A DISSERTATION ON

Fabrication and surface functionalization of Silver Nanoparticles by using *Limonia Acidissima* aqueous fruit pulp extract

SUBMITTED TO THE

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BY

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UNDER THE SUPERVISION OF

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TO WHOM IT MAY CONCERN

This is to certify that **Ms. Nuzhat Fatima**, a student of M.Sc. Biotechnology (IV semester), Integral University has completed her four months dissertation work entitled ***"Fabrication and surface functionalization of Silver Nanoparticles by using Limonia Acidissima aqueous fruit pulp extract"*** successfully. She has completed this work from 2 Feb to 2 June 2022 at the Department of Biosciences, Integral University, under the guidance of **Dr. Salman Khan**.

The dissertation was a compulsory part of her M.Sc. degree. I wish her good luck and a bright future.

(**Dr. Snober S. Mir**)
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IU/DBS/S00192/2022/CIN/221111 **June 2022**

CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Ms. Nuzhat Fatima**, during the months 2 Feb to 2 June 2022 reported in the present thesis was under my guidance and supervision. The results reported by her are genuine and the script of the thesis has been written by the candidate herself. The thesis entitled ***"Fabrication and surface functionalization of Silver Nanoparticles by using Limonia Acidissima aqueous fruit pulp extract"*** is, therefore, being forwarded for acceptance in partial fulfillment of the requirements for the degree award of the student of M.Sc. Biotechnology (IV semester), Department of Biosciences, Integral University, Lucknow, (U.P).

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Nuzhat Fatima **Date**

Contents

Abbreviations

Ag-NPs	Silver Nanoparticles
Se-NPs	Selenium nanoparticles
Mt-NPs	Metal Nanoparticles
NA	Nutrient agar
NB	Nutrient broth
M	Molar
mM	milli Molar
µL	micro litre
DLS	Dynamic light scattering
OD	Optical Density
TEM	Transmission electron microscopy
SEM	Scanning electron microscopy
UV-Vis	Ultraviolet visible spectroscopy
FTIR	Fourier transform infrared spectroscopy
NMR	Nuclear magnetic resonance
ZP	Zeta potential

Introduction

Tiny particles or materials having sizes ranging from 1-100 nm are considered nanoparticles. The prefix nano means a billionth. These possess unique physical and chemical properties due to their large surface area and nanoscale size. Nanoparticles are characterized on basis of their size, properties, and shapes. These are composed of three layers

- 1-the surface layer-which may be functionalized with a variety of small molecules, metals, surfactants, and polymers.
- 2-the shell layer-it is a chemically different material from the core in all aspects.
- 3-the core-it is essentially the central portion of nanoparticles and usually refers to the nanoparticles themselves.

Nanoscience or nanotechnology deals with profuse structures of matter having an average order of a billionth of a meter.

Nanotechnology inquiries into electrical, optical, and magnetic activity as well as structural behavior at the molecular and sub-molecular levels.

Nonidentical metallic nanomaterials are brought out by using copper, zinc, titanium, magnesium, gold, alginate, and silver. Nanoparticles are being used for diverse purposes, from medical treatments, used in various branches of industrial production such as solar and oxide fuel batteries for energy storage, to wide incorporation into diverse materials of everyday use such as cosmetics or clothes. When the dimensions of a material are reduced from a large size, the properties remain the same at first, but then small changes occur. Finally, when the size drops below 100nm, dramatic changes in properties can occur. In modern research, nanotechnology has become an arena with budding applications in electronics and medicines. Nanotechnology is referring to the engineering and exploitation of materials whose size varies from 1 to 100 nM. Due to their relatively large surface area, nanoparticles often exist with distinctive size-dependent properties. Moreover, a particle at the nanoscale has a length smaller than the de Broglie wavelength of the charge carrier or the wavelength of the light. So, at that length, the periodic boundary conditions of crystalline particles vanish.

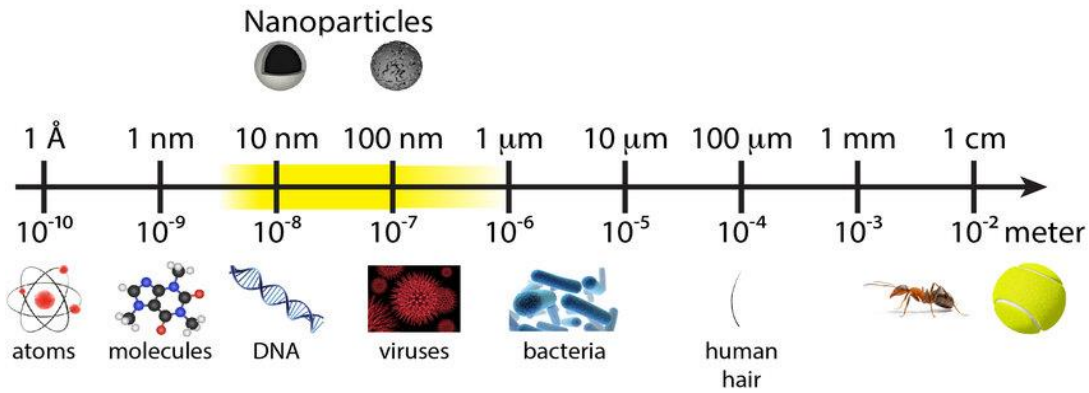

Fig.1. Range of Nanoparticles

History of nanotechnology

Nanotechnology is a characteristically modern scientific field that is constantly developing as commercial and academic interest lengthens to increase and as new research is presented to the scientific community. “In 1979, Eric Drexler encountered Feynman's talk on atomic manipulation and "nano-factories." The Caltech physicist's ideas inspired Drexler to put these concepts into motion by expanding Feynman's vision of the term nanotechnology has been used since the mid-1980s to label a vision first described by Richard Feynman in his classic talk, “There’s Plenty of Room at the Bottom” (R. Feynman, 1961). The Feynman vision projects the development of nanomachines able to build nanomachines and other products with atom-by-atom control (a process termed molecular manufacturing). This vision generalizes the nanomachinery of living systems by promising technology of unprecedented power with commensurate dangers and opportunities. "Molecular assemblers will bring a revolution without parallel since the development of ribosomes, the primitive assemblers in the cell. The resulting nanotechnology can help life spread beyond Earth - a step without parallel since life spread beyond the seas; it can let our minds renew and remake our bodies - a step without any parallel at all (Eric Drexler engines of creation, 1986) Drexler founded the Foresight Institute, the whose stated goal is to "ensure the beneficial implementation of nanotechnology” Drexler used this "institute" as a way to present his vision of molecular manufacturing that he vividly illustrated in Engines of Creation. Thus, this "institute" was used to further propagate research, through his influential yet highly controversial depiction of nanotechnology and its future. As a result, due to the publicity generated by both Drexler's work and the institute, scientists from all over the world began to have a vested interest in the field of nanotechnology.

Nanotechnology and nanosciences

“Nanoscience” is the emerging science of objects that are intermediate in size between the largest molecules and the smallest structures that can be fabricated by current photolithography; that is, the science of objects with smallest dimensions ranging from a few nanometers to less than 100 nanometers. In chemistry, this range of sizes has historically been associated with colloids, micelles, polymer molecules, phase-separated regions in block copolymers, and similar structures—typically, very large molecules, or aggregates of many molecules. More recently, structures such as buckytubes, silicon nanorods, and compound semiconductor quantum dots have emerged as particularly interesting classes of nanostructures. In physics and electrical engineering, nanoscience is most often associated with quantum behavior and the behavior of electrons and photons in nanoscale structures. Biology and biochemistry also have a deep interest in nanostructures as components of the cell; many of the most interesting structures in biology from DNA and viruses to subcellular organelles and gap junctions can be considered as nanostructures (Whitesides, 2005).

Nanoparticles

Nanoparticles are microscopic objects with at least one dimension less than 100 nm (Garg A, et al.) due to their relatively large surface area, nanoparticles often exist with distinctive size-dependent properties. Moreover, a particle at the nanoscale has a length smaller than de Broglie wavelength of the charge carrier or wavelength of light. So, at that length, the periodic boundary condition of the crystalline particle vanishes. As result, the physical properties of nanoparticles become quite different from the bulk materials, which results in yielding new materials. Several types of nanoparticle platforms are differentiated based on their size, shape, composition, and their functionalities. These platforms include liposomes, Albumin-bound, Polymeric, Quantum Dots, and Iron Oxide. The biological method for the synthesis of nanoparticles is easy, cost-effective, and environmentally friendly. Biological systems have the capability of converting metal ions into metal nanoparticles in a very friendly manner. Nanoparticles can be used in Fluorescent biological labels, drug and gene 12 delivery, tissue engineering, etc. Furthermore, nanoparticles have vast applications in daily life (Anwar, 2018).

Nanomaterials

Nanomaterials are materials that have structural components smaller than 1 micrometer in at least one dimension. While the atomic and molecular building blocks 0.2 nm of matter are considered nanomaterials, examples such as bulk crystals with a lattice spacing of nanometers but macroscopic dimensions overall are commonly excluded.

Phyto-nanotechnology

Phyto nanotechnology can be described as the integration between nanotechnology and biotechnology to develop a green and eco-friendly technology for the preparation, characterization, and application of nanomaterials.

Green synthesis

The conventional methods to produce NPs are expensive, toxic, and non-environment friendly. To overcome these problems, researchers have found the precise green routes, i.e., the naturally occurring sources and their products that can be used for the synthesis of NPs. Green synthesis can be categorized as (a) utilization of microorganisms like fungi, yeasts (eukaryotes), bacteria, and actinomycetes (prokaryotes), (b) use of plants and plant extracts (c) use of templates like membranes, viruses DNA, and diatoms. The green synthesis via bacteria, fungi, plants, and plant extracts. Conventionally synthesizing nanoparticles by the chemical method in which chemicals are used because of their general toxicity; engendering the serious concern to develop environment-friendly processes. Thus, to solve the aim; biological approaches are coming up to fill the void; for instant green synthesis using biological as the molecules of extracts derived from plant sources exhibiting superiority over chemical and/or biological methods The biosynthesized nanomaterials have been effectively controlling the various endemic diseases with less adverse effect. The trend of using natural products has increased and the active plant extracts are frequently screened for new drug discoveries. Thus, green synthesis using 13 biological molecules derived from plant sources in the form of extracts exhibits superiority over chemical methods.

