BIOFABRICATION OF SILVER NANOPARTICLES FROM *WITHANIA SOMNIFERA* ROOT EXTRACT AND ITS INVESTIGATION OF ANTI-INFLAMMATORY AND ANTI-BACTERIAL ACTIVITY

A Dissertation submitted to

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In partial fulfilment of the requirements for the award of the Degree of

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The work is original and has not been previously formed the basis for the award of any other Degree, Diploma, Associateship, Fellowship or any other similar title and the dissertation represent entirely an independent work on the part of the candidate.

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DECLARATION

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The work presented in this thesis entitled **"BIOFABRICATION OF SILVER NANOPARTICLES FROM *WITHANIA SOMNIFERA*ROOT EXTRACT AND IT'S INVESTIGATION OFANTI-INFLAMMATORY AND ANTI-BACTERIALACTIVITY"** was carried out by me in the Department of Pharmaceutics, Nandha College of Pharmacy, Erode under the direct supervision and guidance of **Dr. P.AMSA, M.Pharm., Ph.D.,**Professor, Department of Pharmaceutics, Nandha College of Pharmacy, Erode -52.

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ABBREVIATIONS	
µg	Microgram
mg	Milligram
gm	Gram
nm	Nanometer
cm	Centimeter
hrs	Hours
mL	Millilitre
Fig	Figure
NDDS	Novel Drug Delivery System
Ag-NP	Silver Nanoparticle
PVP	Polyvinyl Pyrolidine
FT-IR	Fourier Transform Infra-Red Spectroscopy
SEM	Scanning Electron Microscopy
XRD	X-Ray Diffraction analysis
$AgNO_3$	Silver nitrate
PEG	Polyethyleneglycol
PCE	Polychromatic Erythrocytes
rpm	Rotation Per Minute
Mv	Milli Volt
DLS	Dynamic Light Scattering
SPR	Surface Plasmon Resonance

INTRODUCTION

1. INTRODUCTION

Nanotechnology has changed our lives dramatically and influenced every sector of research and engineering. Nanotechnology is a rapidly growing science of manufacturing and utilizing nano-sized particles, that measure in nanometers. In other words, nanotechnology is the art of characterizing, manipulating, and organizing matter systemically, at the nanometer scale, which has created a revolution in science, engineering, technology, drug delivery, and therapeutics. The size of typically accessible structures is in the sub-micrometer range, being within the limits of optical resolution and barely visible with a light microscope. This scale is about 1/1000 smaller than structures that would be resolved by the microscope, but still 1000 times larger than an atom. Recent developments are addressing the dimensions range below these dimensions and since a typical structure size is within the nanometer range, the methods and techniques are defined as nanotechnology. Nanotechnology may be a significant field of up-to-date research handling design, synthesis, and manipulation of particle structures starting from within the region of 1- 100 nm [1].

Nanotechnology is understood as manipulating matter at the foremost fundamental level and incorporating it into the industry. Nanotechnologies are utilized in drug formulations, disease diagnosis, enhancing access to safe beverages, and boosting energy production. Multiple nanotechnology-based drug formulations have been introduced into the drug market for the treatment of a range of diseases including cancer, central nervous system (CNS) diseases, cardiovascular disease, and infection control. Nanotechnology is additionally utilized for drug delivery where nanoscale particles or molecules are wont to improve the bioavailability and pharmacokinetics of therapeutics, including liposomes, polymer nanoparticles, nano-suspensions, and polymer therapeutics [2].

Nanoscience has potential application within the development of nanoparticles as delivery systems, which exhibit several advantages like low toxicity, sustained and controlled release, capacity for targeting, encapsulation, and delivery of poorly soluble drugs. Various types of nanocarriers such as nanogel, metallic, lipidic, polymeric, and carbon-based nanoparticles have been used as a nano-delivery system.Nanoparticles (NPs) have a broad choice of applications in areas such as fitness care, cosmetics, foodstuff and feed, environmental health, mechanics, optics, biomedical sciences, chemical industries, electronics, space industries, drug-gene delivery, energy science, optoelectronics, catalysis, single-electrontransistors, light emitters, nonlinear optical devices, and photoelectrochemical applications. Nano Biotechnology may be a speedily mounting scientific field of manufacturing and constructing devices, a crucial area of research in nanobiotechnology is the synthesis of Nanoparticles with different chemical compositions, sizes and morphologies, and controlled disparities. Silver nanoparticles (NPs) are the themes of researchers due to their unique properties (e.g., size and shape depending on optical, antimicrobial, and electrical properties). A variety of preparation techniques are reported for the synthesis of silver NPs; notable examples include, laser ablation, gamma irradiation, electron irradiation, chemical reduction, photochemical methods, microwave processing, and biological synthetic methods. This assessment presents a general idea of silver nanoparticle preparation.[3]

Nanotechnology is rapidly gaining importance inseveral areas like health care, cosmetics, food and feed, environmental health, mechanics, optics, biomedical sciences, chemical industries, electronics, space industries, drug-gene delivery, energy science, optoelectronics, catalysis, single-electron transistors, light emitters, nonlinear optical devices, and photoelectrochemical applications. Nanomaterials are seen as a solution to several technological and environmental challenges within the field of solar power conversion, catalysis, medicine, and water treatment. In the context of worldwide efforts to scale back hazardous waste, the continuously increasing demand for nanomaterials must be amid green synthesis methods.

ADVANTAGES

(i) Improved bioavailability,

(ii) Reduced toxicity,

(iii) Sustained and controlled release,

(iv) Ability to target,

(v) Provide effective delivery to the brain and intracellular compartment,

(vi) Improved the permeability

(vii) Faster, safer, and more accurate disease diagnosis,

(viii) More accurate, less invasive surgery,

(ix) Inexpensive

(x) Large-scale production is feasible,

(xi) Smaller dosage form (i.e., smaller tablet),

(xii) Stable dosage forms,

(xiii) Faster dissolution especially in an internal aqueous fluid. Faster dissolution generally equates with greater bioavailability, smaller drug doses, less toxicity.

(xiv) Stability of drugs in biological fluids as it can prevent allergic reactions, pain at the injection site, and/or precipitation of the drug as a result of its' dilution in the blood.

DISADVANTAGES

Despite all that advantages, there have been certain disadvantages in pharmaceutical applications of nanotechnology. Nanoparticles have a very large surface area compared to their volume, so they are active to react quickly, e.g., silver nanoparticles. Their high aggregation in the biological systems is due to their high surface energy, poor solubility, and poor biocompatibility. In the case of carbon nanotubes, it is quickly scavenged by the RES system of the body, leading to low biological half-life, high immunogenicity or foreignness, undefined and unpredictable safety issue, and acute and chronic toxicity due to large absorption. Today, there are many applications of nanotechnology in our lives. Sunscreens containing nanoparticles of flowers of zinc and titanium oxide are the foremost used applications of nanotechnology on the market. They allow the normally white product to be more transparent when applied to the skin. They provide patient compliance; however, studies have shown that with nanoparticles the reactive radical levels will increase. As a result, there is a potential for serious cellular damage [4].

CLASSIFICATION OF NANOPARTICLES:

Pharmaceutical nanotechnology provides two basic types of nanotools; nanomaterials and nanodevices, which play an important role in pharmaceutical nanotechnology and other related fields.

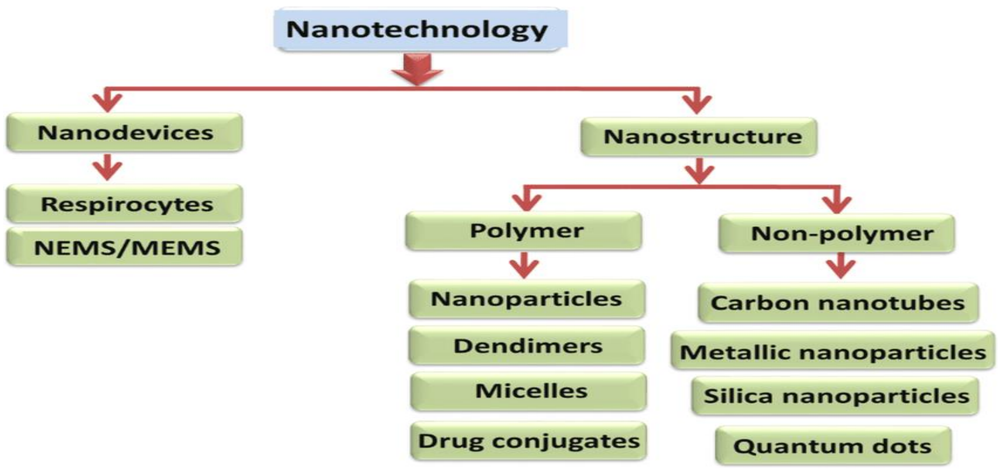


Figure 1:Classification of Nanoparticles

Nanomaterials

Nanomaterials are mainly used as biomaterials, for example in; orthopedic or dental implants or as tissue-engineered products. Their coatings or surface modifications might enhance biocompatibility by enhancing the interaction between the living cells with the biomaterial.

Nanodevices

Nanodevices are mini nature devices in the nanoscale. Most animal cells are 10,000 to 20,000 nanometers in diameter. This means that nanoscale devices (less than 100 nanometers) can enter cells and the organelles inside them to interact with DNA and proteins and to be effective in detection or imaging. Tools developed through nanotechnology may be able to detect disease in a very small number of cells or tissue. They have the power to directly interact with biologically significant molecules and to convert those interactions into directly significantly amplified electrical or electromagnetic signals. It enabled a new generation of early-stage diagnostic techniques. Detection of cancer at early stages is a critical step in improving cancer treatmentidentification [5].

PHARMACEUTICAL NANOSYSTEMS

1. **Carbon Nanotubes (CNTs)**

Carbon nanotubes are hexagonal networks of carbon atoms, 1 nm in diameter and 1–100 nm long, as a graphite layer rolled up into a cylinder. They are existing in two forms: single-walled nanotubes (SWNTs) and multi-wall nanotubes (MWNTs). Both forms differed in their graphene cylinder arrangement. They are small macromolecules that have unique sizes, shapes, and have significant physical properties. Nanotubes offer some distinct advantages over other drug delivery and diagnostic systems due to their interesting physicochemical properties such as ordered structure with high characteristic ratio, ultra-lightweight, high mechanical strength, high thermal and electrical conductivity, high surface area, and metallic or semi-metallic manners.

