly with decreasing size of the nanoparticles. It is reasonable to state that binding of the nanoparticles to the bacteria depend on the surface available for interaction. Smaller particles having the larger surface area available for interaction will give more bactericidal effect than the larger particles **[76].**

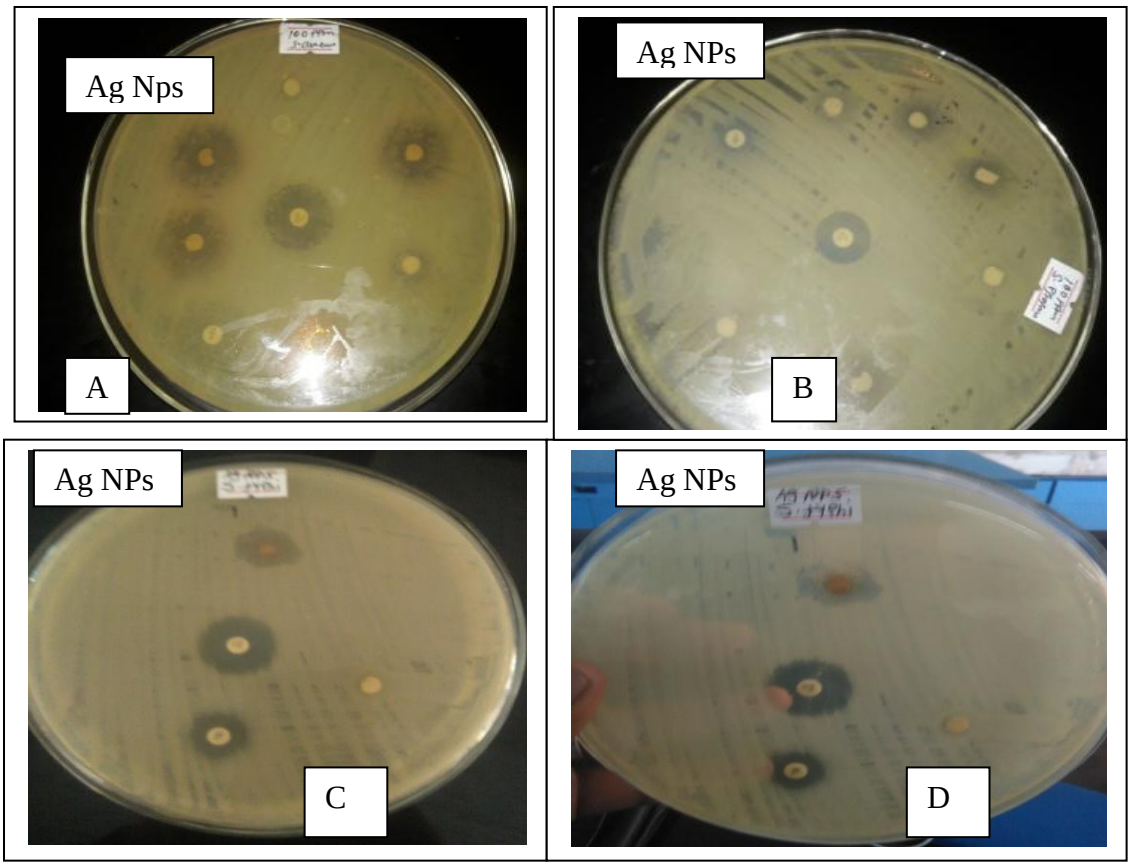


Figure.4.11 Zone of inhibition AgNPs against the two, gramme negative,(A) S.thyphi, (B), E.coil and the two gramme positive,(C), S.auerous, (D), S.phyrogen bacteria.