Characterization

The increasing use of engineered nanoparticles in research and product development in application areas related to medicine, sensing, environmental science, and consumer products engenders a growing need to understand their properties and behaviors as they are synthesized, applied, and evolve (or age) in a particular environment, process, or application. There is also an increasing awareness of the need to understand the health, safety, and environmental impacts of nanoparticles in both their synthesized form and as they evolve through the application or environmental interaction. Nanoparticles are characterized through several methods such as Uv-vis spectroscopy, TEM, SEM, Zeta potential, DLS, X-Ray diffraction, FTIR, etc.(Baer et al., 2010).

Applications of nanotechnology

During the last couple of decades, the rapidly advancing field of nanotechnology has produced a wide palette of nanomaterials, most of which are considered “synthetic” and, among the wider public, are often met with a certain suspicion. Despite the technological sophistication behind many of these materials, “nano” does not always equate with “artificial”. Indeed, nature itself is an excellent 10 nanotechnologist. It provides us with a range of fine particles, from inorganic ash, soot, sulfur, and mineral particles found in the air or wells, to sulfur and selenium nanoparticles produced by many bacteria and yeasts. These nanomaterials are entirely natural, and, not surprisingly, there is a growing interest in the development of natural nanoproducts, for instance in the emerging fields of phyto- and physio-nanotechnology (Griffin et al., 2018). Today, nanotechnology and its diverse products are omnipresent and form an integral part of our products and lifestyle, from nano silver in deodorants and nanoscopic particles with improved release properties in medicines to “nano impregnations” of shower cabins, bathtubs, and washing basins. Innovative materials containing particles with diameters in the one to one hundred nanometer range have emerged in many areas of our daily life, there has also been a feeling that such materials are “not quite natural”. Not surprisingly, therefore, the field of nanotoxicology more recently has attracted particular interest and there has also been mounting concern regarding a possible toxic impact on humans and contamination of the environment with nanomaterial.

Review of literature

Nanotechnology

Nanotechnology has achieved the status as one of the critical research endeavors of the early 21st century, as scientists harness the unique properties of atomic and molecular assemblages built at the nanometer scale. Our ability to manipulate the physical, chemical, and biological properties of these particles affords researchers the capability to rationally design and use nanoparticles for drug delivery, as image contrast agents, and for diagnostic purposes. (McNeil, 2005). Nanotechnology's rapid development worldwide is a testimony to the transformative power of identifying a concept or trend and laying out a vision at the synergistic confluence of diverse scientific research areas.

Nanoparticles

Nanoparticles can be defined as objects ranging in size from 1-100 nm that due to their size may differ from the bulk material. Presently, different metallic nanomaterials are being produced using copper, zinc, titanium, magnesium, gold, alginate, and silver. Nanoparticles are being used for diverse purposes, from medical treatments, used in various branches of industrial production such as solar and oxide fuel batteries for energy storage, to wide incorporation into diverse materials of everyday use such as cosmetics or clothes. Manufactured nanoparticles may find practical applications in a variety of areas, including medicine, engineering, catalysis, and environmental remediation. Most properties of nanoparticles are size-dependent. The novel properties of nanoparticles do not prevail until the size has been reduced to the nanometer scale. The particle size and size distribution of alumina nanoparticles, as a critical property, have been determined by transmission electron microscopy (TEM), photon correlation spectroscopy (PCS), surface area analysis (BET), and x-ray diffraction peak broadening analysis. The particle size plays a crucial role in nanoparticle properties and therefore an essential task in the property characterization of nanoparticles is particle sizing. The particle size and size distribution of nanoparticles can be determined using numerous commercially available instruments. Instruments can be used for the analysis of dry powders and 16 powders dispersed in suspension. In general, there are two basic methods of defining particle size. The first method is to inspect the particles and make actual measurements of their dimensions. Microscopic techniques, for example, measure many-dimensional parameters from particle images. The second method utilizes the relationship between particle behavior and its size. This often implies an assumption of equivalent spherical size developed using a size-dependent property of the particle and relating it to a linear dimension (Akbari B, et al,)

Properties of nanoparticles

The most important physicochemical aspects of nanoparticles are:

- Composition and surface composition
- Crystalline phase
- Particle size distribution
- Agglomeration/aggregation
- Shape
- Specific surface area
- Roughness/Porosity
- Water solubility/dispersibility or hydrophobicity/hydrophilicity
- Zeta potential or surface charge
- Surface chemistry
- Catalytic/Photocatalytic activity
- Magnetic properties
- Optical properties

Composition and Surface Composition

Nanoparticles can be made of a single material or composite materials. Many nanoparticles will oxidize in the presence of air and form a thin film at their surface. Nowadays nanoparticles comprising different composite materials are easily fabricated using a physical vapor deposition process at grazing incidence over a nano-seeded pattern, involving the manipulation of substrate motion and temperature. This technique of deposition at a glancing angle incidence can be used to manipulate the morphology of single materials as well. (Robbie et al., 2004).

Crystalline Structure

Crystalline structure is of paramount importance in deciding a nanoparticle's mechanical, chemical, and physical properties. The same material in a different crystalline form can have very different properties and application

Shape or Morphology

Nanoparticles can now be fabricated in almost any shape and contain various combinations of composite materials. (Mark et al.) demonstrate nanoparticles with complex three-dimensional morphologies comprising a variety of different functional materials. Their fabrication method combines a physical vapor deposition process at grazing incidence over a nano-seeded pattern, involving the manipulation of substrate motion and temperature. Nanoparticles with different morphologies have been demonstrated, such as:

- Nanorods (Robbie et al., 2004)
- Nano zigzags (Robbie et al., 2004)
- Nano hooks (Chen et al., 2013)
- Nano stars(Das Jana et al., 2020)
- Nano cubes(Kumar et al., 2012)
- Nano helices (Das Jana et al., 2020)
- Nanoplates (Li et al., 2007)

Surface Area

Particle size and surface area are important parameters that play a major role in the interaction of nanomaterials with the exterior. Decreasing the size of the nanoparticle leads to an exponential increase in surface area relative to volume, resulting in an enhanced reactivity.

Water Solubility-Dispersibility or Hydrophobicity-Hydrophilicity

Nanoparticles can be insoluble or repelling water, also called hydrophobically. Water-soluble nanoparticles are called hydrophilic. Many of the engineered nanoparticles are hydrophobic and do not form stable suspensions in aqueous media. They are nanoparticles made of metals and their compounds (such as titanium dioxide or quantum dots), nonmetals (carbon-based nanoparticles including fullerenes C60, carbon nanotubes, carbon soot, and silicon-based nanoparticles). To improve their dispersibility via electrostatic repulsion, nanoparticles can be coated with different substances. For example, gold nanoparticles can be functionalized with citrate groups, while multi-walled carbon nanotubes are usually functionalized via a selective breaking of C = C bonds achieved through oxidation. (Kole et al., 2013).

Catalytic/Photocatalytic Activity

Nanoparticles that have surface facets sufficiently large will have catalytic properties that approximate those of their bulk counterparts with crystalline surfaces. With decreasing size, usually smaller than 3 nm, a nanoparticle catalytic activity starts to deviate from its bulk behavior due to the increasing contribution of the corner and edge atoms of the nanoparticle. Spherical nanoparticles with a size of about 3 nm have half of their atoms on the surface. In the case of nanoparticles with sizes between *1 and 2 nm, even slight changes in their structure, the deletion or addition of a few atoms, can have an important impact on its catalytic properties. Nanoparticles with such small sizes are usually hard to characterize experimentally because their structure may be altered during catalytic reactions. Furthermore, for a given crystalline structure, the different crystalline surfaces may show a different 19 reactivity toward the same reaction. It has been shown that different crystal surfaces of Pt have very different electronic structures and atomic arrangements, consequently having a different reactivity toward the same reaction (Chen et al., 2013).

Magnetic Properties of Nanoparticles

There are two types of materials that exhibit magnetism in nanoform. There are magnetic nanomaterials that are magnetic in bulk form and nanoparticles that show magnetic behavior only in nanoform, while in bulk are nonmagnetic. Magnetic properties of nanoparticles that are magnetic in bulk form Magnetic materials are defined as materials that change their magnetic behavior as a result of an external magnetic field. There are five main types of magnetic materials: ferromagnetic, paramagnetic, diamagnetic, antiferromagnetic, and ferromagnetic. In ferromagnetic materials (such as Fe, Ni, Co) the magnetism arises due to atomic net magnetic moments as a result of unpaired electrons (Huber, et al., 2005). The material has domains containing large numbers of atoms with parallel magnetic moments, each domain having a net magnetic moment pointing in a specific direction. Due to the random orientation of the magnetic moments of the domains, the net magnetic moment of the material is zero. In an external magnetic field, the domains of the ferromagnetic material align along the direction of the applied magnetic field, resulting in a large net magnetic moment. After the removal of the external field, there is a residual magnetic moment left. In paramagnetic materials (Gd, Mg, Li, Ta) magnetic domains are absent even though atoms have net magnetic moment due to unpaired electrons. After the application of an external magnetic field, the atoms align along the direction of the field resulting in a weak net magnetic moment. After the removal of the field, the paramagnetic materials do not have a remanent magnetization. Diamagnetic materials (bulk Cu, Ag, Au, and most of the rest of the elements) have atoms with no unpaired electrons and show a zero net magnetic moment. Their magnetic response in an external magnetic field is very weak and after the removal of the field, they do not retain any magnetic moment. Antiferromagnetic materials (Mn, NiO) have two different atom types occupying different lattice positions. The two types of atoms have opposite magnetic moments, resulting in zero net magnetic moments. Ferrimagnetic 20 materials (magnetite Fe_3O_4) have also different atom types occupying different lattice sites with antiparallel magnetic moments, but different magnitudes. Hence the magnetic moments do not cancel out, which results in a net spontaneous magnetic moment. In an external magnetic field, both antiferromagnetic and ferrimagnetic materials behave similarly to ferromagnetic materials.