2. Polymeric Nanoparticles

Polymeric nanoparticles are the most used nanoparticles in drug synthesis. That was due to its biocompatibility-like properties, non-immunogenicity, non-toxicity, and biodegradability. In polymeric nanoparticles, the drug is dissolved, encapsulated (nanocapsules), and entrapped in matrix systems (nanospheres). Nanocapsules are systems during which the drug is enclosed and surrounded by a polymeric membrane while nanospheres are matrix systems during which the drug is physically and uniformly dispersed. Various natural polymers like albumin, gelatin, and alginate are used to prepare the nanoparticles; however, they have certain disadvantages like poor batch-to-batch variability, highly degradable, and potential antigenicity. Synthetic polymers are preferable.

3. Metallic Nanoparticles

Metallic nanoparticles are emerging as a good delivery carrier for drugs and biosensors. They're used for diagnostic use or treatment use as they provide a quick, highly defined snapshot of the living system. The main metallic nanoparticles of various metals have been made yet were silver and gold nanoparticles. They have prime importance for biomedical use, especially in cancer treatment (tumor-targeting ligands). Their large surface area to volume ratio provides an opportunity for surface modification. Various ligands have been linked to nanoparticles to decorate the surface, including sugars, peptides, proteins, and DNA. Therefore, they have been used as an active bioactive delivery and in drug discovery, bioassays, imaging, detection, and many other applications. The most common applications of metallic nanoparticles were silver nanoparticles and gold nanoparticles.

Gold nanoparticles (AuNPs) exhibit a mixture of physical, chemical, optical, and electronic properties unique from other biomedical nanotechnologies. They provide a highly multifunctional platform to image and diagnose diseases, to deliver therapeutic agents selectively, to sensitize cells and tissues to treatment regimens, to monitor and guide surgical procedures, to preferentially administer electromagnetic radiation to disease sites, and to deliver compounds that are intrinsically susceptible to enzymatic degradation, as well as those that exhibit poor intracellular penetration (e.g., siRNA).

Also, AuNPs can be coated with polyethylene glycol chains via Au-S bonds to reduce their uptake by the liver and spleen and prolong their half-life in the blood. Other biomolecules such as peptides, small-molecular-weight compounds aptamers, and monoclonal antibodies have also been attached to the surface of AuNPs to increase the particles' tumor-targeting potential. That means the multi-functionality of AuNPs, gives future advances in AuNP-mediated cancer therapy which could include chemotherapy and chemoradiotherapy.

Silver nanoparticles (AgNPs) have antibacterial properties due to the presence of silver ions. They may enter the living organism's body in food, and also through the skin or the respiratory system, and even pass the blood-brain barrier. Silver nanoparticles have a typically cytotoxic effect. As they enhanced the antioxidative defense proteins of genes coding expression which is a typical feature of the response to oxidative stress[6].

BIOPRODUCTION OF NANOPARTICLES:

Due to incredible properties nanoparticles became significant in many fields within recent years, like energy, health care, environment, agriculture, etc. The preparation of nanoparticles is carried out either by (i) Nanoparticle synthesis or by (ii) the Processing of nanomaterials into nanostructure particles.

The silver nanoparticles are prepared by using physical, chemical, and biological methods. The physical and chemical methods are very expensive. Biological methods of nanoparticles synthesis would help to get rid of harsh processing conditions by enabling the synthesis at physiological pH, temperature, pressure, and at an equivalent time at a lower cost. A large number of microorganisms have been found capable of synthesizing inorganic nanoparticles composite either intra or extracellularly.

There are numerous methods available using various approaches including chemical, physical, and biological protocols for the synthesis of nanoparticles.

1. Physical Method:

In the physical approach, metal nanoparticles are synthesized by either evaporation -condensation method or laser ablation method. In the evaporation condensation method, the reaction is carried out using a tube furnace at atmospheric pressure. The target material is kept within a boat centered at the furnace is vaporized into a carrier gas. The particles synthesized through the laser ablation method depend upon the wavelength of the laser, the duration of the laser pulses the laser fluency, the ablation time duration, and the effective liquid medium which may or may not contain the surfactant.

2. Chemical Method:

The chemical method is the most commonly used method for the synthesis of silver nanoparticles. The most commonly used reducing agents are sodium borohydride, hydrazine hydrate, potassium auro chlorate, and sodium citrate. The reduction of various complexes with Ag+ ions lead to the formation of silver atoms (Ag0), which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to the formation of colloidal Ag particles. The function of the protective agent is to protect the nanoparticles from agglomeration. Sol-gel technique is used for the synthesis of metal oxides from a chemical solution which acts as a precursor for an integrated network of discrete particles or polymers. The precursor can be either deposited on the substrate to form a film cast into an appropriate container having desired shape or can be used to synthesize powders. Solvothermal synthesis is a flexible low-temperature route in which polar solvents under pressure and at temperatures above their boiling points are used. The reaction of the reagents under the solvothermal conditions increases significantly and enabling the reaction to take place at lower temperatures.

3. Biosynthesis of Nanoparticles:

Although chemical & physical methods are very successful to produce well-defined nanoparticles, they have certain limitations such as increased cost of production, the release of hazardous by-products, long time for synthesis, and difficulty in purification. Global warming & climate change has induced a worldwide awareness to reduce toxic & hazardous waste materials, thus, the green synthesis route has raised actively the progress in the fields of science & industry. Biosynthesis of nanoparticles as the name indicates help in the synthesis of very complex reactions within a fraction of minutes has now taken up the attention towards synthesis grievance the need for environmentally benign technologies in material science.

The use of biological organisms such as microorganisms, plant extracts, and biomass could be the best alternative method of the physical and chemical method for the synthesis of nanoparticles because the biological or green synthesis route is very spontaneous, economic, environmentally friendly, and non-toxic. Therefore, biological sources such as bacteria, fungi, yeasts, algae, and plants can materials catalyze specific reactions as a part of modern & realistic biosynthetic strategy.

Nature has devised various processes for the synthesis of nano- and micro-length scaled inorganic materials which have contributed to the development of the relatively new and largely unexplored areas of research based on the biosynthesis of nanomaterials. The synthesis and assembly of nanoparticles would benefit from the development of clean, non-toxic, and environmentally acceptable ‘‘green chemistry’’ procedures, probably involving organisms ranging from bacteria to fungi and even plants[7].

GREEN SYNTHESIS:

Science and technology are moving at a rapid pace today. Environmental engineering science is witnessing drastic changes. Similarly, nanotechnology and green nanotechnology are moving from one visionary paradigm towards another. Environmental regulations, frequent environmental catastrophes, and loss of ecological biodiversity have urged the scientific community to move forward towards a newer vision and newer innovations. Green nanotechnology is the frontier of science and engineering today. Green synthesis is defined as the use of environmentally compatible materials such as bacteria, fungi, and plants in the synthesis of nanoparticles. These attractive green strategies are free of the shortfalls associated with conventional synthetic strategies, i.e., they are eco-friendly. Alternatively, synthesis from biologically derived extracts offers several advantages such as rapid synthesis, high yields, and importantly, the lack of costly downstream processing required to produce the particles. Hence, nanoparticle synthesis from plant extracts tentatively offers a route for the large-scale production of commercially attractive nanoparticles.

Plant extracts are reported to have antioxidant and reducing properties which are responsible for the reduction of metal salt to their respective nanoparticles. Plant-basedmethods of nanoparticles synthesis eliminate the elaborate process of nanoparticles synthesis and are considered beneficial because of the presence of a wide range of biomolecules.

A new era of 'green synthesis' approaches/methods is gaining great attention in current research and development on materials science and technology. Green synthesis of materials/ nanomaterials, produced through regulation, control, clean up, and remediation process will directly help uplift their environmental friendliness [8].

Green synthesis methodologies based on biological precursors depend on various reaction parameters such as solvent, temperature, pressure, and pH conditions (acidic, basic, or neutral). For the synthesis of metal/metal oxide nanoparticles, plant biodiversity has been broadly considered due to the availability of effective phytochemicals in various plant extracts. The basic features of such nanomaterials have been investigated for use in biomedical diagnostics, antimicrobials, catalysis, molecular sensing, optical imaging, and labeling of biological systems. The green synthesis of metal/metal oxide nanoparticles with their advantages over chemical synthesis methods [9].

Production of NPs using extracts from natural substances is emerging as an important area in nanotechnology. The use of natural resources for the production of NPS is sustainable, eco-friendly, inexpensive, and free of chemical contaminants for biological and medical applications where the purity of NPs is of major concern. Useful and common nanomaterials can be produced easily on large scale. The biological methods do not need harsh or toxic chemicals.

Advantages of Green Synthesis Include:

a. Energy efficiency

b. Economical

c. Less waste

d. Fewer accidents

e. Lower cost of production and regulation

f. Competitive

g. Safer products

h. Healthier workplaces and communities

i. Protects human health and the environment

j. Compatible for pharmaceutical and other biomedical applications

i. Can be used for large-scale production of nanoparticles and external experimental conditions like high energy and high pressure are not required, leading to substantial energy-saving processes.

The waste products of plant extracts are non-toxic and easier to dispose of. Furthermore, NPs synthesized via the green route are more stable and effective in comparison with those produced by physicochemical methods. The biological pathways involve the use of microorganisms (bacteria, fungi, yeast, algae, etc.) or plants, and using microorganisms is riskier because of the pathogenicity issue; it also requires maintenance of large cultures. Therefore, the synthesis of nanoparticles with greener methods is preferred[10].

Biological Components for "Green" Synthesis

Innumerable physical and chemical synthesis approaches require high radiation, highly toxic reluctant, and stabilizing agents, which can cause pernicious effects to both humans and marine life. In contrast, green synthesis of metallic nanoparticles is a one-pot or single-step eco-friendly bio-reduction method that requires relatively low energy to initiate the reaction. This reduction method is also cost-efficient.

Bacteria in Synthesis:

Bacteria have been most extensively researched for the synthesis of nanoparticles because of their fast growth and relative ease of genetic manipulation. The Silver producing bacteria isolated from the silver mines Pseudomonas stutzeri AG259 where the silver nanoparticles were accumulated in the periplasmic space but the particles size ranges from 35 to 46 nm. The Lactobacillus strains present in the milk were exposed to a larger concentration of nanoparticles to produced silver, gold, and alloy crystals of defined morphology. Bacteria have also been used to synthesize gold nanoparticles. The PH was an important factor in controlling the morphology of bacteriogenic nanoparticles and the location of the deposition. These nanoparticles were used in many applications e.g., direct electrochemistry of proteins. The most important application of bacterium would be in industrial silver recovery.