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusions

The green chemistry approach used in the present work for the synthesis of AgNPs using mango leaves extract as reducing, stabilizing and capping agent is simple, cost effective and environmentally friendly way unlike chemical and physical methods. The formation of AgNPs is affected by volume of mango leaf extract, concentration of silver nitrate solution and reaction time. The synthesized NPs are highly stable and reproducible. The synthesized AgNPs were characterized using Visual observations (colour change), UV-Vis absorption and FT-IR spectrometer which confirmed the formation of the AgNPs using mango leaves aqueous extract. The SPR property of synthesized NPs was studied by UV-Vis spectroscopy and the peak intensity was found at 418 nm. The synthesized AgNPs has been found to have a stable maximum absorbance at a wave length of 418 nm (pH of 11), which was very much different from the 366 nm of the mango leaves extract. The FT-IR also showed that there were (3430, 2333, 2102, 1638 and 647) cm^{-1} newly formed peaks that is different from the spectrum of the pure mango leaves of (3484, 1627 and 703) cm^{-1}. This indicates that the formation of the silver nanoparticle.

Antibacterial potential of AgNPs as a function of nanoparticles volume ratio of silver nitrate solution to mango leaf extract was tested against four different bacteria like *Escherichia coli*, *Salmonella typhi, Staphylococcus aureus* and *Streptococci pyrogens*. The test was performed by disc diffusion method. From the study, AgNPs were observed to have good antimicrobial potential. These nanoparticles showed significant antibacterial activity against *E. coli*, *S.thyphi*, *S.pyrogens* and *S.auerous* and inhibition zone range from 22 to 7 mm.

5.2 Recommendations

In this work the AgNPs strong peak has been obtained using fresh mango leaf. Therefore fresh mango leaf is recommended to be used. The effect of extracted mango leaf on the size of AgNPs formation in green method has been studied. To do so; AgNPs was synthesized by differing the concentration of extracted mango leaf, the concentration of silver nitrate solution ,the effect on PH and the formation of reaction time. Moreover, the effect of AgNPs on bacteria was studied by disc diffusion method. Based on the results of this study it is recommended that:

- Further studies should be done on the effect of temperature on the synthesis of Ag nanoparticles.
- The synthesized AgNPs should be characterized by other techniques like XRD and SEM to interpret its crystal structure, the size of nanoparticles and morphology, respectively.

REFERENCES

[1]. Yu, W.W., Qu, L., Guo, W. and Peng, X., 2003. Experimental determination of the extinction coefficient of CdTe, CdSe, and CdS nanocrystals. *Chemistry of Materials*, *15*(14), pp.2854−2860.

[2]. Sun, Y. and Xia, Y., 2002. Shape-controlled synthesis of gold and silver nanoparticles. *Science*, *298*(5601), pp.2176−2179.

[3]. Gref, R., Couvreur, P., Barratt, G. and Mysiakine, E., 2003. Surface-engineered nanoparticles for multiple ligand coupling. *Biomaterials*, *24*(24), pp.4529−4537.

[4]. Park, S.W., Jang, J.T., Cheon, J., Lee, H.H., Lee, D.R. and Lee, Y., 2008. Shape-dependent compressibility of TiO2 anatase nanoparticles. *The Journal of Physical Chemistry C*, *112*(26), pp.9627−9631.

[5]. Kowalczyk, B., Lagzi, I. and Grzybowski, B.A., 2011. Nanoseparations: strategies for size and/or shape-selective purification of nanoparticles. *Current Opinion in Colloid & Interface Science*, *16*(2), pp.135−148.

[6]. Kalishwaralal, K., Deepak, V., Pandian, S.R.K., Kottaisamy, M., BarathManiKanth, S., Kartikeyan, B. and Gurunathan, S., 2010. Biosynthesis of silver and gold nanoparticles using Brevibacterium casei. *Colloids and Surfaces B: Biointerfaces*, *77*(2), pp.257−262.

[7]. Nakanishi, H. and Grzybowski, B.A., 2010. Supercapacitors based on metal electrodes prepared from nanoparticle mixtures at room temperature. *The Journal of Physical Chemistry Letters*, *1*(9), pp.1428−1431.