Optical Properties

The optical properties of nanoparticles vary with their composition, morphology, and size (Khlebtsov & Dykman, 2010)(Chen et al., 2013). The optical properties of nanoparticles pertain to the way that they interact with light. If one exposes matter to light, several processes can occur (Huang, et al., 2007).

- ❖ Absorption of light
- ❖ Scattering of light at the same frequency as the incoming light (Mie or Rayleigh scattering)
- ❖ Reemission of the absorbed light (fluorescence)

Enhancing the local electromagnetic field of the incoming light, thus enhancing spectroscopic signals from molecules at the material surface. Au nanoparticles exhibit an enhancement of all the above-mentioned processes due to a unique interaction of the nano particle-free electrons and light. When a gold nanoparticle is exposed to an external optical field, the electrical component of the light acts upon the conduction-band electrons and displaces them, creating uncompensated charges at the nanoparticle surface (Jain et al., 2007). Because the polarization occurs at the nanoparticle surface, these oscillations are called surface plasmons. Plasmons are defined as collective excitations of conductive electrons in metals (Khlebtsov & Dykman, 2010). There are several types of plasmons: bulk plasmons (or 3D plasma), surface propagating plasmons polaritons (in 2D films), and surface localized plasmons (in nanoparticles). The surface plasmons have a well-defined resonance frequency, the coherent oscillations of the nanoparticle electrons in resonance with the electromagnetic field are called surface plasmon resonance (SPR). In the case of Au nanospheres, the surface plasmon resonance is in the visible spectrum at around 520 nm, hence the red color of solutions of spherical Au nanoparticles.

Melting temperature of nanoparticles

Large cluster nanoparticles. The melting temperature decrease with decreasing particle size is a thermodynamic consequence of the change in the surface-to-volume ratio that is confirmed experimentally(Asoro et al., 2009). For example, the melting temperature of gold nanoparticles with a diameter of 3.8 nm (approximately 1,700 atoms) is about 1000 K, while for 2.5 nm nanoparticles (about 500 atoms) is around 500 K, and the bulk melting point is 1,337 K.

Types of nanoparticles

Nanoparticles can be classified into different types according to their size, morphology, and physical and chemical properties. Some of them are carbon-based nanoparticles, ceramic nanoparticles, metal nanoparticles, semiconductor nanoparticles, polymeric nanoparticles, and lipid-based nanoparticles.

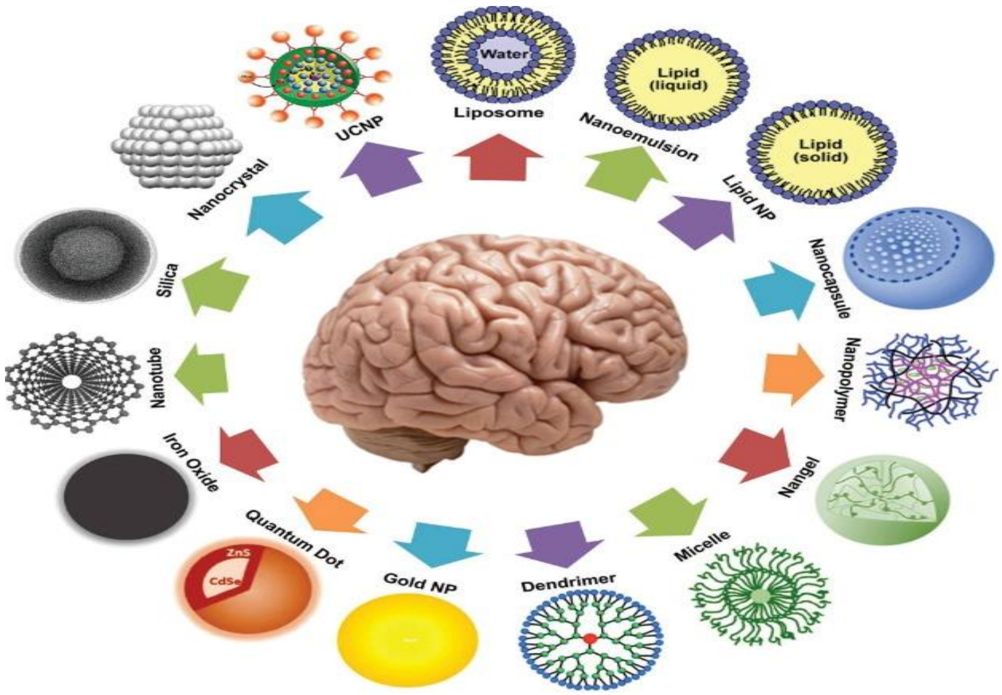

Fig. 2. Types of nanoparticles

Carbon-Based Nanoparticles

Carbon-based nanoparticles include two main materials: carbon nanotubes (CNTs) and fullerenes. CNTs are nothing but graphene sheets rolled into a tube. These materials are mainly used for structural reinforcement as they are 100 times stronger than steel. Carbon-based nanoparticles can be classified into single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). CNTs are unique in a way as they are thermally conductive along the length and nonconductive across the tube. Fullerenes are the allotropes of carbon having a structure of a hollow cage of sixty or more carbon atoms. The structure of C-60 is called Buckminsterfullerene and looks like a hollow football. The carbon units in these structures have a pentagonal and hexagonal arrangement. These have commercial applications due to their electrical conductivity, structure, high strength, and electron affinity.

Ceramic Nanoparticles

Ceramic nanoparticles are inorganic solids made up of oxides, carbides, carbonates, and phosphates. These nanoparticles have high heat resistance and chemical inertness. They have applications in photocatalysis, photodegradation of dyes, drug delivery, and imaging. 22 By controlling some of the characteristics of ceramic nanoparticles like size, surface area, porosity, the surface volume ratio, etc, they perform as a good drug delivery agent. These nanoparticles have been used effectively as a drug delivery system for several diseases like bacterial infections, glaucoma, cancer, etc.

Metal Nanoparticles

Metal nanoparticles are prepared from metal precursors. These nanoparticles can be synthesized by chemical, electrochemical, or photochemical methods. In chemical methods, the metal nanoparticles are obtained by reducing the metal ion precursors in a solution with chemical reducing agents. These can adsorb small molecules and have high surface energy. These nanoparticles have applications in research areas, detection and imaging of biomolecules, and environmental and bioanalytical applications.

Silver nanoparticles

Silver nanoparticles have unique optical, electrical, and thermal properties and are being incorporated into products that range from photovoltaics to biological and chemical sensors. Silver nanoparticles have attracted increasing interest due to their chemical stability, catalytic activity, localized surface plasma resonance, and high conductivity. In addition, previous reports showed that the reactive oxygen species(ROS) formed at the surface of the silver nanoparticles or by the released free silver ions under certain conditions may induce cell death of either mammalian cells or microbial cells, which endows the silver nanoparticles with unique antibacterial and antifungal effects (M. wang, et al.,) Silver nanoparticles have received increased attention as potential new agents, due to the believed broad-spectrum antimicrobial efficacy of silver, and its established use in burn and wound treatment. It is hypothesized that the antimicrobial activity of silver nanoparticles (Ag-NP) would be enhanced, compared to metallic silver, by the high specific surface area and a high fraction of surface atoms. Silver has always been used against various diseases; in the past, it found used as an antiseptic and antimicrobial against Gram-positive and Gram-negative bacteria due to its low cytotoxicity. The synergistic action of AgNPs and antibiotics resulted in enhanced antibacterial effects; therefore, the simultaneous action of antibiotics and AgNPs can hamper the resistance development by pathogenic 23 bacteria, also given the reduced amount of antibiotics administered. (Fayaz et al., 2010). Applications Silver nanoparticles are being used in numerous technologies and incorporated into a wide array of consumer products that take advantage of their desirable optical, conductive, and antibacterial properties.

- ❖ Diagnostic Applications: Silver nanoparticles are used in biosensors and numerous assays where the silver nanoparticle materials can be used as biological tags for quantitative detection.
- ❖ Antibacterial Applications: Silver nanoparticles are incorporated in apparel, footwear, paints, wound dressings, appliances, cosmetics, and plastics for their antibacterial properties.
- ❖ Conductive Applications: Silver nanoparticles are used in conductive inks and integrated into composites to enhance thermal and electrical conductivity.
- ❖ Optical Applications: Silver nanoparticles are used to efficiently harvest light and for enhanced optical spectroscopies including metal-enhanced fluorescence (MEF) and surface-enhanced Raman scattering (SERS).