The bacterial synthesis of nanoparticles has been adopted due to the relative ease of manipulating the bacteria. Some examples of bacterial strains that have been extensively exploited for the synthesis of bio-reduced silver nanoparticles with distinct size/shape morphologies include *Escherichia coli*, *Lactobacillus case*, *Bacilluscereus*, *Aeromonas* sp. SH10 *Phaeocystis Antarctica*, *Pseudomonasproteolytic*, *Bacillus amyloliquefaciens*, *Bacillusindicus*, *Bacillus cecembensis*, *Enterobacter cloacae*, *Geobacter* spp., *Arthrobacter gangotriensis*, *Corynebacterium* sp. SH09, and *Shewanella oneidensis*.

Fungi in Synthesis:

Fungi-mediated biosynthesis of metal/metal oxide nanoparticles is also a very efficient process for the generation of monodispersed nanoparticles with well-defined morphologies. They act as better biological agents for the preparation of metal and metal oxide nanoparticles, due to the presence of a variety of intracellular enzymes.

The fungi taking the center stage of studies on the biological generation of nanoparticles because of the tolerance and bioaccumulation. The advantages of using fungi in their scale-up process (e.g., using a thin solid substrate fermentation method). Fungi are efficient secretors of extracellular enzymes they can easily obtain large-scale production of enzymes. Further advantages of using the fungal mediated green approach for the synthesis of metallic nanoparticles include economic viability and ease in handling biomass.

Algae in Synthesis:

Algae in Nanoparticles Synthesis: Algae are similar to yeast for the biosynthesis of nanoparticles, still very few reports used algae as a “Biofactory” for the nanoparticle’s synthesis. The marine algae used for the biosynthesizing highly stable extracellular gold nanoparticles in a relatively short periodcompared to another biosynthesizing process. Palladium and platinum nanoparticles starting with their corresponding metallic chloride-containing salts have been investigated.

Virus in Synthesis:

The biological synthesis of nanoparticles has been extended to biological particles like viruses, proteins, peptides, and enzymes. Cowpea chlorotic mottle virus and cowpea mosaic virus have been used for the mineralization of inorganic materials. Tobacco mosaic virus has been shown to direct successfully the mineralization of sulphide and crystalline nanowires. One step further, peptides capable of nucleating Nanocrystal growth have been identified from combinatorial screens and displayed on the surface of the M13 bacteriophage

Yeast in Synthesis:

Yeasts are single-celled microorganisms present in eukaryotic cells. A total of 1500 yeast species have been identified. The MKY3, a silver-tolerant yeast species, when challenged with soluble silver in the log phase of growth, majority of silver precipitate extracellularly as elemental nanoparticles. When challenged with soluble silver in the log phase of growth, based on differential thawing of the sample, for separation of the Metallic nanoparticles from the medium. Conditions have also been standardized for the synthesis of large quantities of silver nanoparticles by using silver-tolerant yeast strain MKY3. The procedure for separation of these silver particles has also been documented that was based on differential thawing of the samples. Recently, yeast strains have been identified for their ability to produce gold nanoparticles, whereby controlling growth and other cellular activities-controlled size and shape of the nanoparticles was achieved[11].

Plants in Synthesis:

Plants have the potential to accumulate certain amounts of heavy metals in their diverse parts. Consequently, biosynthesis techniques employing plant extracts have gained increased consideration as simple, efficient, cost-effective, and feasible methods as well as an excellent alternative means to conventional preparation methods for nanoparticle production. Various plants can be utilized to reduce and stabilize the metallic nanoparticles in the “one-pot” synthesis process. Many researchers have employed a green synthesis process for the preparation of metal/metal oxide nanoparticles via plant leaf extracts to further explore their various applications.

The relative amount of triangle and spherical nanoparticles led to significant control over the optical properties of the nanoparticle solution. The key role in the formation of gold nano triangles was played by the slow reduction of aqueous gold ions (HAuCl4) along with the shape-directing effects of constituents (carbonyl compounds) of the plant extract. On the other hand, aqueous silver ions (AgNO3) when incubated with Aloe vera extract produce only spherical Ag nanoparticles. The appearance of brownish-redcolor and faint yellow color in the reactions indicate the formation of gold and silver nanoparticles, respectively.

Adding to the list of plants that are showing potential for nanoparticle production, Cinnamomum camphora leaf extract has been identified very recently for the production of gold as well silver nanoparticles. The marked difference in shape control between gold and silver nanoparticles was attributed to the comparative advantage of protective biomolecules and reductive biomolecules. The polyol components and the water-soluble heterocyclic components were mainly found to be responsible for the reduction of silver ions or chloroauric ions and the stabilization of the nanoparticles, respectively[12].

METHOD OF PREPARATION

Solvent System-Based "Green" Synthesis

Solvent systems are a fundamental component in the synthesis process, whether it is a "green" synthesis or not. Water is always considered an ideal and suitable solvent system for synthesis processes. According to Sheldon, "the best solvent is no solvent, and if a solvent is desirable then water is ideal". Water is the cheapest and most commonly accessible solvent on earth. Since the advent of nanoscience and nanotechnology, the use of water as a solvent for the synthesis of various nanoparticles has been carried out. For instance, synthesized Au and Ag nanoparticles at room temperature using gallic acid, a bifunctional molecule, in an aqueous medium. Gold nanoparticles were produced via a laser ablation technique in an aqueous solution. The oxygen present in the water leads to partial oxidation of the synthesized gold nanoparticles, which finally enhanced its chemical reactivity and had a great impact on its growth. In the literature, "green" synthesis consists of two major routes:

- Wherein water is used as a solvent system.
- Wherein a natural source/extract is utilized as the main component.

Both of these routes have been covered in the coming section according to the present literature. Hopefully, our efforts will help researchers gain a better knowledge of 'green' synthesis methods, the role of toxic/non-toxic solvents (or components), and renewable resources derived from natural sources. Ionic and supercritical liquids are one of the best examples in this emerging area. Ionic liquids (ILs) are composed of ions that have melting points below 100 °C. Ionic liquids are also acknowledged as "room temperature ionic liquids." Several metal nanoparticles (e.g., Au, Ag, Al, Te, Ru, Ir, and Pt) have been synthesized in ionic liquids. The process of nanoparticle synthesis is simplified since the ionic liquid can serve as both a reductant and a protective agent. I can be hydrophilic or hydrophobic depending on the nature of the cations and anions benefits of using ionic liquids instead of other solvents include the following:

(a) Many metal catalysts, polar organic compounds, and gases are easily dissolved in ILs to support biocatalysts.

(b) ILs have constructive thermal stabilities to operate in a broad temperature range. Most of these melts below room temperature and begin to decompose above 300 or 400 °C. As such, they allow a broader synthesis temperature range (e.g., three to four times) than that of water.

(c) The solubility properties of IL can be modulated by modifying the cations and anions associated with them.

(d) Unlike other polar solvents or alcohols, ILs are non-coordinating. However, they have polarities comparable to alcohol.

(e) I do not evaporate into the environment like volatile solvents because they have no vapor pressure.

(f) ILs have dual functionality because they have both cations and anions.

The problems associated with the biodegradability of ionic liquids make them not acceptable for the synthesis of metallic nanoparticles. To diminish these non-biodegradability issues, many new potentially benign ionic liquids are being developed with maximum biodegradation efficiency. Likewise, ordinary solvents can be converted into supercritical fluids at temperatures and pressures above the critical point. Suggested that decreasing the solubility of metal oxides around the critical point can lead to supersaturation and the ultimate formation of nanoparticles[13].

METAL NANOPARTICLES

Metal nanoparticles and their technological applications in various fields of science have become a topic of importance for the global research community. The selective and reproducible synthesis of Metal nanoparticles is imperative for wide applications. Various chemical and biological methods are used for the synthesis of metal nanoparticles. Chemical methods use toxic chemicals while biological methods are free from toxic materials. Greener synthesis of metal/metal oxides nanomaterials is a trustworthy, viable, and environmentally amicable method. Fundamentally, greener synthesis appears to be an important mechanism to curtail the lethal effects associated with the traditional methods of synthesis of nanoparticles commonly utilized in laboratories and industry. The stabilization of these metal nanoparticles is of great concern to avoid their agglomeration during their applications. The stability of metal nanoparticles in ionic liquids is also discussed here. In the field of nanotechnology, Metallic nanoparticles have shown several properties and it has unlocked many new pathways in nanotechnology. Metallic nanoparticles have a specialty with appropriate functional groups. It can be synthesized and modified that would allow them to bind with ligands, antibodies, drugs. A metallic nanoparticle is a nanosized metal with a size range of 10- 100 nm. Metallic nanoparticles have unique characteristics such as surface Plasmon resonance and optical properties. The gold solution does have a golden yellow color, for example, a solution of 20 nm gold nanospheres has red ruby colorwhereas 200 nm nanospheres havea bluish color. The noble metals, especially silver and gold, have gained much attention to researchers in various branches of science and technology namely catalysis, photography, medical field as anticancer and anti-microbial agents. The existence of metallic nanoparticles in solution and Mie gave the quantitative explanation of their color.

In the medieval era, metallic nanoparticles were used to decorate cathedral windows. Due to the unique properties of noble metal nanoparticles, it has made a special place in the field of nanotechnology. The most important feature of nanoparticles is their surface area to volume ratio, which easily allows them to interact with other particles. In nanoparticles, a high surface area to volume ratio makes diffusion faster and is feasible at lower temperatures. And this field has been found more interesting, without disturbing and poisoning healthy cells, we can directly treat affected cells and tissues. In fluorescence enhancement and surface-enhanced Raman spectroscopy and environment refractive index sensing nanoparticles have found additional application in the enhancement of field-sensitive optical processes. The optical properties of metal nanoparticles play a key role due to the localized surface Plasmon with resonance wavelength in the visible region. Silver and gold nanoparticles are effective in inhibiting the growth of gram-positive and gram-negative bacteria. For the production of nanodevices, a living organism has huge potential. However, it requires much more experimentation. There is a drawback such as the involvement of toxic chemicals makes it difficult for the synthesis of metallic nanoparticles [14].

So, there is an alternate way of synthesizing metallic nanoparticles by using living organisms such as fungi, bacteria, plants. Several studies have shown that metallic nanoparticles characteristics like (size, stability, physical, chemical properties, morphology) are strongly influenced by the experimental conditions, adsorption process of stabilizing agent, the kinetics of interaction of metal ions with reducing agents.

In various industrial applications, metallic nanoparticles have been attracted, because of their different physical and chemical properties from bulk metals. Various properties like mechanical strengths, high surface area, low melting point, optical properties, and magnetic properties. Catalysts which are used in metallic nanoparticles are selective and highly active, havea long life for many chemical reactions.