[8]. Roy, I., Ohulchanskyy, T.Y., Pudavar, H.E., Bergey, E.J., Oseroff, A.R., Morgan, J., Dougherty, T.J. and Prasad, P.N., 2003. Ceramic-based nanoparticles entrapping water-insoluble photosensitizing anticancer drugs: A novel drug− carrier system for photodynamic therapy. *Journal of the American Chemical Society*, *125*(26), pp.7860−7865.

[9]. Kalsin, A.M., Kowalczyk, B., Smoukov, S.K., Klajn, R. and Grzybowski, B.A., 2006. Ionic-like behavior of oppositely charged nanoparticles. *Journal of the American Chemical Society*, *128*(47), pp.15046−15047.

[10]. Vestal, C.R. and Zhang, Z.J., 2002. Synthesis of CoCrFeO4 nanoparticles using microemulsion methods and size-dependent studies of their magnetic properties. *Chemistry of materials*, *14*(9), pp.3817−3822.

[11]. Narayanan, R. and El-Sayed, M.A., 2004. Shape-dependent catalytic activity of platinum nanoparticles in colloidal solution. *Nano letters*, *4*(7), pp.1343−1348.

[12]. Kreibig, U. and Vollmer, M., 2013. *Optical properties of metal clusters* (Vol. 25). Springer Science & Business Media.

[13]. Linic, S., Christopher, P. and Ingram, D.B., 2011. Plasmonic-metal nanostructures for efficient conversion of solar to chemical energy. *Nature materials*, *10*(12), p.911.

[14]. Swami, A., Selvakannan, P.R., Pasricha, R. and Sastry, M., 2004. One-step synthesis of ordered two-dimensional assemblies of silver nanoparticles by the spontaneous reduction of silver ions by pentadecylphenol Langmuir monolayers. *The Journal of Physical Chemistry B*, *108*(50), pp.19269−19275.

[15]. Kumar, S.A., Abyaneh, M.K., Gosavi, S.W., Kulkarni, S.K., Pasricha, R., Ahmad, A. and Khan, M.I., 2007. Nitrate reductase-mediated synthesis of silver nanoparticles from AgNO3. *Biotechnology letters*, *29*(3), pp.439−445.

[16]. Roy, S. and Das, T.K., 2015. Plant Mediated Green Synthesis of Silver Nanoparticles-A. *Int J Plant Biol Res*, *3*(3), p.1044.

[17]. Pandey, S., Oza, G., Mewada, A. and Sharon, M., 2012. Green synthesis of highly stable gold nanoparticles using Momordica charantia as nano fabricator. *Arch. Appl. Sci. Res*, *4*(2), pp.1135−1141.

[18]. Sigamoney, M., Shaik, S., Govender, P. and Krishna, S.B.N., 2016. African leafy vegetables as bio-factories for silver nanoparticles: a case study on Amaranthus dubius C Mart. Ex Thell. *South African Journal of Botany*, *103*, pp.230−240.

[19]. Ko, S.H., Park, I., Pan, H., Grigoropoulos, C.P., Pisano, A.P., Luscombe, C.K. and Fréchet, J.M., 2007. Direct nanoimprinting of metal nanoparticles for nanoscale electronics fabrication. *Nano letters*, *7*(7), pp.1869−1877.

[20]. Williams, D.H. and Bardsley, B., 1999. The vancomycin group of antibiotics and the fight against resistant bacteria. *Angewandte Chemie International Edition*, *38*(9), pp.1172−1193.

[21]. Suriya, J., Raja, S.B., Sekar, V. and Rajasekaran, R., 2012. Biosynthesis of silver nanoparticles and its antibacterial activity using seaweed Urospora sp. *African Journal of Biotechnology*, *11*(58), pp.12192−12198.

[22]. Tien, D.C., Tseng, K.H., Liao, C.Y. and Tsung, T.T., 2009. Identification and quantification of ionic silver from colloidal silver prepared by electric spark discharge system and its antimicrobial potency study. *Journal of alloys and compounds*, *473*(1), pp.298−302.