Selenium nanoparticles

Selenium, a nutrient element that has a massive function in biological systems, is one of the interesting compounds to integrate with antibacterial agents. Selenium is an essential trace element in the diet, required for the maintenance of health and growth. Given that the least toxic form of Se is elemental Se7, its nano form has attracted significant attention. Selenium nanoparticles have found applications and are used extensively in food science and biomedicine because of their higher biocompatibility and low toxicity nature. Selenium is originally an important factor in the process of seleno-protein formation as the vital antioxidant-like agent. Selenium nanoparticles have strong free radicals suppressing impacts in vitro and in vivo and can protect nucleic acids and DNA against oxidative damage. In addition to this, selenium nanoparticles have been shown to have 24 anti-carcinogenic behavior against some cancers and reported cases of antimicrobial properties. Moreover, they are used as nutritional supplements, diagnostics, nanotherapeutics, and even as medical devices. Selenium nanoparticles have industrial applications as photocopiers xerography rectifiers and solar cells. Selenium nanoparticles can be synthesized following chemical, physical and biological methods. The chemical and physical methods involve the synthesis at higher temperatures and acidic ph. The biological methods, however, have no hazards and toxicity as the chemical methods. Due to natural coatings by agents, the particles synthesized according to the biological procedure are more stable and have no agglomeration. Applications

- Anticancer activity
- Protective role of SeNPs in drug-induced toxicity
- Role of SeNPs in inflammatory diseases
- Diabetes and associated complications

Gold nanoparticles

Gold nanoparticles have been utilized for centuries by artists due to the vibrant colors produced by their interaction with visible light. More recently, these unique optoelectronic properties have been researched and utilized in high technology applications such as organic photovoltaics, sensory probes, therapeutic agents, drug delivery in biological and medical applications, electronic conductors, and catalysis. The optical and electronic properties of gold nanoparticles are tunable by changing the size, shape, surface chemistry, or aggregation state. Application The range of applications for gold nanoparticles is growing rapidly and includes.

Electronics

Gold nanoparticles are designed for use as conductors from printable inks to electronic chips. As the world of electronics becomes smaller, nanoparticles are important components in chip design. Nanoscale gold nanoparticles are being used to connect resistors, conductors, and other elements of an electronic chip.

Photodynamic Therapy

Near-IR absorbing gold nanoparticles (including gold nanoshells and nanorods) produce heat when excited by light at wavelengths from 700 to 800 nm. This enables these nanoparticles to eradicate targeted tumors. When light is applied to a tumor containing gold nanoparticles, the particles rapidly heat up, killing tumor cells in a treatment also known as hyperthermia therapy.

Therapeutic Agent Delivery

Therapeutic agents can also be coated onto the surface of gold nanoparticles. The large surface area-to-volume ratio of gold nanoparticles enables their surface to be coated with hundreds of molecules (including therapeutics, targeting agents, and antifouling polymers).

Sensors

Gold nanoparticles are used in a variety of sensors. For example, a colorimetric sensor based on gold nanoparticles can identify if 26 foods are suitable for consumption. Other methods, such as surface-enhanced Raman spectroscopy, exploit gold nanoparticles as substrates to enable the measurement of vibrational energies of chemical bonds. This strategy could also be used for the detection of proteins, pollutants, and other molecules label-free.

Probes

Gold nanoparticles also scatter light and can produce an array of interesting colors under dark-field microscopy. The scattered colors of gold nanoparticles are currently used for biological imaging applications. Also, gold nanoparticles are relatively dense, making them useful as probes for transmission electron microscopy.

Diagnostics

Gold nanoparticles are also used to detect biomarkers in the diagnosis of heart diseases, cancers, and infectious agents. They are also common in lateral flow immunoassays, a common household example being the home pregnancy test.

Catalysis

Gold nanoparticles are used as catalysts in several chemical reactions. The surface of a gold nanoparticle can be used for selective oxidation or in certain cases the surface can reduce a reaction (nitrogen oxides). Gold nanoparticles are being developed for fuel cell applications. These technologies would be useful in the automotive and display industry.

Zinc nanoparticles

Zinc (Zn) Nanoparticles, nanodots, or nanopowder are spherical or faceted high surface area metal particles. Nanoscale Zinc Particles are typically 20-40 nanometers (nm) with a specific surface area (SSA) in the 30 - 50 m2/g range and are also available with an average particle size of 100 nm range with a specific surface area of approximately 7 m2/g. Nano Zinc Particles are also available in Ultra high purity and high purity and coated and dispersed forms. They are also available as a dispersion through the AE Nanofluid production group. Nanofluids are generally defined as suspended nanoparticles in solution either using surfactant or surface charge technology. Nanofluid dispersion and coating selection technical guidance is also available. Other nanostructures include nanorods, nanowhiskers, nano horns, nanopyramids, and other nanocomposites. 27 Development research is underway in Nano Electronics and Photonics materials, such as MEMS and NEMS, Bio Nano Materials, such as Biomarkers, Bio Diagnostics & Bio Sensors, and Related Nano Materials, for use in Polymers, Textiles, Fuel Cell Layers, Composites, and Solar Energy materials. Nanopowders are analyzed for chemical composition by ICP, particle size distribution (PSD) by laser diffraction, and Specific Surface Area (SSA) by BET multi-point correlation techniques. Novel nanotechnology applications also include quantum dots. High surface areas can also be achieved using solutions and using thin film by sputtering targets and evaporation technology using pellets, rods, and foil Applications for zinc nanocrystals include as an anti-microbial, antibiotic, and anti-fungal (fungicide) agent when incorporated in coatings, bandages, nanofiber, nanowire, plastics, alloy and textiles and further research for their potential electrical, dielectric, magnetic, optical, imaging, catalytic, biomedical and bioscience properties.

Applications

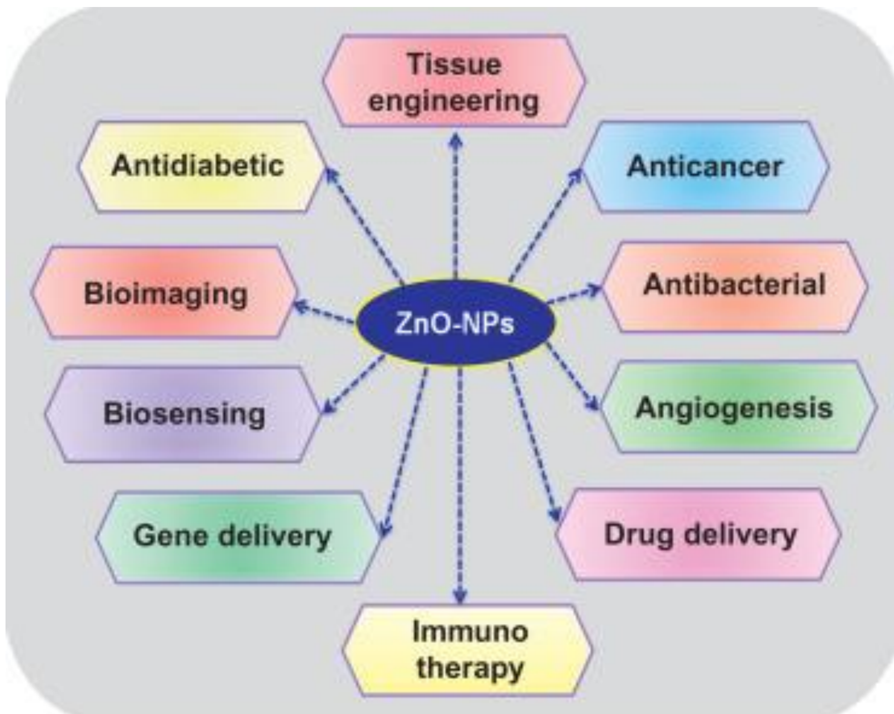

Fig. 3. Applications of zinc nanoparticles

Types of nanoparticles

Based on dimensions Nanoparticles are broadly classified into three dimensions based on dimensions.

One-dimension nanoparticles

one dimensional system (thin film or manufactured surfaces) has been used for decades. Thin films (sizes 1–100 nm) or monolayer is now commonplace in the field of solar cells offering, different technological applications, such as chemical and biological sensors, information storage systems, magneto-optic and optical devices, and fiber-optic systems.

Two-dimension nanoparticles

Carbon nanotubes

Three-dimension nanoparticles

Dendrimers, Quantum Dots, Fullerenes (Carbon 60), (QDs)

Synthesis of nanoparticles

There are two different ways by which metallic nanoparticles can be formed. These are the “bottom-up" approach and the "top-down" approach. The two can be distinguished in the sense that while the bottom-up method is sourced from scientific research including nanoscience, the top-down is not In terms of the size of objects, both methods are very similar. Both approaches tend to converge in terms of the size range of objects. The former approach, however, tends to be more abundant based on the type of material, design varieties, and nanometric control, while the latter approach only acquires materials of more importance, however, control may not be as strong.

When it comes to the synthesis of metallic Nanoparticles, two distinct approaches are utilized. The first is the top-down strategy and the second approach is referred to as the bottom-up strategy.

While the former deals with the reduction in the size of current technological devices, the latter performs an opposite role, which is the building of even more complex molecular devices on an atomic arrangement.

While the top-down approach is beneficial in the production of technological structures in a far-reached order and for connecting macroscopic devices, the bottom-up is suitable for the production and arrangement of short-range order at the nanoscale aspect.

The combination of both strategies is expected to form the best integration of equipment for nano-based fabrication. Furthermore, the top-down technique is built up for architectural structures at the micrometer scale (um). The bottom-up strategy is also built up for bringing small collections of atoms together measured in nanometers (nm). What is left is to integrate both approaches to create elongated forms at the nanoscale.

The commonest form of the top-down approach to fabrication is the lithographic technique that utilizes enhanced visual sources of a short wavelength. One main benefit of the top-down technique in the fabrication of joint circuits is the fact that all of its parts are created and structured in an orderly form so that no further assemblage is required.

The high level of polishing makes visual lithography developed especially in the production of the micro-electric chip with the wavelength reaching a level below 100 nanometers (going by the traditional method). On the other hand, the sources of shorter wavelengths like intense UV and X-ray, are created to permit the techniques for printing lithography to attain a level between 10-100 nanometers.

Beams like the electron lithographic beam make provision for a model reaching 20 nanometers. In this technique, the model is stated by flushing a finely patterned electron beam across the surface. Other mire concentrated ionized beams are utilized for the direct processing and modeling of wafers with a lesser effect compared to electron beam lithography. Printing methods of a mechanical nature, also known as the nanoscale imprinting, stamping, and molding— expand to cover small measurements of 20 to 40 nanometers.