Advantages of Metallic Nanoparticle:

a. Enhance Rayleigh scattering

b. Surface-enhanced Raman scattering

c. Strong plasma absorption

d. Biological system imaging

e. Determine chemical information on the metallic nanoscale substrate.

f.No photo bleaching

g. Less invasive

h. Increased contrast

Disadvantages of Metallic Nanoparticles

a. Particle instability: Nanomaterials cantransform, as they are thermodynamically unstable and lie in the region of high energy local minima. This leads to deterioration of quality, poor corrosion resistance, and the main concern is retaining the structure becomes difficult.

b. Impurity: While synthesizing nanoparticles, nitrides, oxides, the formation can be aggravatedby the impure environment. As nanoparticles are highly reactive, there can also be high chances of impurity as well. In solution form, nanoparticles should be synthesized in form of encapsulation. So, it becomes a challenge to overcome impurity in nanoparticles.

c. Biologically harmful: nanomaterials have been reported toxic, carcinogenic, and irritate as they become transparent to the cell dermis.

d. Explosion: exothermic combustion can lead to an explosion, as fine metal particles act as strong explosives.

e. Difficulty in synthesis: while synthesizing nanoparticles, it should be encapsulated, because it is extremely challenging to retain the size of nanoparticles in solution form [15].

Characteristics of Metallic Nanoparticles

a. large surface energies

b. As compared to bulk they have alarge surface area to volume ratio

c. Quantum confinement

d. Plasmon excitation

Points Need to Be Considered While Preparing Metallic Nanoparticles

Ideally, metallic nanoparticles should be prepared by a suitable method which is

a. Easily reproducible

b. Easily available, economical

c. Use a minimum number of reagents

d. May control the particle shape

e. Use a reaction temperature close to room temperature

f. Minimizing the quantities of generated by-products and waste [16].

Metal Nanoparticles as Catalyst

It is already known that metallic nanoparticles function as a catalyst for various kinds of chemical reactions. On its surface metals, catalytic sites are located. So, by this, we can mean that metallic nanoparticles of 1-10 nm size can work as effective catalysts. Concerning all atoms in a particle, the ratio of surface atoms increases with decreasing particle size. Metallic nanoparticles should be stabilized under the catalytic condition when it is used as catalysts. Otherwise, what will happen is, it will easily coagulate in solution and lead to form aggregates, which are less effective as catalysts. Advantages of using metal nanoparticles as catalysts are the following:

a. The temperature applied to the catalyst where the metallic nanoparticles dispersed in solution is below the boiling point of the solvent.

b. Metallic nanoparticles dispersed in solutions can be used as photocatalysts as they are transparent to light.

c. By the preparation, metallic nanoparticles' size and shape can be easily controlled.

d. Metallic nanoparticles immobilized on solid supports act as catalysts even for the reactions in a gaseous phase.

e. Bimetallic and trimetallic nanoparticles can be prepared by modifying the structures and composition [17].

Synthesis of Metallic Nanoparticles:

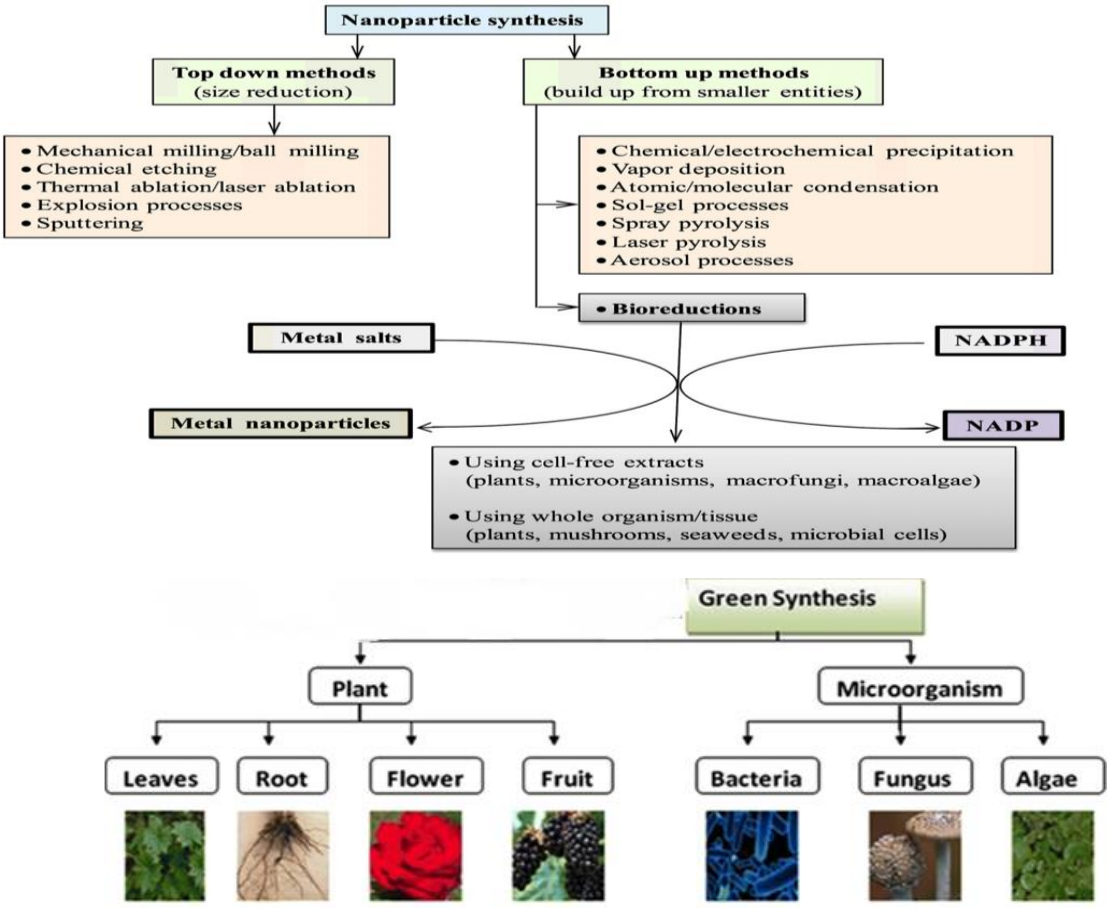


Figure 2: Showing the methods for synthesis of metal nanoparticles

In the synthesis of nanoparticles, which can be the natural or synthetic origin and exhibit unique properties at the nanoscale, two basic approaches that include various preparation methods and are known from early times are used. The first approach is the "top-down" method which calls for breaking down solid materials into small pieces by applying external force. In this approach, many physical, chemical, and thermal techniques are used to provide the necessary energy for nanoparticle formation. The second approach, known as "bottom-up", is based on gathering and combining a gas or liquid atoms or molecules. These two approaches have advantages and disadvantages relative to each other. In the up-down approach, which is costlier to implement, it is impossible to obtain perfect surfaces and edges due to cavities and roughness that can occur in nanoparticles; whereas excellent nanoparticle synthesis results can be obtained by the bottom-up approach. In addition, with the bottom-up approach, no waste materials that need to be removed are formed, and nanoparticles having smaller sizes can be obtained thanks to the better control of the sizes of the nanoparticles.

Bottom-upApproach:

These approaches include the attenuation of materials components with further self-assembly process which leads to the formation of nanostructures. During self-assembly, the physical forces operating at the nanoscale are used to combine units into large stable structures. Typical examples include Quantum dots and the formation of nanoparticles from colloidal dispersion.

Top-downApproach:

These approaches include macroscopic structures which can be externally controlled in the processing of nanostructures. Typical examples are ball milling, the application of severe plastic deformation.

Top-down method v/s bottom-up methods, Top-down method starts with a pattern generated on a large scale, then reduced to the nanoscale, quick to manufacture, slow, and not suitable for large scale production. The bottom-up approach begins with atoms or molecules and builds up to nanostructures, fabrication is much less expensive. Attrition/ milling is a top-down type of method and the bottom-up method is the production of colloidal dispersion [18].

1. Chemical Reduction Method

Gold Nanoparticle

For centuries, gold nanoparticles have been intensively studied. When chitosan capped gold nanoparticles coupled with ampicillin, two-fold enhancement of antibacterial activity was observed Chemical reduction is the most common and widely used method for the preparation of gold nanoparticles. This method includes, in the presence of a reducing agent, gold salt is reduced.

Michael Faraday first studied gold colloids synthesis in the solution phase, wherein an aqueous medium gold chloride was reduced with phosphorus. One article has been reported in 1951 which discovered the citrate reduction method. Synthesis of AuNPs was based on a single-phase reduction of gold tetra chloroauric acid by sodium citrate in an aqueous medium and produced particles about 20 nm.

Platinum Nanoparticles

In the synthesis of platinum nanoparticles, the platinum metal precursor either in an ionic or a molecular state is taken. By the reducing agents, chemical changes are made to convert the precursor to platinum metal atoms. These metal atoms then combine into stabilizers or support materials to form nanoparticles. By introducing decomposition, displacement, reducing agent, electrochemical reactions, the dissolved metal precursor can be converted into the solid metal [19].

Silver Nanoparticles

Silver nanoparticles have been used extensively in anti-bacterial agents in the health industry, food storage, textile coating, and several environmental applications. Important to note, despite decades of use, the evidence of the toxicity of silver is not still clear. The antibacterial property of silver nanoparticles has allowed its wide range of applications from disinfecting devices and home appliances to water treatment. Catalytic activities of nanoparticles differ from chemical properties.Silver nanoparticles are one of the most attractive inorganic materials because of theirenvironmentally free nature. Moreover, it has several applications in various fields like photography, diagnostics, catalysis, biosensor, antimicrobial.

a. Reduction by citrate anion

From the pioneering studies; it is now well known that citrate acted in both ways. The first is to stabilize the nanoparticles and reduce the metal cation. To determine the particle's growth this reactant played a major role. Citrate controls the size and shape of silver nanoparticles. This function was investigated by Pillai and Kamat. At different citrate concentrations, by using the boiling method, silver nanoparticles with Plasmon maximum absorbance at 420nm were produced. By increasing the concentration 1 to 5 times of sodium citrate to silver cation. i.e., [citrate] / [Ag+], the elapsed time for silver nanoparticles formation was 40 to 20min reduced respectively, which indicates that a fraction of the Ag+ was not reduced under equimolar conditions.

b. Reduction by Gallic acid

At room temperature, reduction of Ag+ in water can be achieved by using Gallic acid (GA) whose oxidation potential is 0.5V. In benzoic acid structure the hydroxyl group at determined Positions play an important role in the synthesis of metal nanoparticles. When hydroxyl groups are located at the Meta position, nanoparticles synthesis was not successful but it was achieved when hydroxyl groups are present at ortho and para positions. Here carboxylic group act as a stabilizer and hydroxyl isthe reactive part.