[23]. Philip, D., 2011. Mangifera indica leaf-assisted biosynthesis of well-dispersed silver nanoparticles. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *78*(1), pp.327−331.

[24]. Arya, V., 2010. Living Systems: eco-friendly nanofactories. *Digest Journal of Nanomaterials & Biostructures (DJNB)*, *5*(1).

[25]. Arivalagan, K., Ravichandran, S., Rangasamy, K. and Karthikeyan, E., 2011. Nanomaterials and its potential applications. *Int. J. ChemTech Res*, *3*(2), pp.534−538.

[26]. Sathya, K., Saravanathamizhan, R. and Baskar, G., 2017. Ultrasound assisted phytosynthesis of iron oxide nanoparticle. *Ultrasonics Sonochemistry*, *39*, pp.446−451.

[27]. Lateef, A., Akande, M.A., Ojo, S.A., Folarin, B.I., Gueguim-Kana, E.B. and Beukes, L.S., 2016. Paper wasp nest-mediated biosynthesis of silver nanoparticles for antimicrobial, catalytic, anticoagulant, and thrombolytic applications. *3 Biotech*, *6*(2), pp.1−10.

[28]. Banerjee, P., Satapathy, M., Mukhopahayay, A. and Das, P., 2014. Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresources and Bioprocessing*, *1*(1), p.3.

[29]. Luo, X., Morrin, A., Killard, A.J. and Smyth, M.R., 2006. Application of nanoparticles in electrochemical sensors and biosensors. *Electroanalysis*, *18*(4), pp.319−326.

[30]. Iravani, S., 2011. Green synthesis of metal nanoparticles using plants. *Green Chemistry*, *13*(10), pp.2638−2650.

[31]. Auffan, M., Rose, J., Bottero, J.Y., Lowry, G.V., Jolivet, J.P. and Wiesner, M.R., 2009. Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. *Nature nanotechnology*, *4*(10), pp.634−641.

[32]. Das, M., Shim, K.H., An, S.S.A. and Yi, D.K., 2011. Review on gold nanoparticles and their applications. *Toxicology and Environmental Health Sciences*, *3*(4), pp.193−205.

[33]. Lee, D.G., Park, S.J., Park, Y.O. and Ryu, J.I., 2007. Development of Membrane Filter with Nanostructured Porous Layer by Coating Metal Nanoparticles onto a Micor-Filter. *Korean Chemical Engineering Research*, *45*(6), pp.591−595.

[34]. Pelley, A.J. and Tufenkji, N., 2008. Effect of particle size and natural organic matter on the migration of nano-and microscale latex particles in saturated porous media. *Journal of Colloid and Interface Science*, *321*(1), pp.74−83.

[35]. Auffan, M., Rose, J., Proux, O., Borschneck, D., Masion, A., Chaurand, P., Hazemann, J.L., Chaneac, C., Jolivet, J.P., Wiesner, M.R. and Van Geen, A., 2008. Enhanced adsorption of arsenic onto maghemites nanoparticles: As (III) as a probe of the surface structure and heterogeneity. *Langmuir*, *24*(7), pp.3215−3222.

[36]. Schmid, G. ed., 2011. *Nanoparticles: from theory to application*. John Wiley & Sons.

[37]. Mie, G., 1908. Articles on the optical characteristics of turbid tubes, especially colloidal metal solutions. *Ann. Phys*, *25*(3), pp.377−445.

[38]. Lei, G., 2007. Synthesis of nano-silver colloids and their anti-microbial effects.

[39]. Chan, W.C. and Nie, S., 1998. Quantum dot bioconjugates for ultrasensitive nonisotopic detection. *Science*, *281*(5385), pp.2016−2018.

[40]. shaker, k.s., 2016. synthesis and characterizations of silver nano particles using chemical reaction method. *synthesis*, *9*(01), pp.113−120.