Though the details differ, the main aim of this is to create a massive "stamp" by utilizing a high pixel method like the electron beam lithography thereafter adding the stamp or the following ones to the surface layer, thus, producing a model.

Each variant comprises the coating of the surface layer of the stamp with the "ink" and then emptying directly on the surface of the 36 stamp's model. Given an example, the model under the control of a molecule monolayer can be obtained successfully by depositing the ink directly on the coated surface. Using another technique, the stamp is utilized to mechanically press the model to the tiny layer of the element.

Typically, the surface layer is a polymeric element that has been patterned for molding by heating during the stamping process. Etching of plasma is then used for masking under the stamped layers; polymers are subsequently removed, while a nanoscale lithography model remains on the surface. Relief models are equally formed from photoresist on a wafer by visual or electro-beam lithography and then emptied on a watery precursor.

The effect of this is a solid rubber-like substance that can be easily detached and utilized as a stamp. They can be utilized in any of the ways produced above. A distinguishing feature of the latter technique is the flexibility of the stamp.

Top-down approach

The top-down approach usually involves the following processes:

Evaporation/condensation

This has to do with metal evaporation through heating and then condensing the obtained vapor to obtain nanopowders. This method is based on the pressure caused by vaporizing the metal, which is its capacity to vaporize depends on its chemical strength. The surface oxidation Fe, Ni, Co, Cu, Pd, and Pt equally create sufficient steam through radiation and inductive heating (1200 and 2000 degrees), respectively. A certain amount of the material is made in the laboratory. Oxygen (A1, C8, T1, Zr) and refractory metals (with low pressure in vapor, Mo, Hf, Ta, w) demand greater modes of heating. An example is electron bombardment heating of 3000 degrees and combined with electric arc (3000 and 14000 degrees). If the atmosphere in which the metallic elements are placed is reactive (oxygen), the effect of this would be oxidation of the nanoparticles. The challenge with this process is the loss of control of the nanometric size. The powders are derived by swift cooling of the escaped vapor, making sure the formation of a huge number of nanoparticles, then restricting their growth and other processes. The technique is particularly used in factory production of 37 ceramic and metal nanopowders and production is made in very large tons a year. The nanopowders are prone to fire and explosion in the air.

Laser Pyrolysis

The method is easy and effective in the synthesis of nanopowders. It is produced by a form of exchange between the release of carbon dioxide laser and the movement of reagents. The energy current between the two leads to a quick increase in temperature in the reactive zone by arousing the vibration degrees of molecules. There is a disconnection of reactors and a flame that manifests where the nanoparticles are produced and then go through quenching once the flames disappear. The powders are deposited where they will be obtained. The above technique is an easy way for particle synthesis of 15 – 20 nanometers at 100g/h. One of the benefits of this technique is the flow of reaction. Other benefits are a high level of purity and excellent chemical and physical properties. Many nanopowders have been formed through this process.

Ionic/electronic irradiation

Through the use of irradiations, nanoparticles can be formed originally. The technique is hinged on the following: The features of energy production by heavy ions facilitators including GANIL, which introduces local changes in the material in a circumference of 10nm around the ion). The method is suitable for the production of polymer membranes nanopowder. To achieve a ‘matrix’ production, the material can be developed in the membrane pores of the polymer; irradiation makes the attainment of mass possible.

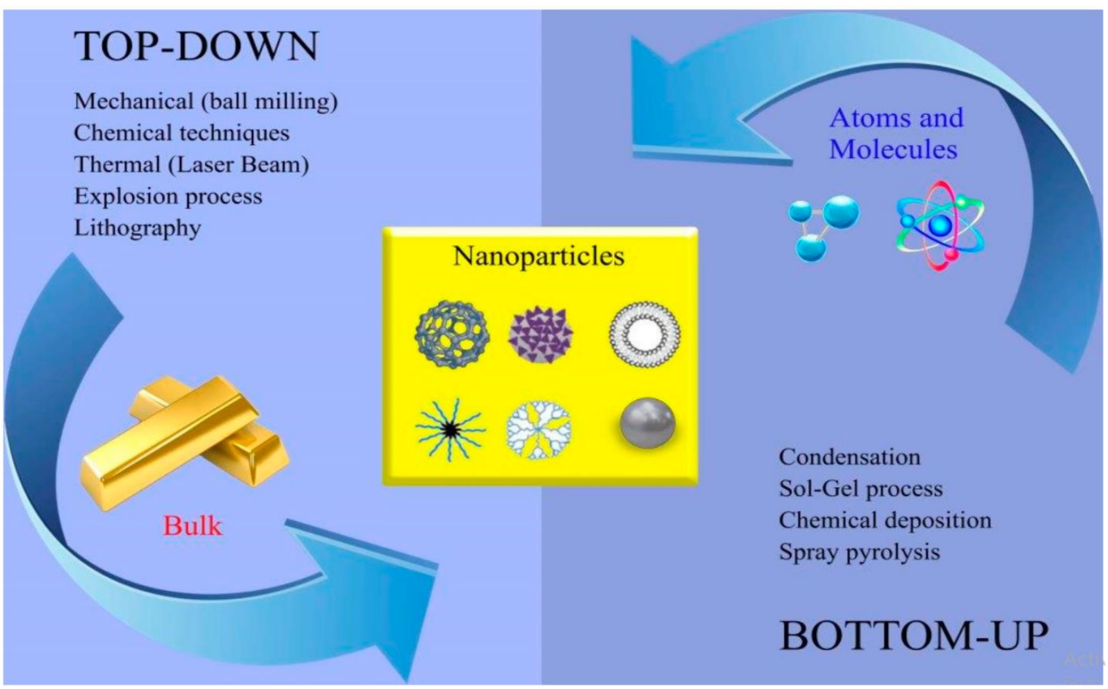

Fig. 4. Types of synthesis of nanoparticles

Bottom-up approach

The bottom-up approach utilizes physical and chemical processes that function at the nanoscale to integrate main components into bigger structures. With a reduction in unit size, the approach yields a relevant consummation to the top-down approach. Biological systems motivate this approach in which natural forces of life harness their chemical counterparts to produce the structures. Although, scientists are working to produce the same forces as nature to self-integrate into bigger structures.

Gold-palladium alloy nanoparticles which were based on carbon treated with acid and breaking down hydrogen peroxide are formed from the combination of white hydrogen and red oxygen when disallowing unwanted decay. This approach has been used in the production of nanoparticles from condensation to coalescence of atomic vapors and liquids, respectively.

Chemical techniques

a. sol-gel techniques

This allows the production of nanomaterials from alkoxides or colloidal-based solutions. They take the form of monoliths, crystallized nano pigments, and thin layers. They are centered on reactions in polymerization. The three types of this are sol-gel, metal alkoxide, and Cecchini. The sol-gel method focuses on the growth of chemical compounds in the solution. Following movements between the species and liquid, the sol is changed into some sort of three-sided channel increased in the solvent. The process is then frozen and the sol-gels are transformed to deformed solid material by the removal of liquid from the air-gel or by evaporation One benefit of the process is the ability to manage the size and oneness of the material spread out. They make the production of large parts of fibers possible. But, one main disadvantage of the technique is that the precursors are too pricy with very low gain; low weight products, and very dangerous to human health.

b. Supercritical fluid with a chemical reaction

The main goal of having a chemical conversion in a supercritical solvent is to consistently improve the physical and chemical processes of the precursors by improving the temperature on small mediums as well as the pressure. This control allows easy control of the chemical reaction. The method permits the production of divided materials of inorganic nature, such as oxide metal and nitrate.

Green synthesis/ biosynthesis

Green synthesis is an emerging area in the field of bio nanotechnology and provides economic and environmental benefits as an alternative to chemical and physical methods. In this method, nontoxic safe reagents which are eco-friendly and safe are used. Various natural resources available in nature such as plant extracts, and many more have been studied for the synthesis of metal oxide 43 nanoparticles. The use of plant extracts in the green synthesis of metal oxide nanoparticles has drawn considerable attention as a straightforward approach.

Synthesis of Nanoparticles from Plants

Syntheses of nanoparticles from plants are useful because it produces a large number of nanoparticles. The reducing and stabilizing agents are present in plants by nature. It has been reported that polymorphic gold nanoparticles can be synthesized from Citrus limon, Murraya koenigii Linn. leaves, Canna indica (red), and *Quisqualis indica* pink owers. These nanoparticles were stable and 30-130 nm in size (Ramanathan et al., 2013). Gold and silver nanoparticles were synthesized from Lonicera japonica plant leaf extract. Among them, silver nanoparticles were 36-72 nm in size and their shape was spherical to plate-like poly-shaped, while gold nanoparticles synthesized were poly-shaped nanoplates of 40-92 nm in size. It was found that carbohydrates, polyphenols, and proteins were responsible for the reduction of metal ions into nanoparticles (Vankar & Shukla, 2012). Magnolia Kobus leaf extract was used as a reducing agent to reduce copper ions to nanoparticles and their antibacterial activity was evaluated. Ag, Au, and Pt nanoparticles can be synthesized from gum kondagogu (Cochlospermum Gossypium). Among them, silver nanoparticles had significant antibacterial action on both the Gram classes of bacteria.(Kora & Arunachalam, 2011).