2. Physical Method

To form AuNPs, photochemical reduction of gold salts has been used. This formulation uses continuous-wave UV irradiation (250-400nm), PVP as the capping agent, ethylene glycol as the reducing agent. Glycol concentration and viscosity of the solvent mixture are the two factors where AuNPs formation is dependent. The process was further improved by the addition of Ag+ to the solution, leading to an increase in the production of Au nanoparticles. To synthesize platinum nanoparticles, irradiation and laser ablation techniques have also been used. In one method irradiation was combined with ultra-sonication.

3. Biological Method

Plant-mediated synthesis has gained more popularity due to being eco-friendly. Zingiber Officinale extract acts as a reducing agent, as well as a stabilizer with particles ranging from 5-15nm in diameter. To synthesize metallic nanoparticles, several bacteria,fungus-like prokaryotic bacteria, and eukaryotic fungus. Plant extract may have been employed for the reduction of aqueous metal ions. Biological methods may have a wide distribution in particle size but have a slow reaction rate. At room temperature, the extract is mixed with a metal salt solution, within minutes the reaction is complete. By this method, gold, silver nanoparticles have been synthesized. The rate of nanoparticles production, their quantity can be affected by the plant extract concentration, its nature metal salt concentration, temperature, the pH. Preparing metallic nanoparticles by the use of plant extract is environmentally friendly,it brings controlled size and morphology of nanoparticles[20].

Characterization of Metallic Nanoparticle

a) Absorbance Spectroscopy: Spectroscopy is useful to characterize metal nanoparticles because they possess bright color which is visible by the naked eye. By this technique, qualitative information about the nanoparticle can be obtained. By applying Beer’s law absorbance is measured. Depending on path length (l), nanoparticle conc. (c), extinction coefficient (A) can be obtained.

b) Infrared Spectroscopy: This method can provide information on organic layers surrounding metallic nanoparticles. It also gives valuable information to understand the surface structure of the metal nanoparticles.

c)SEM: (Scanning Electron Microscopy) it is a powerful technique for imaging any material surface with a resolution down to about 1nm. The interaction of an incident electron beam with the specimen produces secondary electrons, with energies smaller than 50ev. SEM can give information about the purity of nanoparticle samples.

d)TEM: (Transmission electron microscope) is also widely used to characterize nanomaterials to gain information about particle size, shape, crystallinity, and interparticle interaction. TEM is a high spatial resolution structural and chemical characterization tool. It can directly image atoms in crystalline specimens at resolutions close to 0.1nm, smaller than interatomic distance. An electron beam can also be focused to a diameter smaller than ~0.3nm, allowing quantitative chemical analysis from a single nanocrystal.

e) AFM: (Atomic Force Microscopy)It is a better choice for nonconductive nanomaterials. Typically, it has a vertical resolution of less than 0.1nm and a lateral resolution of around 1nm.It gives detailed information on the atomic scale, which is important for understanding the electronic structure and chemical bonding of atoms and molecules.

f) XRD: (X-Ray Diffraction)It is a useful and widely used technique for determining the crystal structures of crystalline materials. Diffraction line widths are closely related to the size and their distribution, strain in the nanocrystal. The line width is broadened, as the size of the nanocrystal decreases, due to loss of long-range order relative to the bulk. XRD line can be used to determine the particle size by the Debye-Scherrer method.

$D= 0.9\,\lambda / b\cos\Theta$ Where,

D= nanocrystal diameter

λ=light wavelength

b=full width half at max. Of the peak (radians)

Θ=Bragg angle

g) FT-IR: (Fourier Transform Infrared Spectroscopy)It is a widely adopted technique compared to IR spectroscopy. Functional groups attached to the metallic nanoparticle surface show a different FT- than those of free groups.

h) EXAFS: (Extended X-ray Absorption Fine Structure) this is one of the most reliable and powerful characterization techniques to evaluate the structure of metallic nanoparticles; especially it is useful to determine bimetallic nanoparticles. To gain appropriate information about the structure, the sample of metallic nanoparticles should be homogeneous. This method provides the no. Of atoms surrounded the x-ray absorbing atom and their interatomic distances involved in the shells.

i) XPS: (X-ray Photoelectron Spectroscopy) is used to provide information on the mental state. Suppose the oxidation state of metal on the surface. It is often oxidized by air. So, by using this method, the 0-valency pattern of surface metal must be confirmed[21].

General Application of Metallic Nanoparticles

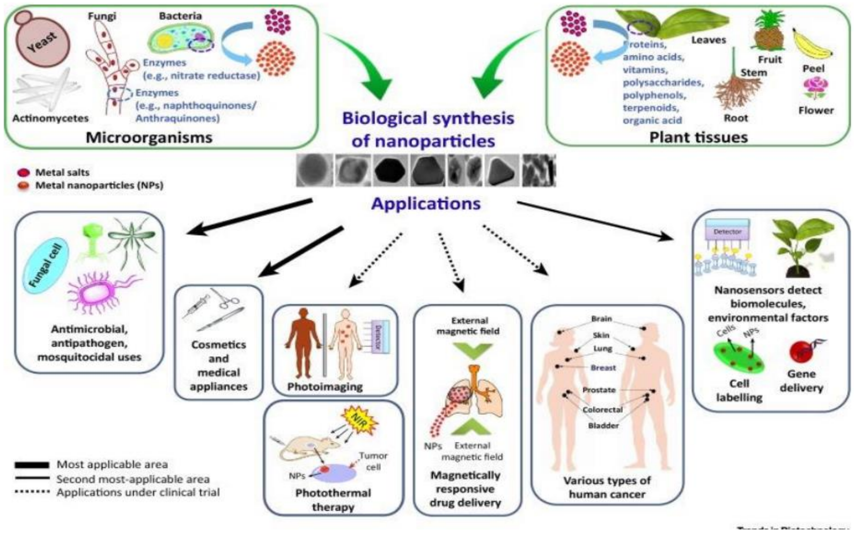


Figure 3: Application areas of metallic nanoparticles synthesized by biological methods

1. Optical Function

Imaging sensor, display, solar cell, Photocatalysis, biomedicine, optical detector, laser-this are the applications based on the optical properties of metal nanoparticles. It is mainly dependent on some factors such as shape, size, surface area, doping, and interaction with the surrounding. The optical properties of CdSe semiconductor nanoparticles can change with size. For different samples of gold nanospheres, the optical properties changes with the enlargement of metallic nanoparticles. Surface absorption Plasmon Au & Ag can change into various colors by changing the particle size, form, and shape of the particle and condensation rate

2. Thermal Function

When nanoparticle diameter is less than 10nm, the melting point is also lower than a bulk metal. With the low boiling point, electronic wiring can be made with nanoparticles.

3. Electrical Function

Can be used to make high-temperature superconductivity material. In conductance, one step can be shown, at constant applied voltage, the mechanical thinning of a nanowire and electric current measurement. So, the main point here to be noted is that the number of electron wave modes supporting the electrical conductivity is becoming smaller with decreasing diameter of the wire. Only one electron wave mode is observed in electrically conducting carbon nanotubes which transport the electrical current. Electrically conducting carbon nanotubes touch the mercury surface at different times, as their length and orientation are different and this leads to the transport of electrical current. This gives two types of information i) different nanotubes resistance ii) the effect of carbon nanotubes length on the resistance.

4. Mechanical Function

Polymers filled with nanotubes lead to improvement in their mechanical properties. And this progress is purely dependent on the filler type and the way with which the filing is conducted. The larger the particle size of the filler, the poorer is the properties obtained. Polymer matrix and defoliated phyllosilicates consisting of components provide excellent mechanical properties. The mechanical property of metallic nanoparticles can be improved by mixing the nanoparticles with metals or ceramics.

5. Magnetic Function

At the nanosized level, pt and gold nanoparticles exhibit magnetic properties but as bulk they are non-magnetic. By capping, the nanoparticle surface and bulk atoms can be improvised by interaction with other chemical species. So, capping with appropriate molecules; gives the chance to modify the physical property of nanoparticles.

6. Catalysis

Catalysts based on metallic nanoparticles are-selective, highly active, exhibit a long lifetime for several kinds of reactions. So, there are two types of catalysts- Heterogeneous catalysts- which are immobilized on an inorganic support. Applications- Oxidation reactions, synthesis of H2O2, water gas shift, hydrogenation. Homogenous catalysts- metallic nanoparticles surrounded with stabilizers. Applications- Nitrile hydrogenation, olefin hydrogenation[22].

Therapeutic Applications of Metallic Nanoparticles

1. As Anti-Infective Agents

The anti-viral properties of silver nanoparticles are more effective than chemically synthesized silver nanoparticles. In one study, metallic nanoparticles have been described as an HIV preventative therapeutic. In a couple of studies, it has been shown that as virucidal agent silver acts directly on the virus by binding to the glycoprotein gp120. This binding, in turn, prevents the CD4 dependent virion binding which effectively decreases HIV- 1's infectivity and it has also been reported that metallic nanoparticles have been effective antiviral agents against herpes simplex virus, influenza, respiratory syncytial viruses.

2. As anti-Angiogenic

It is well known that angiogenesis is the development of new blood vessels and occurs during normal development and in some disease states. It plays a main role in several diseases such as cancer, rheumatoid arthritis. In normal conditions, angiogenesis is tightly regulated between various pro-angiogenic growth factors (VEGF, PDGF, and TGF-B) and anti-angiogenic factors (platelet factor 4, TSP-1). Under diseased conditions, angiogenic is turned on. Some reviews have reported that these agents have serious toxicities such as fatal hemorrhage, thrombosis, and hypertension. It may be overcome if these nanoparticles alone can be efficacious as an anti-angiogenic agent.

3. In Tumor Therapy

It has been studied that naked gold nanoparticles inhibited the activity of heparin-binding proteins such as VEGF165 and bFGF *in vitro* and VEGF-induced angiogenesis *in vivo*. Further work in this area has been reported that onto the surface of AuNPs heparin-binding proteins are absorbed and were subsequently denatured. The researchers also showed that surface size plays the main role in the therapeutic effect of AuNPs.

4. In Multiple Myeloma

Researchers (Washington university school of Medicine in St. Louis, journal Molecular cancer Therapeutics) have designed a nanoparticle-based therapy that is effective in treating mice with multiple myeloma. Multiple myeloma is cancer that affects plasma cells. Mukherjee and the group demonstrated that a gold nanoparticle inhibits the VEGF and bFGF dependent proliferation of multiple myeloma cells.