[41]. .Van Oss, C.J., Chaudhury, M.K. and Good, R.J., 1988. Interfacial Lifshitz-van der Waals and polar interactions in macroscopic systems. *Chemical Reviews*, *88*(6), pp.927−941.

[42]. Poulose, S., Panda, T., Nair, P.P. and Theodore, T., 2014. Biosynthesis of silver nanoparticles. *Journal of nanoscience and nanotechnology*, *14*(2), pp.2038−2049.

[43]. Lok, C.N., Ho, C.M., Chen, R., He, Q.Y., Yu, W.Y., Sun, H., Tam, P.K.H., Chiu, J.F. and Che, C.M., 2006. Proteomic analysis of the mode of antibacterial action of silver nanoparticles. *Journal of proteome research*, *5*(4), pp.916−924.

[44]. Barber, D.J. and Freestone, I.C., 1990. An investigation of the origin of the colour of the Lycurgus Cup by analytical transmission electron microscopy. *Archaeometry*, *32*(1), pp.33−45.

[45]. Khalil, K.A., Fouad, H., Elsarnagawy, T. and Almajhdi, F.N., 2013. Preparation and characterization of electrospun PLGA/silver composite nanofibers for biomedical applications. *Int J Electrochem Sci*, *8*, pp.3483−3493.

[46]. Singh, M., Singh, S., Prasad, S. and Gambhir, I.S., 2008. Nanotechnology in medicine and antibacterial effect of silver nanoparticles. *Digest Journal of Nanomaterials and Biostructures*, *3*(3), pp.115−122.

[47]. Elumalai, E.K., Prasad, T.N.V.K.V., Venkata, K., Nagajyothi, P.C. and David, E., 2010. Green synthesis of silver nanoparticle using Euphorbia hirta L and their antifungal activities. *Archives of Applied Science Research*, *2*(6), pp.76−81.

[48]. Kalaycı, Ö.A., Cömert, F.B., Hazer, B., Atalay, T., Cavicchi, K.A. and Cakmak, M., 2010. Synthesis, characterization, and antibacterial activity of metal nanoparticles embedded into amphiphilic comb-type graft copolymers. *Polymer Bulletin*, *65*(3), pp.215−226.

[49]. Kumar, R., Howdle, S. and Münstedt, H., 2005. Polyamide/silver antimicrobials: effect of filler types on the silver ion release. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, *75*(2), pp.311−319.

[50]. Sondi, I. and Salopek-Sondi, B., 2004. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. *Journal of colloid and interface science*, *275*(1), pp.177−182.

[51]. Bhattacharya, D. and Gupta, R.K., 2005. Nanotechnology and potential of microorganisms. .....*Critical reviews in biotechnology*, *25*(4), pp.199−204.

[52]. Tian, J., Wong, K.K., Ho, C.M., Lok, C.N., Yu, W.Y., Che, C.M., Chiu, J.F. and Tam, P.K., 2007. Topical delivery of silver nanoparticles promotes wound healing. *ChemMedChem*, *2*(1), pp.129−136.

[53]. Pollini, M., Paladini, F., Catalano, M., Taurino, A., Licciulli, A., Maffezzoli, A. and Sannino, A., 2011. Antibacterial coatings on haemodialysis catheters by photochemical deposition of silver nanoparticles. *Journal of Materials Science: Materials in Medicine*, *22*(9), pp.2005−2012.

[54]. Magnusson, M.H., Deppert, K., Malm, J.O., Bovin, J.O. and Samuelson, L., 1999. Gold nanoparticles: production, reshaping, and thermal charging. *Journal of Nanoparticle Research*, *1*(2), pp.243−251.

[55]. Jain, P. and Pradeep, T., 2005. Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter. *Biotechnology and bioengineering*, *90*(1), pp.59−63.

[56]. Nikawa, H., Yamamoto, T., Hamada, T., Rahardjo, M.B., Murata, H. and Nakanoda, S., 1997. Antifungal effect of zeolite-incorporated tissue conditioner against Candida albicans growth and/or acid production. *Journal of oral Rehabilitation*, *24*(5), pp.350−357.