Limonia acidissima

Limonia acidissima, belonging to the family Rutaceae synonymical known as Feronia *Limonia Swingle*, *Feronia elephant*, *Schinus Limonia* and commonly also called wood apple and elephant apple is native and common in the wild in dry plains of India and Ceylon and cultivated along roads and edges of fields and occasionally in orchards. It is also grown throughout Asia tropical, Asia temperate, Southern America, and northern Malaysia. *Limonia acidissima* (L.) of the family Rutaceae (Citrus family) belongs to the monotypic genus Limonia, confined to India, Pakistan, Sri Lanka, and Southeast Asia. (Donald Moore Press Ltd. Singapore, 1967). It is also known as wood apple, elephant-apple, monkey fruit, curd fruit, Kath bel, and Kaitha. This plant is given as a medicine for the treatment of various disorders. (Khare, 2008).*L. acidissima* is a deciduous, slow-growing, erect tree with a few upward-reaching branches bending outwards near the summit where they are subdivided into slender branchlets drooping at the tips. Wood apple is useful in preventing and curing scurvy and in relieving flatulence. Mashed seedless pulp of the raw fruit is beneficial in the treatment of dysentery, diarrhea, and piles. *L. acidissima,* considered to be a hepato protectant, possesses different biological activities namely adaptogenic activity against blood impurities, leucorrhoea, dyspepsia, and jaundice. Traditionally, all parts of the plants are given as natural medicine as a cure for various ailments. (JF, 1987).

Deciduous trees, to 20 m high, bark dark-grey or black, deeply cracked longitudinally; thorns straight, to 2.5 cm, axillary. Leaves imparipinnate, alternate, 1-3 in a cluster, estipulate; rachis 60-80 mm long, stout, glabrous, often narrowly winged; leaflets 4-7, opposite, sessile, stipellate; lamina 1.3-3.8 × 1.3 cm, obovate, base cuneate or acute, apex obtuse, margin entire, glabrous, pellucid-punctate, coriaceous; lateral nerves pinnate, obscure, intercostal obscure. Flowers polygamous, dull red, 1.3 cm across, in axillary cymes; calyx small, flat, 5- toothed, pubescent without, deciduous; petals 5, free, spreading; stamens 10-12, inserted around the disc; filaments dilated below, villous on face and margins; anthers linear-oblong; disc thick, annular, pubescent; pistillode short; ovary superior, oblong, 5-6-celled, at length 1- celled, ovules many; stigma oblong, fusiform. Fruit a berry, 5-7.6 cm across, globose, whitish-brown, rind hard and woody; seeds many.

Fig. 5. *Limonia acidissima*

Classification

Family:	Rutaceae
Kingdom:	Plantae
Sub-kingdom:	Tracheobionta
Super division:	Spermatophyta
Division:	Magnoliophyta
Class:	Magnoliospida
Subclass:	Rosidae
Order:	Sapindales
Genus:	Limonia L.
Species:	*L. acidissima* (Bhandari, 1978)

Medicinal Properties

- The fruit contains fruit acids, vitamins, and minerals. (Chevallier, 1996).
- It is used mainly as a liver tonic to stimulate the digestive system. (Chevallier, 1996).
- The fruit is also astringent, especially when unripe, and a cardiac tonic. (Chakraborty, 1959).
- The pulp of the fruit, especially when unripe, is used in the treatment of diarrhea and dysentery. (Chevallier, 1996).
- The fruit is also seen as an effective treatment for hiccoughs, sore throat, and diseases of the gums.
- Both the fruit pulp and the powdered rind can be poulticed onto bites and stings of venomous insects.
- The fruit is part of a formula that is applied as a paste to the breasts to tone them. (Chevallier, 1996)
- The leaves contain tannins and essential oil. (Chevallier, 1996).
- They are astringent and are used internally, often combined with milk and sugar, in the treatment of indigestion, flatulence, diarrhea, dysentery (especially in children), and hemorrhoids. (Chevallier, 1996)
- An oil derived from the crushed leaves is applied on itchy skins.
- The powdered gum, mixed with honey, is given to overcome dysentery and diarrhea in children.
- The spines are crushed with those of other trees and an infusion is taken as a remedy for menorrhagia.
- The bark is chewed with that of Barringtonia and applied on venomous wounds.

Phytochemicals constituents

The preliminary phytochemical analysis of *Limonia acidissima* plant parts showed the presence of alkaloids, flavonoids, phenols, terpenoids, tannins, fats steroids, saponins, glycosides, and gum, mucilage, and fixed oils15-18. The unripe fruits contain stigmasterol. Fruit pulp contains a large quantity of citric acid and other fruit acids, mucilage, and minerals. Alkaloids, coumarins, fatty acids, and sterols have been detected in the pericarp. It also contains umbelliferone, dictamnine, xanthotoxol, scoparone, xanthotoxin, isopimpinellin, iso-imperatorin, and marmin. (Chakraborty, 1959).

Leaves contain stigmasterol, psoralen, bergapten, orientin, vitexin, saponarin, tannins, and an essential oil. (Patra et al., 1988). Marmesin, feronolide, and feronone have been isolated from the bark. (Patra et al., 1988) .Seeds contain fixed oil, carbohydrates, proteins, and amino acids. Roots contain Feronia lactone, geranium umbelliferone, bergapten, osthol, isopimpinellin, marmesin, and marmin.

Pharmacological activity

Antidiarrhoeal activity

Plants have various useful chemical constituents which are used in the treatment of diarrhea.

Antidiabetic activity

Plants have various useful chemical constituents such as Phenols, and flavonoids which are used in the treatment of diabetes. The anti-diabetic activity was performed by using Methanolic extract, Aqueous extract of stem bark, and fruit. (Priya et al., 2012)

Anticancer activity

The fruit extract of *L. acidissima Linn.* shows an anti-cancer effect.[13] Fruit extracts from fractions 1 to 4 and also the crude extract (ethanolic extract) were used to determine the ED50 value (50% inhibition of cancer cell growth) in two different breast cancer cell lines, SKBR3 and MDA-MB-435. The bio-assays of extracts from L. acidissima Linn. showed that a fraction (fraction 3) from an ethanolic extract had an anticancer effect on SKBR3 and MDA-MB-435 human breast cancer cells. After 48 h of exposure, this fraction at a concentration of 100µg/ml, significantly reduced cell proliferation in both cancer cells. In MDA-MB-435 cells, cell cycle analysis showed that the fruit extract fraction 3 induced the accumulation of cells inG2/M phase, whereas no significant change in the cell cycle was detected in SKBR3 cells. (Priya et al., 2012)

Antibacterial activity

It was found that ethanolic extract of *Limonia acidissima*. leaves possess an abroad spectrum of activity against Gram-positive and Gram-negative bacterial strains responsible for the most common bacterial diseases 36,18,15. The antibacterial activity was evaluated against Gram-negative and Gram-positive bacteria by the agar well diffusion method. Methanol extract showed good antibacterial activity with the high inhibition zones while chloroform extract exhibited mild to moderate activity and hexane extract was found to be less active (NAIDU et al., 2014).

Antifungal activity

The different extracts (petroleum ether, chloroform, methanol, and aqueous) of Feronia Limonia Linn fruit pulp exhibited antifungal activity against some pathogenic fungus. The essential oil from the leaves of the plant exhibited antifungal activity against eight tested fungi.

Hepatoprotective

The hepatoprotective activity of the ethanolic extract of fruit pulp of L. acidissima (MELA) was investigated against carbon tetra chloride (CCl4) induced hepatic injury in rats. MELA exhibited a significant dose-dependent protective effect against CCl4-induced liver damage which can be mainly attributed to the antioxidant property of the extract (Ilango & Chitra, 2010)

Antioxidative property

The crude methanol extract of the stem bark of *Limonia acidissima L*. and its different organic soluble partitions were screened for antioxidant activities. (Nanasombat et al., 2012). The antioxidant (free radical scavenging) activity of the partitions on the stable radical 1,1-diphenyl- 2- picrylhydrazyl (DPPH) was determined. The chloroform soluble fraction (CL) of the crude methanolic extract showed the highest free radical scavenging activity. At the same time, the pet ether soluble fraction (PE) also exhibited strong antioxidant potential. The methanolic extract of Limonia fruit was also screened for their free radical scavenging properties by Ferric reducing antioxidant power (FRAP) assay and DPPH radical scavenging assay. (Nanasombat et al., 2012).In vitro antioxidant activity of different extracts from leaves of *Limonia acidissima* has been well documented. (Attarde et al., 2011)

Objectives

Objectives

- Fabrication of Silver Nanoparticles by using *limonia acidissima* aqueous fruit pulp extract.
- Characterization of Silver Nanoparticles by various physical techniques.
- To check the antibacterial potential of the above-mentioned nanoparticles.

Materials

All chemicals used in this experiment were of the highest purity and obtained from Sigma (Bangalore, India) and Merck (Mumbai, India). *L. Acidissima* Fruit was collected from a vendor near the campus of Integral University Lucknow. Phosphate buffer salts (Na_2HPO_4) and (NaH_2PO_4) were purchased from HIMEDIA. Double distilled water has been used as an aqueous medium for all experiments. All buffers were filtered with 0.2µm filter paper immediately after they were prepared. Microbiological media and ingredients were purchased from Himedia, India. All solvents and chemicals were of analytical grade and used as obtained from Merck and Sigma Aldrich (St. Louis, MO, USA)

Methods

Preparation of plant extract

For the preparation of *Limonia acidissima* (fruit) extract, the fruit was cut into small pieces. They were thoroughly washed, 60 grams of pulp weighed, and crushed in pestle mortar with 85ml of PBS buffer added to it respectively. Then extract was transferred to the centrifuge tube and then the tubes were placed in the centrifuge at 6000 rpm at 4°C for 10 min. Then the pellet was removed from the extract and supernatant was taken in another centrifuge tube. The extract is stored in a refrigerator for future purposes.