5. In Leukemia

B-chronic Lymphocytic Leukemia (CLL) is an incurable disease predominantly characterized by apoptosis resistance, by co-culture with an anti-VEGF antibody, found induction of more apoptosis in CCL B cells. In CLL therapy, gold nanoparticles were used to increase the efficacy of these agents. Gold nanoparticles were chosen based on their biocompatibility, very high surface area, surface functionalization, and ease of characterization. To the gold nanoparticles, VEGF antibodies were attached and determined their ability to kill CLL B cells.

6. In Rheumatoid Arthritis

Scientists from the University of Wollongong (Australia) have built a new class of anti-arthritic drugsthat could be used by gold nanoparticles and it has fewer side effects. Rheumatoid arthritis is an autoimmune disease that occurs when the immune system does not function properly and attacks a patient's joints. New research has shown that gold particles can invade macrophages, and stop them from producing inflammation without killing them. Journal of inorganic biochemistry has been published that reducing the size of gold into smaller nanoparticles (50nm) was able to cause more gold to immune cells with lesser toxicity.

7. In Photo Thermal Therapy

Gold nanoparticles absorb light strongly as they convert photon energy into heat quickly and efficiently. Photo-thermal therapy (PTT) is an invasive therapy in which photon energy is converted into heat to kill cancer.

8. In Radiotherapy

Tumors loaded with gold;absorb moreamountof X-rays and gold is an excellent absorber of X-rays. Thus, deposition of more beam energy results in a local dose which increases specifically to tumor cells. Gold nanoparticles have been more useful to treat cancer[23].

Potential Side Effects of Metal Nanoparticles

Argyria is one of the reported side effects in patients which results from prolonged contact with or ingestion of silver salts. Argyria is characterized by gray, black staining of the skin and mucous membrane produced by silver deposition. Silver may be deposited in the skin either from silver salts containing medications or from industrial exposure. One report was suggested where a patient ingested colloidal silver 3 times a year over 2 years resulted in diabetics, hypertension, hyperlipidemia. Mouse brains exposed to Nanosilver reported apoptosis and gene modulation. Due to inhalation, workers also had tarnished corneas and conjunctiva. Without any reported side effects, gold colloid has been used for centuries in the therapeutic field. It has also been reported that gold nanoparticle causes thrombosis, immunogenic reactions, and hemolysis. The enzyme present in saliva can transform gold (0) to gold (I), which is subsequently engulfed by immune cells.

Some side effects related to this are erythema nodosum, allergic reactions, macular and papular rash. While injected with gold complexes leads to a very low incidence of nephrotoxicity with minor proteinuria. Due to the teratogenicity of gold complexes, it is not recommended for pregnant women because it can lead to hematological disorders. In a healthy human body, gold is present in the range of 0-0.001ppm. It is found in small amounts in the skin (0.03ug/g), hair (0.3ug/g), and nails (0.17ug/g). It has been reported that Au, Ag, and Pt nanoparticles toxicity over 72-hour period using the Zebra fish model. It causes delayed hatching, crippled backbone, cardiac disorders, and platinum accumulation in the brain when they found polyvinyl alcohol capped at particles (3- 10nm). It has been reported that the neurotoxicity of Cu and Mn nanoparticles in PC-12 cell lines[24].

Metallic Nanoparticle as Drug Delivery

Most of the chemotherapeutics agents distribute to the whole-body results in toxicity and it gives poor compliance by patients, so targeted delivery of therapeutic agents to tumor cells is a challenge. By active and passive targeting, imaging of tumor cells is done by metallic nanoparticles. Both at the surface and inside cells, metallic nanoparticles can interact with biomolecules because of their small size which gives better targeting for therapeutics. Between 10-100nm of different shapes, sizes of gold, nickel, silver, iron metallic nanoparticles have been checked out as diagnostics and drug delivery systems. Gold nanoparticles utility in cancer cells and xenograft tumor mouse models were experimented with and reported the use of non-toxic PEG gold nanoparticles for tumor targeting (in vivo) which were biocompatible and were characterized by SERS (surface-enhanced Raman scattering). But the use of metallic nanoparticles for drug delivery is a concern because, after drug administration, some fraction of metallic particles can be retained in the body even though it is inert and biocompatible.

These metallic nanoparticles can be easily conjugated with various agents such as peptides, antibodies, and DNA/RNA to specifically target different cells, with polymers (polyethylene glycol) which are biocompatible to prolong their circulation *in vivo* for drug and genedelivery applications. They can also transform light into heat, thus enabling thermal ablation of targeted cancer cells. For the delivery of anticancer drugs such as Paclitaxel or cisplatin, oxaliplatin (platinum-based drugs); Au nanoparticles have been used as vehicles.

This has investigated 2nm Au nanoparticles covalently bind with the chemotherapeutic drug paclitaxel. Gold-gold sulfideNanoshells have been produced as a photothermal modulated drug delivery system. These Nanoshellsare covered by a hydrogel matrix which isthermosensitive. These Nanoshells were designed to absorb NIR light and to release multiple bursts of any soluble material held within the hydrogel matrix in response to repeated NIR irradiation[25].

SILVER NANOPARTICLES

Ag-NPs are widely known for their antimicrobial properties against microbes such as bacteria, fungi, and viruses. Due to their proven antimicrobial properties, Ag-NPs are widely used in daily used commercial products such as plastics, food packaging, soaps, pastes, food, and textiles, which has increased their market value to a great extent. Its importance can be judged from the fact that Ag- NPs can be used in various forms, such as colloidal (enamel, coating, and in paints), in liquid form (shampoo), or solid form (blending Ag-NPs with a solid material such as polymer scaffolds) and even can be found suspended in materials like soap and nonwoven fabrics. Ag-NPs importance cannot be neglected even in the textile industry, where Ag-NPs are used in the water filtration membranes. The idea behind the use of Ag-NPs in the water filtration membrane is based on the utilization of their proven antimicrobial properties and slow-release rate of Ag-NPs from the membrane. The slow-release rate prolongs the capability of the membrane to be used as a protective barrier against various bacterial and other pathogenic microbes present in the water[26].

Synthetic Routes Adapted for the Synthesis of Ag-NPs

Currently, a variety of methods, such as chemical, physical, photochemical, and biological, has been employed for the synthesis of Ag-NPs. Each method has its merits and demerits. The synthetic routes with commonly associated problems, that is, costs, stability, scalability, particle sizes, and size distribution for the Ag-NPs, have been described as follows.

1.Chemical Method. Among the existed reported methods, so far, chemical methods are preferred for the preparation of Ag-NPs due to the ease in synthesizing them in solution. Many research groups and academia are using these methods to synthesize Ag-NPs in various sizes and shapes. For example, one research group synthesized monodisperse silver nanocubes by simply reducing Ag (NO3) with ethylene g in the presence of polyvinylpyrrolidone (PVP) polymer the process was called the polyothe llycol process. In this process, it has been revealed that ethylene glycol works as both the solvent and the reducing agent. Furthermore, the size and shape of the nanocubes were dependent on the molar ratio of Ag (NO3) and PVP. Thus, by controlling the experimental Parameters, the geometry (size and shapes) of the Ag-NPs can be tailored. Round-shaped Ag-NPs with a controlled size and monodispersity were synthesized by modifying the polyol method using precursor injection. In this method, particles with 20 nm or smaller sizes were prepared. The governing factors of the precursor injection method were precursor injection rate and in situ conditions (inside the reaction mixture). The injection precursor method proved to be effective in synthesizing particles with brilliant control on the size of the monodisperse. In another reported method, monodispersed Ag-NPs were synthesized using Ag (NO3), oleylamine, and liquid paraffin. Oleylamineparaffin system was used for controlling high temperature which paid a pivotal role in determining the particle size of the synthesized Ag-NPs. Liquid paraffin was used to sustained high temperatures and secondly helped in avoiding the use of solvents that would hamper the whole synthetic process. Normally, the synthesis of Ag-NPs by chemical method banks on three factors (stages): (a) Ag precursor, (b) reducing agents, and (c) stabilizing agent. Furthermore, the synthesis and geometry of Ag-NPs rely on the nucleation and subsequent stacking of the Ag nuclei.

2. Physical Method. Along with the chemical methods used, various alternative techniques were also adopted by researchers for the synthesis of Ag-NPs. Usually in those techniques, evaporation, and condensation processes are implemented for the synthesis of Ag-NPs. Similar to the chemical methods, these methods have their own merits and demerits. One of the most common drawbacks of these methods is the higher energy requirement and time-consuming. Therefore, researchers have reported numerous alternative physical methods for the synthesis of Ag-NPs instead of implementing conventional condensation and evaporation methods. These methods not only reduced the preparation time but also are energy-friendly. For example,the thermal-decomposition method was implemented for the synthesis of Ag-NPs in solid form. In such a method, a complexation reaction between Ag and oleate at elevated temperature was carried out for the synthesis of Ag-NPs with particle size less than 10 nm. The heating system was used to evaporate the precursor used in the preparation of Ag-NPs. The technique was proposed to be an alternative for chemical techniques with the added advantage of saving time. Ag- NPs were also prepared by sputtering of metal into the reaction mixture, that is, physical deposition of Ag into glycerol.

3. Photochemical Synthesis.In the photochemical techniques mainly two different methodologies are implemented for the synthesis of Ag-NPs: (a) photophysical and
(b) Photochemical techniques.

In photochemical techniques, Ag-NPs are synthesized by photoreduction of precursor or Ag ions using photochemically activated intermediates such as radicals. In one of the methods, Ag-NPs were synthesized using UV radiation and an aqueous solution containing Triton x-100 which acts as a stabilizing agent. The surfactant used in the study helped in maintaining the stability, monodispersed, and uniform size of the synthesized Ag-NPs. In another reported method, Ag-NPs were synthesized from an aqueous solution of alkali containing AgNO3 and carboxymethylated chitosan (CMCTS) using UV radiation. CMCTS was used as a reducing agent with the added advantage of enhancing the stability of the synthesized Ag-NPs. The diameter of the synthesized Ag-NPs was less than 10 nm. The stability of synthesized Ag-NPs was more than 6 months in the alkali/CMCTS alkali solution. They used dyes as photoactive agents. The merits of photochemical techniques for the preparation of Ag-NPs were (a) high purity of the synthesized Ag-NPs and easy processing; (b) Ag-NPs were prepared by UV-radiation with the help of reducing agents; (c) a wide range of reaction medium can be used that can be glass, polymer, micelles, emulsion, and so forth.