[57]. Mafune, F., Kohno, J.Y., Takeda, Y., Kondow, T. and Sawabe, H., 2000. Structure and stability of silver nanoparticles in aqueous solution produced by laser ablation. *The Journal of Physical Chemistry B*, *104*(35), pp.8333−8337.

[58]. Sylvestre, J.P., Kabashin, A.V., Sacher, E., Meunier, M. and Luong, J.H., 2004. Stabilization and size control of gold nanoparticles during laser ablation in aqueous cyclodextrins. *Journal of the American Chemical Society*, *126*(23), pp.7176−7177.

[59]. Thirumurugan, A., Tomy, N.A., Ganesh, R.J. and Gobikrishnan, S., 2010. Biological reduction of silver nanoparticles using plant leaf extracts and its effect on increased antimicrobial activity against clinically isolated organism. *Der Pharma Chemica*, *2*(6), pp.279−284.

[60]. Merga, G., Wilson, R., Lynn, G., Milosavljevic, B.H. and Meisel, D., 2007. Redox catalysis on "naked" silver nanoparticles. *The Journal of Physical Chemistry C*, *111*(33), pp.12220−12226.

[61]. Evanoff, D.D. and Chumanov, G., 2004. Size-controlled synthesis of nanoparticles. 2. Measurement of extinction, scattering, and absorption cross sections. *The Journal of Physical Chemistry B*, *108*(37), pp.13957−13962.

[62]. Sharma, V.K., Yngard, R.A. and Lin, Y., 2009. Silver nanoparticles: green synthesis and their antimicrobial activities. *Advances in colloid and interface science*, *145*(1), pp.83−96.

[63]. Aboelfetoh, E.F., El-Shenody, R.A. and Ghobara, M.M., 2017. Eco-friendly synthesis of silver nanoparticles using green algae (Caulerpa serrulata): reaction optimization, catalytic and antibacterial activities. *Environmental Monitoring and Assessment*, *189*(7), p.349.

[64]. Shah, M., Fawcett, D., Sharma, S., Tripathy, S.K. and Poinern, G.E.J., 2015. Green synthesis of metallic nanoparticles via biological entities. *Materials*, *8*(11), pp.7278−7308.

[65]. Tamuly, C., Hazarika, M., Borah, S.C., Das, M.R. and Boruah, M.P., 2013. In situ biosynthesis of Ag, Au and bimetallic nanoparticles using Piper pedicellatum C. DC: green chemistry approach. *Colloids and Surfaces B: Biointerfaces*, *102*, pp.627−634.

[66]. Marshall, A.T., Haverkamp, R.G., Davies, C.E., Parsons, J.G., Gardea-Torresdey, J.L. and van Agterveld, D., 2007. Accumulation of gold nanoparticles in Brassic juncea. *International journal of phytoremediation*, *9*(3), pp.197−206.

[67]. Muralikrishna, T., Malothu, R., Pattanayak, M. and Nayak, P.L., 2014. Green Synthesis of Gold Nanoparticles Using Mangifera Indica (Mango Leaves) Aqueous Extract. *World J. Nano Sci. Technol., 3(2), pp. 66−73*

[68]. Nadeem, M., Imran, M., Iqbal, Z., Abbas, N. and Mahmud, A., 2017. Enhancement of the oxidative stability of butter oil by blending with mango (mangifera indica L.) Kernel oil in ambient and accelerated oxidation. *Journal of Food Processing and Preservation*, *41*(3).

[69]. Pino, J.A., Mesa, J, Munoz Y, Marti MP, Marbot R. Volatile components from Mango (Mangifera indica L.) cultivars.J Agric Food Chem. 2005; 53, pp.2213−2223.

[70]. Wetungu, M.W., Tarus, P.K., Segor, F.K., Cheseto, X. and Omolo, M.V.O., 2015. Essential oil chemistry of some Mangifera Indica varieties from Kenya. *American Journal of Essential Oils and Natural Products*, *3*(2), pp.01−06.