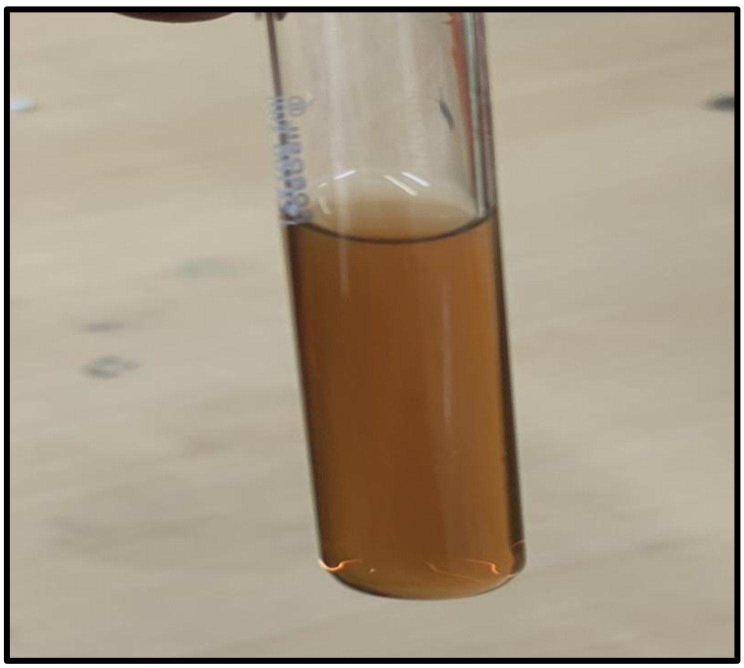

Fig. 6. Aqueous fruit pulp extract of *Limonia acidissima*.

Biosynthesis of silver nanoparticles

5ml of the prepared fruit extract was taken in a 15 ml of centrifuge tube and 5mM of silver nitrate (stock 1mM) was added to the fruit extract. Keep the reaction tube in an incubator at about 40°C for 48 hours. After 48 hours plant extract changes its color due to silver salt from pale yellow to reddish-brown indicating the synthesis of silver nanoparticles.

Results

The alkalinized extract of *Limonia acidissima* possesses a broad variability of metabolites that may help in the reduction of silver nitrate. The preliminary confirmation for the formation of AgNPs was the visual observation of the color change of the reaction mixture. The color, noted by visual observation, increased in intensity resulting in a brownish-yellow color from the original yellow after 24h of incubation (figure 7). The color change was noticed a few minutes after the addition of $AgNO_3$, but the intensity increased with time. Similar color changes have been observed in previous studies thereby confirming the completion of the reaction between the alkalinized extract and $AgNO_3$. Characterization of AgNPs using various techniques such as UV-Vis Spectroscopy, Dynamic Light Scattering, Zeta Potential, and Transmission Electron Microscopy.

Fig. 7. Synthesized silver nanoparticles show reddish-brown color.

Characterization technique

The formation of silver nanoparticles in the reaction media was analyzed by diluting small aliquots of the sample with sterile distilled water using a UV-visible spectrophotometer (Labomed Model UV- D3200) and Its optical property was evaluated by recording emission and excitation spectrum with an aid of Luminescence Spectrophotometer (Perkin Elmer – Model LS45). The color of the reaction medium prepared with the fruit pulp of *Limonia acidissima* was initially pale yellow and turns to brown due to the reduction.

Uv-visible analysis

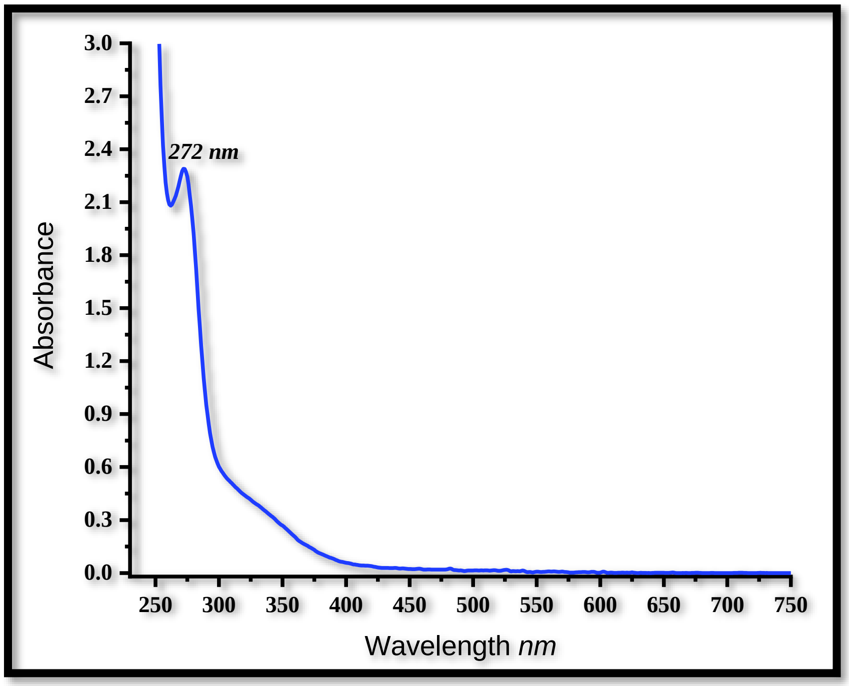

Fig. 8. UV-visible spectroscopy of aqueous fruit pulp extract of *limonia acidissima* showing absorption at 272 nm.

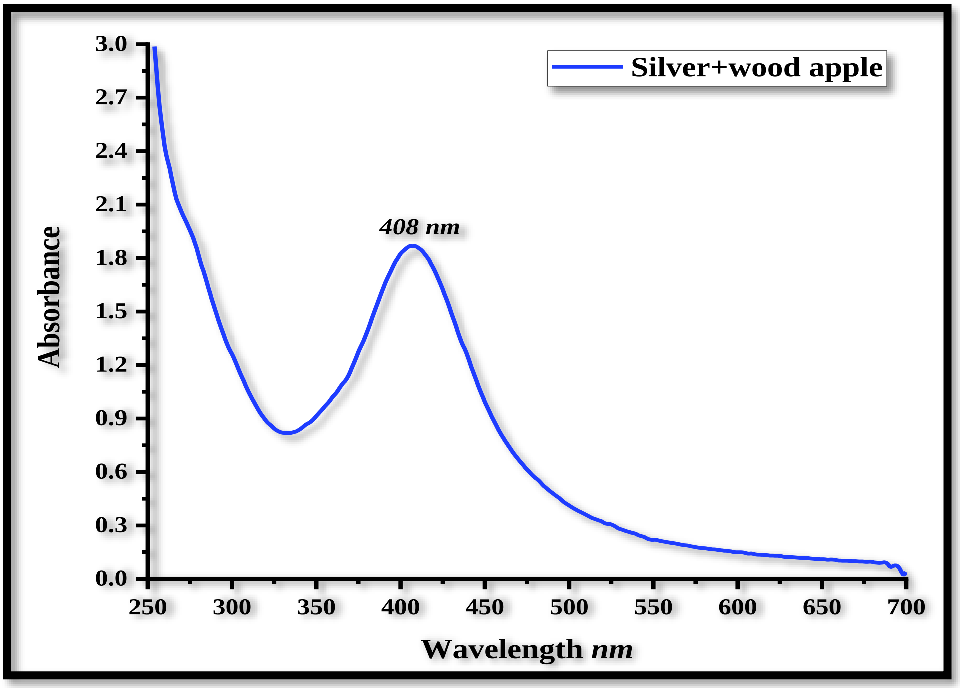

Fig. 9. UV-Visible spectroscopy of *limonia acidissima* synthesized Ag-NPs showing absorption at 408 nm.

DLS (Dynamic Light Scattering)

The thin electric dipole layer of the solvent adheres to the surface of a dispersed nanoparticle when it moves through a liquid medium; therefore, the hydrodynamic diameter estimated by DLS provides us information about the inorganic core along with coating material and the solvent layer attached to the particle. AgNPs had an average particle size of 65 d.nm and polydispersity index (PDI) of 0.214, showing a homogenous size distribution.

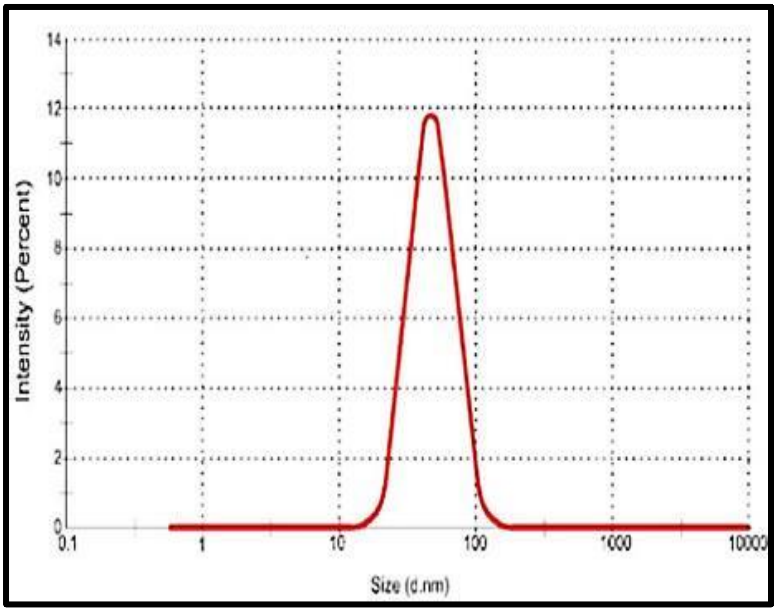

Fig. 10. Dynamic light scattering values of SNPs (65 d.nm)

Zeta Potential

The determination of Zeta Potential is considered an effective, simplest, and most straightforward method to predict the stability and understand the surface properties of the nanoparticles. Information regarding the concentration, distribution, exposure, or shielding of charged moieties, ionization, and adsorption could be drawn from the analysis of zeta potential. In most situations, nanoparticle colloidal stability necessitates a zeta value of -20mV. The synthesized AgNPs have zeta potentials of -17mV, indicating high particle strength.