4. Biological Synthesis.Using conventional methods for the synthesis of Ag-NPs requires (a) Ag precursors, (b) reducing agent, and (c) stabilizer/capping agent (PVP) (for avoiding agglomeration of the newly synthesized Ag-NPS). However, in biological techniques, biomolecules replaced the conventional reducing and stabilizing agents. In biological methods, Ag-NPs are synthesized using plants (such as algae, yeast, fungi, and bacteria) as reducing and stabilizing agents. The synthesized Ag-NPs size was less than 15 nm, with uniform dispersion, spherical shape, enhanced stability, and large surface area. Such methods used for the synthesis of Ag-NPs are highly economical and reproducible and consume lesser energy contrary to the conventional methods. In another study, *Trichoderma viride* fungus was used for the biosynthesis of Ag-NPs from Ag (NO3) precursor.The geometry of the biosynthesized Ag-NPs was highly variable with particles size less than 50 nm. Furthermore, stable Ag-NPs with size of less than 20nm were synthesized by using airborne bacteria (*Bacillus* sp.) using Ag (NO3) as the precursor. The biosynthesized Ag-NPs were collected from the periplasmic region (a space between the outer and inner Membrane) of the bacterial cell. Moreover, spherical Ag- NPs synthesis was reported by the reduction of

Ag (NO3) Phyllanthus extract at room temperature. The size and shape of the Ag-NPs were governed by the concentration of Phyllanthus extract[27].

Effect of Shape, Size, and Chemical Forms of Ag-NPs

Many scientists have reported on the particle size-dependent activity of Ag-NPs, although it is not a rule. Powerset al. presented size-dependent properties of PVP-capped Ag-NPs. In others studies, researchers suggested that the smaller is the size the more is the cytotoxicity. This could be attributed to the fact that small-sized Ag-NPs have easier uptake, easy dissolution, and smooth release of Ag-NPs ions along with increased surface area. Some of the studies emphasized the mass-based property of Ag-NPs without taking particle size and surface area into consideration. It has also been proposed that surface modification of Ag-NPs enhances the dissolution and stability of these nanoparticles (for a longer time). As biomolecules, coating and capping agents can affect the size, shape, and interfacial properties of the Ag-NPs. Therefore, researchers have highlighted the fact that size shape, and surface area are the governing factors in determining the extent of cytotoxicity of Ag-NPs. For this reason, numerous techniques such as dynamic light scattering (DLS), transmission electron microscope (TEM), and centrifugation have been employed to facilitate accurate measuring of the size and dispersion of these nanoparticles[28].

Application of Silver Nanoparticles

Silver nanoparticles have been used extensively in anti-bacterial agents in the health industry, food storage, textile coating, and number of environmental applications. Important to note, despite decades of use, the evidence of the toxicity of silver is not still clear. The antibacterial property of silver nanoparticles has allowed its wide range of applications from disinfecting devices and home appliances to water treatment. The size and geometry of Ag-NPs are dependent on the synthetic route adopted for its synthesis; however, it can be found in spherical, rod, and triangular shape, or coated with a polymer, biomolecules, and sugars. Ag-NPs have numerous chemical, physical and biological functions which are explained point by point.

1. Textiles. Fabrication of functionalized fabrics with Ag- NPS has a fair share in the functionalized fabricated materials. Various techniques are adopted for the fabrication of fabric functionalized with Ag-NPs; for example, the most famous and talked about techniques used for the fabrication of silver containing fabrics are by blending Ag-NPs with the fabricated material so that the Ag-NPs are embedded inside the functionalized fabrics or secondly by the surface immobilization of fabrics (to be functionalized) with Ag-NPs. However, along with the merits, such fabrics do have demerits such as the release of Ag ions during washing which is considered to be the prime concern regarding the durability of such Ag-NPs functionalized materials.

2. Food Packaging. As already discussed, the merits of Ag- NPs functionalized fabrics likewise Ag-NPs have also made their mark in the food packaging industry and are known to be one of the important components in the packaging materials used for preservation of food for a longer duration. For example, materials whose surfaces are coated with Ag-NPs can be useful in preventing the preserved food from contamination (preventing contamination caused by microbes) due to slow release of Ag-NPs from the coated surface along with preventing the growth of the microbes on the surface of the packaging material (effect of Ag-NPs against microbes will be discussed in detail in the latter part of this review). Researchers working in the field of food preservation have reported sonochemical coating, a simple and versatile technique used for the preparation of coating materials from the colloidal solution containing Ag-NPs using ultrasonication. The coating proved to be effective against various strains of bacteria (gram-negative *E. coli* and gram-positive *S. aureus* bacteria). The method proved to be a step forward towards the synthesis of materials with the help of which food can be preserved for a longer duration. Cushen reported that Ag-NPs have significance in research relating food preservation and packaging industry[29].

3. Plastic Coatings.The importance of Ag-NPs in the preparation of medical devices can be judged from the fact that a wide range of medical devices is prepared from it due to its potency against various strained of bacteria (gram-positive and gram-negative). Catheters are usually prepared from materials that contain Ag-NPs to avoid infection and contamination. These catheters are useful in sustaining and targetingthe release of Ag ions from the Ag-NPs which ultimately prevents microbial activity.

4. Nanoprism Preparation. Recently scientist has diverted their attention towards the synthesis and optical activity of Ag-NPs nanoprisms. Ag-NPs havea unique optical activity which results in surface-plasmon resonance (SPR) peaks at relatively long wavelengths. Focusing on silver, various physical and chemical processes have been adopted for the fabrication of nano prisms. One of the processes for making Ag-NPs nanoprism is a lithographic technique (nanosphere lithography (NSL)). In this technique, Ag-NPs are synthesized and are kept on solid substrates that are capable of producing nanoprisms with control over their geometry. The gold coating provided stability to Ag-NPs nano prisms against etching, the nano prisms had clean surfaces, and the purity endows those gold-coated Ag-NPs nano prisms to be used in fields of biosensing and bioimaging[30].

Role of Ag-NPs in Environment

Silver nanoparticles (Ag-NPs) are world widely famous for their versatility which they exhibit in terms of applications such as electrical, thermal, and antimicrobial properties. In the past, people used Ag for ornamental, medicinal, crockery, clothing, building materials, and coins making and as a disinfectant.

1. Water.Ag-NPs are widely known for their potent properties against pathogens, preventing infections, and antimicrobial activity. With durability and their antimicrobial activity, Ag- NPs are considered to be a useful tool against infectious diseases caused by the microbes present in the environment. Therefore, its high potency against various infectious microbes can help in avoiding and curing microbial infections. Recently researchers reported the disinfectant ability of colloidal Ag-NPs for the treatment of gastrointestinal bacterial infections. One of the other reported studies proposed that core-shell magnet nanoparticles comprised of Ag-NPs are effective disinfectants in water purification systems. These core-shell magnetic nanoparticles exhibited excellent antimicrobial and ant pathogenic properties. Most importantly these core/shell magnets can be successfully recovered from the system with the help of an external magnet field.

2.Air. There is concern over the impact of Ag-NPs on mankind and animals, as several reported studies revealed that Ag-NPs have great penetration ability in different environments. Humans and animals are exposed to these Ag-NPs via breathing, ingestion, through skin, and so forth. Therefore, concerns arise regarding the impact of Ag-NPs on human and animal's health. Due to the small size, these nanoparticles can easily penetrate through different organs and ultimately reach the cytoplasm of the cell. With different exposure time to the Ag-NPs, the rats did not exhibit any abnormal behavior and no change in terms of weight was observed because of inhalation of Ag-NPs. However, some scientists have reported that the lungs are the primary target thatis affected by prolonged exposure to Ag-NPs.

3.Release of Ag-NPS from Functionalized Materials. Consumer product comprised of any form of silver (Ag) is the main cause of silver released into the environment. Several articles have been published concerning the release of silver from a wide range of materials that use silver nanoparticles (Ag-NPs) in their products such as functionalized fabrics paints. Usually inductively coupled plasma (ICP), energy dispersive spectroscopy (EDS), and transmission electron microscopy (TEM) is used for the detection of released silver from the materials to be investigated. The authors revealed that the most important factor for Ag release from the Ag- NPs functionalized fabrics is the processes used for their incorporation into the fabrics during fabrication [31].

Mechanism of Action of Ag-NPs against Microbes

Silver (Ag) and especially silver nanoparticles (Ag-NPs) antimicrobial activity, irrespective of the strains of bacteria whether they are antibiotically resistant or not, is famous around the globe in the scientific community. The confirmed mechanism is yet to be discovered but can be related to the mechanism of silver (Ag) ions action on bacteria strains such as trypanosomes and yeasts, where accumulation of Ag- NPs occurs from the aqueous solution that ultimately causes saturation of enzymes and protein in the cell. In a published report the authors proposed the mechanism which states that the changes caused by the Ag-NPs in the cell wall and nuclear along with DNA and RNA are the main cause of retarding the bacterial cell growth. Mechanisms that can cause bacterial cells death using Ag-NPs particle.

(1) The first proposed mechanism states that the bacterial cell growth and proliferation are inhibited by the adhesion of Ag-NPs onto the cell wall (due to the fine particles size) of the bacteria thus causing changes in the cell wall in which intern is unable to protect the internal part of the cell.

(2) In the second proposed mechanism the authors stated the penetration of the Ag-NPs into the bacterial cell causing changes in the DNA retarding its normal function thus ultimately causing its death. Silver nanoparticles penetrate through bacterial cell wall, resulting in DNA damage.

(3) In the third proposed mechanism they stated that when Ag^+ ions interact with the proteins containing Sulphur present in the cell wall of the bacteria this ultimately leads to the malfunctioning of the bacterial cell wall. This process is assumed to be the main mechanism in explaining the antibacterial activity[32].

Ag-NPs Role in Medicine

1. Antibacterial Properties.The Ag-NPs are famous for its potent antibacterial activity against various strains of bacteria including highly pathogenic bacteria species (gram positive and gram-negative bacteria). The *E. coli* bacterial strains were used as representative species for gram negative bacteria. At optimized experimental parameters, they found out that Ag-NPs were adhered to the cell wall of the gram-negative bacteria (*E.coli*) that caused the destruction of the bacterial cell.

In another reported study scientists conducted experiments on the size related properties of the Ag-NPs on different species of gram-negative bacterial strains. The results obtained from their study suggested that size of Ag-NPs is an important factor in preventing the bacterial cells from their normal functions. Furthermore, in another published study, researchers conducted experiments for elucidating the dose dependent properties of Ag-NPs on gram negative and gram-positive bacteria; the authors reported that gram-negative bacteria (*E. coli*) can be inhibited at relatively low concentration as compared to the gram-positive bacteria (*S. aureus*).