[71]. Raudone, L., Raudonis, R., Liaudanskas, M., Viskelis, J., Pukalskas, A. and Janulis, V., 2016. Phenolic profiles and contribution of individual compounds to antioxidant activity of apple powders. *Journal of food science*, *81*(5).

[72]. Krenek, K.A., Barnes, R.C. and Talcott, S.T., 2014. Phytochemical composition and effects of commercial enzymes on the hydrolysis of gallic acid glycosides in mango (Mangifera indica L. cv.'Keitt') pulp. *Journal of agricultural and food chemistry*, *62*(39), pp.9515−9521.

[73]. Pierson, J.T., Monteith, G.R., Roberts-Thomson, S.J., Dietzgen, R.G., Gidley, M.J. and Shaw, P.N., 2014. Phytochemical extraction, characterisation and comparative distribution across four mango (Mangifera indica L.) fruit varieties. *Food chemistry*, *149*, pp.253−263.

[74]. Abdullah, A.S.H., Mohammed, A.S., Abdullah, R. and Mirghani, M.E.S., 2015. Identification and Quantification of Phenolic Compounds in Mangifera Indica Waterlily Kernel and Their Free Radical Scavenging Activity. *Journal of Advanced Agricultural Technologies Vol*, *2*(1).

[75]. Srikar, S.K., Giri, D.D., Pal, D.B., Mishra, P.K. and Upadhyay, S.N., 2016. Green synthesis of silver nanoparticles: a review. *Green and Sustainable Chemistry*, *6*(01), p.34.

[76]. Banala, R.R., Nagati, V.B. and Karnati, P.R., 2015. Green synthesis andcharacterization of *Carica papaya* leaf extract coated silver nanoparticles through X-ray diffraction, electron microscopy and evaluation of bactericidal properties. *Saudi Journal of Biological Sciences*, *22*, *pp.*637−644.

[77]. Geethalakshmi, R. and Sarada, D.V.L., 2010. Synthesis of plant-mediated silver nanoparticles using Trianthema decandra extract and evaluation of their anti microbial

activities. *International Journal of Engineering Science and Technology*, *2*(5), pp.970−975.

[78]. Umair, M., Javed, I., Rehman, M., Madni, A., Javeed, A., Ghafoor, A. and Ashraf, M., 2016. Nanotoxicity of inert materials: the case of gold, silver and iron. *Journal of Pharmacy & Pharmaceutical Sciences*, *19*(2), pp.161−180.

[79]. Srikar, S.K., Giri, D.D., Pal, D.B., Mishra, P.K. and Upadhyay, S.N., 2016. Green synthesis of silver nanoparticles: a review. *Green and Sustainable Chemistry*, *6*(01), p.34.

[80]. Singh, P., Kim, Y.J., Zhang, D. and Yang, D.C., 2016. Biological synthesis of nanoparticles from plants and microorganisms. *Trends in biotechnology*, *34*(7), pp.588−599.

[81]. Tagad, C.K., Dugasani, S.R., Aiyer, R., Park, S., Kulkarni, A. and Sabharwal, S., 2013. Green synthesis of silver nanoparticles and their application for the development of optical fiber based hydrogen peroxide sensor. *Sensors and Actuators B: Chemical*, *183*, pp.144−149.

[82]. Shanmuga, P.P., Vasantha, V.S., Jeyasundari, J. and Brightson, Y.A., 2015. Synthesis of Ag nanoparticles using Ficus microcarpa leaf extract and their antibacterial activity. *European Chemistry Bulletin*, *4(*3), *pp.*116−120.

[83]. Kundu, S., Maheshwari, V., Niu, S. and Saraf, R.F., 2008. Polyelectrolyte mediated scalable synthesis of highly stable silver nanocubes in less than a minute using microwave irradiation. *Nanotechnology*, *19*(6), p.065604.