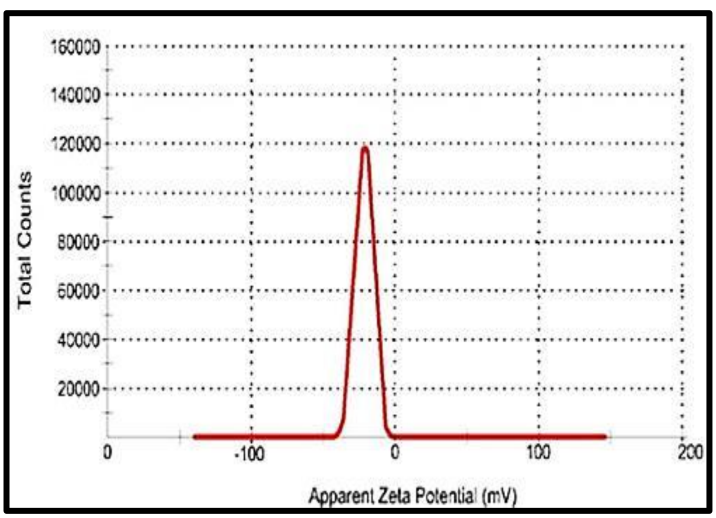

Fig. 11. The Zeta potential shows peak at -17mV.

Transmission electron microscopy

The high-resolution image was acquired using the transmission electron microscope (TEM), which confirmed the average size of AgNPs as 26 ± 2 nm using a Gatan digital micrograph and showed the spherical form of AgNPs. The TEM micrographs did not expose the agglomeration of the synthase. The transmission electron microscope(TEM) was utilized to identify the precise size, shape, and 2-D morphology of AgNPs, which were found to be 17d nm in diameter, spherical in form, and monodispersed in parameter.

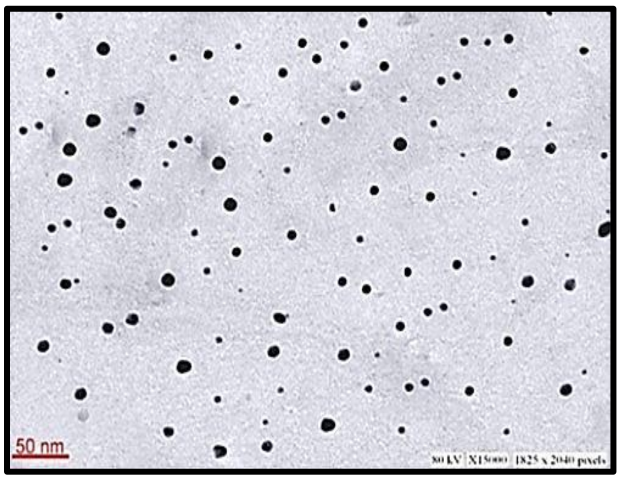

Fig. 12. Transmission Electron Microscope (TEM) micrograph illustrates the size (16 d.nm) of AgNPs.

Antibacterial activity of synthesized silver Nanoparticles

The antibacterial action of synthesized AgNPs against both gram-positive and gram-negative bacterial strains was found to be satisfactory. Using the agar well diffusion method, the antibacterial potential of synthesized AgNPs was evaluated against normal strains of *Escherichia coli, S. Abony, Staphylococcus aureus, and Bacillus subtilis*. The antibacterial potential was confirmed by a clear zone of inhibition surrounding the inoculated region. The maximum zone of inhibition was found against Escherichia coli.

Fig. 13. Synthesized AgNPs shows Antibacterial Activity against *S. aureus, S. abony, E. coli, and Bacillus subtilis* (well diffusion method).

S. No.	Species	Silver Nanoparticle (Zone of Inhibition in mm)		
		AgNPs (50µg/well)	Antibiotic	Aqueous Fruit pulp extract
1.	*Escherichia coli*	**19**	**24**	**7**
2.	*S. abony*	**17**	**26**	**8**
3.	*Staphylococcus aureus*	**12**	**20**	**6**
4.	*Bacillus subtilis*	**15**	**18**	**5**

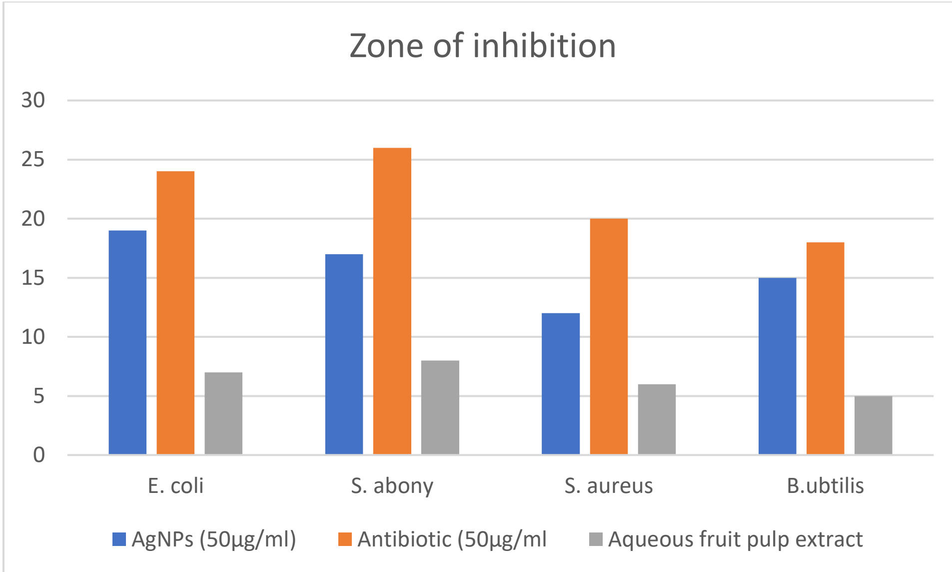

Fig. 14. Table and graph showing zone of inhibition of AgNPs against above mentioned pathogens .

The antibacterial studies with AgNPs showed a profound antibacterial effect against both gram-positive and gram-negative strains. The results of the present study suggest that plants and silver in their nano form possess certain constituents with antibacterial properties that may be used as antibacterial agents in new drugs against common bacterial pathogens. The synthesized nanoparticle was active on all the organisms tested. The highest activity against the tested bacteria was obtained in *S. aureus***,** *S.abony, E. coli, and Bacillus subtilis* gram-positive and negative bacteria.

MIC (Minimum Inhibitory Concentration)

We found that biogenic LA-AgNPs synthesized by *Limonia acidissima* fruit extract show strong antibacterial activity against both Gram-positive and Gram-negative pathogenic bacterial strains. The MIC_{50} of LA-AgNPs was evaluated against different pathogenic bacterial strains that included 14.5 µg/mL against *S. aureus*, 8.6 µg/mL against *S. abony*, 6.063 µg/mL against *E. coli*, and 13.4 µg/mL against *Bacillus subtillis*, indicating its broad-spectrum feature. However, we found that AgNPs were more effective against *E. coli* and *S.abony* than other pathogenic strains. A thick peptidoglycan layer in Gram-positive bacteria prohibited the entry of LA-AgNPs into the cytoplasm, and a higher AgNPs concentration is required to inhibit the growth of Gram-positive than Gram-negative bacteria.

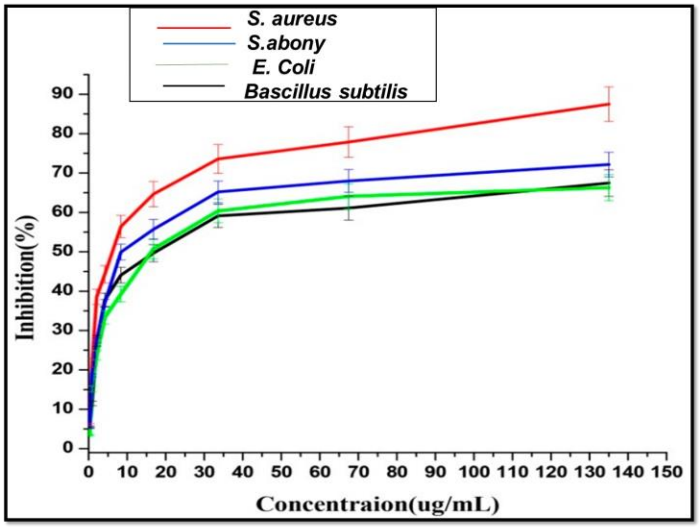

Fig. 15. Antibacterial potential of LA-AgNPs. (8µg/ml) against *Staphylococcus aureus, Escherichia coli*, *S. abony*, and *bacillus subtilis* (96 well plate method).

Discussion and conclusion

Conclusion

A simple, one-step green approach was developed for the synthesis of AgNPs using acidissima (wood Apple) fruit extract, the fruit extract acts as both a reducing and stabilizing agent. Silver nanoparticles showed significant antibacterial activity against the selected Gram-negative and Gram-positive pathogens. Thus, AgNPs might be a good alternative to develop as an antibacterial agent against the multidrug-resistant strains of bacteria. The applications of AgNPs may lead to valuable findings in various fields such as medical devices and antimicrobial systems.

Discussion

Green synthesis of nanoparticles makes use of environment-friendly, non-toxic and safe reagents. Nanoparticles synthesized using biological techniques or green technology have diverse natures, with greater stability and appropriate dimensions since they are synthesized using a one-step procedure. In this study, *Limonia acidissima* (fruit extract) was used for the synthesis of LA-AgNPs Silver is an important essential element. The idea behind this approach was to utilize food industrial waste to obtain Nanoparticles that can aid in several applications such as to perform therapeutic analysis for Antibacterial, Antifungal, and Multiple drug resistance as well as study their impact on cell lines. Our study is in the direction to develop a new improved and highly accurate drug delivery system with enhanced bioavailability of the drugs. The formation of silver nanoparticles was confirmed by their representative surface plasmon resonance (SPR) band measured as optical density in UV–VIS absorption spectroscopy. The SPR absorption band for AgNPs for fruit extract appeared at 408 nm. Plasmon resonance surface phenomenon “after attachment of any ligand at surface significantly affects the absorption intensity and full width at half maximum (FWHM) of absorption band”.

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