2. Antifungal.Fungi are considered to play a vital role in causing fungal infections, especially in hospitals. Along with the antibacterial activities of the Ag-NPs numerous studies had been reported on the antifungal activities of Ag- NPs, which reveals that Ag-NPs could be used as effective antifungal agent because Ag-NPs exhibit excellent antifungal properties against various species of fungi. The possible mechanism for the antifungal activity of Ag-NPs, which states that Ag-NPs cause abnormalities in the cell wall of the fungi which results in the abnormal functions (retarding the normal budding process) of the fungal cells. In another published paper researchers found out that catheters coated with Ag-NPs can result in complete inhibition of fungi (*C. albicans*). Another reported work states that Ag-NPs have exhibited antifungal activities against different strains of fungi such as *C. albicans* and *C. glabrata Trichophytonrubrum (T. rubrum)* but the activity is dose dependent. In short due to the reported literature available on the antifungal activity of Ag-NPs, it can be concluded that silver nanoparticles can be used as antifungal agent against various strains (species) of fungi and can be helpful in overcoming various fungal infections caused by fungi.

3. Antiviral Agent.Recently, the increase in infectious diseases caused by virus such as SARS-Cov, influenza A/H5N1, influenza A/H1N1, Dengue virus, HIV, HBV, and new encephalitis viruses, is of prime concern. These infections can create havoc in no time because of the rapid proliferation (glimpses of destruction caused by these viral infections have been observed in some of the countries and the most dangerous of these viral infections were bird flu, swine flu, and dengue), ultimately resulting in causing severe damage to health and wealth of human beings. Ag-NPs are famous for their antimicrobial activities; therefore, researchers have diverted their attention and started evaluating the importance of Ag-NPs in controlling infectious diseases caused by pathogens and viruses. However, the number of reported works using Ag-NPs for controlling viral infections is very low but still it can pave the way for other researchers to show their interest in dealing against viral infections using nanoparticle specifically Ag-NPs.

4.Wound Dressing:Wound dressings (bandages) functionalized with silver nanoparticles (Ag-NPs) are commercially available and are frequently used for medicinal purpose for curing various infections such as burn wounds, toxic epidermal necrolysis, Steven-Johnson syndrome, chronic ulcers, and pemphigus. In typical bandages (wound dressings) Ag-NPs are coated on the surface of polyethylene layer. Experiments have proven the superior wound healing properties of the Ag-NPs coated bandages (wound dressing) as compared to the wound dressing without Ag-NPs. Such wound dressing has the ability to prevent infections along with minimizing the healing time without any side effect. In an article published on Ag-NPs, researchers concluded that bandages (wound dressing) containing Ag-NPs minimize the healing time of burns wounds[33].

Future Prospects

The use of nanoparticles already established for some medical applications like wound infections, dressing, and treatment of preclinical stages. Recent research has revealed exciting new biological properties of NS that could be translated into new therapeutic and pharmacological treatments. The full potential of this technology has yet to be discovered. The antibacterial, antifungal and antiviral properties of silver ions, silver compounds and silver nanoparticles have been extensively studied. Silver is also found to be non-toxic to humans in minute concentrations[34].

SILVER NANOPARTICLES FROM *WITHANIA SOMNIFERA* ROOT EXTRACT BY GREEN SYNTHESIS

WITHANIA SOMNIFERA **PLANT**

Withania Somnifera, also known as Ashwagandha, Indian ginseng, Winter cherry, is a plant in genus *Withania* is one of the genera found in the family Solanaceae or nightshade family. It grows as a short shrub (35-75 cm) with a central stem from which branch extend radially in a star pattern (stellate) and covered with a dense matte of wooly hairs (tomentose).

It has been an important herb in the Ayurvedic and indigenous medical systems for over 3000 years. The flowers are small and green, while the ripe fruit is orange-red and has milk-coagulating properties. The plant also has long brown tuberous roots that are used for medicinal purposes. It is cultivated in many of the drier regions of India such as Manasa, Neemuch, and Jawad tehsils of the Mandsaur District of Madhya Pradesh, Punjab, Sind and Rajasthan. In Ayurveda, the roots of *W. somnifera* are used to prepare many herbal medicines. It is claimed to possess aphrodisiac, sedative, rejuvenative and life prolonging properties. It is traditionally used to treat the symptoms and conditions associated with chronic fatigue, dehydration, bone weakness, muscle weakness and tension, loose teeth, thirst, impotency, premature ageing, emaciation, debility, constipation, senility, rheumatism, nervous exhaustion, memory loss, neurodegenerative disorders, spermatorrhoea[35].

The roots of the plant are categorized as rasayanas, which are reputed to promote health and longevity by augmenting defense against disease, arresting the ageing process, revitalizing the body in debilitated conditions, increasing the capability of the individual to resist adverse environmental factors and by creating a sense of mental wellbeing.

It is in use for a very long time for all age groups and both sexes and even during pregnancy without any side effects. Clinical trials and animal research support the use of *Withania Somnifera* for anxiety, cognitive and neurological disorders, inflammation, hyperlipidemia and Parkinson’s disease. *The* chemo preventive properties make it a potentially useful adjunct forthe patients undergoing radiation and chemotherapy treatments. Recently, *Withania. Somnifera* is also used to inhibit the development of tolerance and dependence on chronic use of various psychotropic drugs.

Various research has been conducted on the genus to study different biological properties, especially on *W. somnifera* that has been under the study since the 1980s. Historically, the plant has been used as an antioxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory agent, astringent and more recently to treat ulcers, bacterial infection, venom toxins and senile dementia. Recently, more research has been conducted on other species such as *W. adpressa, W. begoniifolia* and *W. qaraitica*. However, no profound research on other species has been found. This review will primarily comprise all the traditional uses, phytoconstituents, and biological activities of *Withania* genus, aiming to improve new researches on various other species[36].

Table no 1: Parts and uses of plant *Withania Somnifera*

PLANT	PART OF THE PLANT	USES
W. Somnifera	Leaf	Reduce stress, fertility treatment, sign of aging, anti-inflammatory
	Gum	Anemia, asthma, dental decay
	Seeds	Antianxiety, depression, joint pains, GERD
	Oil	Insomnia, antifungal, acute rheumatism, swelling
	Flowers	Tumor, inflammation, hysteria, cholesterol - lowering, cytotoxic
	Roots	Diabetes, hypertension, stress, arthritic diseases, and cancer
	Barks	Aphrodisiac, liver tonic, ant inflammatory agent, astringent, Aiding digestion, stomach pain,

		poor vision, ulcer, hypertension, joint pain, anemia, diabetes
	Leaves	Diabetes, hypertension, parasitic diseases, cuts, contraceptive remediesulcers, painful swelling, external pains,

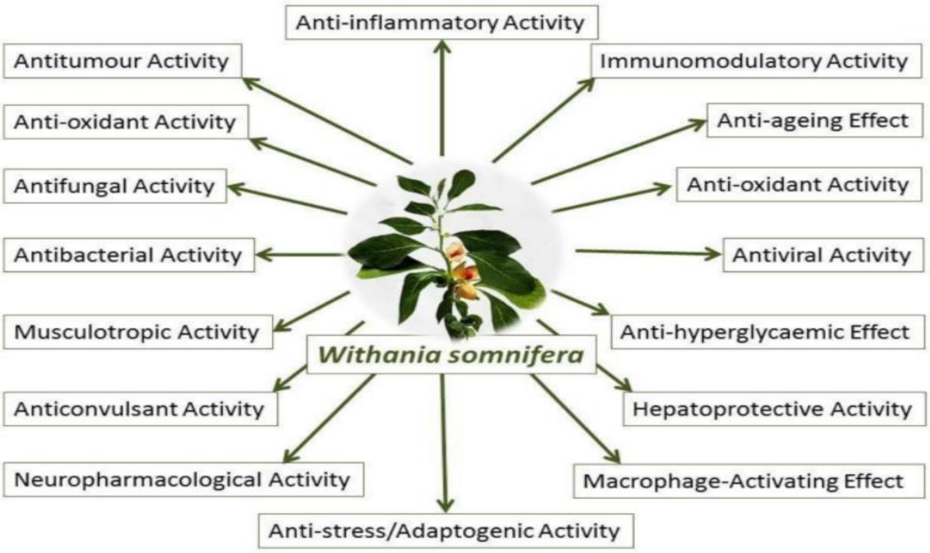


Figure no4: shows the uses of *Withania Somnifera*

PHYTOCONSTITUENTS

The roots of *Withania Somnifera* consist primarily of compounds known as withanolides, which are believed to account for its extraordinary medicinal properties. Withanolides are steroidal and bear a resemblance, both in their action and appearance, to the active constituents of Asian ginseng (Panax ginseng) known as ginsenosides. Ashwagandha's withanolides have been researched in a variety of animal studies examining their effect on numerous conditions, including immune function and even cancer. Much of Ashwagandha's pharmacological activity has been attributed to two main withanolides, withaferin A, D and withanolide. Withanolide A The withanolides serve as important hormone precursors that can convert into human physiologic hormones as needed. Regardless of the high phytochemical contents of the genus, the constituents of only specific species had been explored, namely *W. adpressa, W. begoniifolia, W. qaraitica* and *W. somnifera,* most of the studies are focused on the roots of the plants[37].

BIOLOGICAL ACTIVITIES

Antioxidant

The brain and nervous system are relatively more vulnerable to free radical damage than other tissues because they are rich in lipids and iron, both known to be important in generating reactive oxygen species. Free radical damage of nerve tissue may be involved in normal aging and neurodegenerative diseases, e.g., epilepsy, schizophrenia, Parkinson's, Alzheimer's, and other diseases. The active principles of WS, sitoindosides VII-X and withaferin A (glycowithanolides), have been tested for antioxidant activity using the key free-radical scavenging enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) levels in the rat brain frontal cortex and striatum.

Decreased activity of these enzymes leads to accumulation of toxic oxidative free radicals and resulting degenerative effects. An increase in these enzymes would represent increased antioxidant activity and a protective effect on neuronal tissue. Active glycowithanolides of WS were given once daily for 21 days, dose-related increased in all enzymes were observed; the increases comparable to those seen with deprenyl (a known antioxidant) administration. This implies that WS does have an antioxidant effect in the brain, which may be responsible for its diverse pharmacological properties.

Anxiety and depression

Anxiolytic and antidepressant actions of the bioactive WSG, isolated from WS roots, in rats were assessed. WSG was administered orally once daily for 5 days and the results were compared by those elicited by the benzodiazepine lorazepam for anxiolytic activity, and by the tricyclic antidepressant, imipramine. WSG induced an anxiolytic effect was comparable to lorazepam, in the elevated plus-maze, social interaction and feeding latency in an unfamiliar environment, tests. WSG also exhibited an antidepressant effect, comparable with that induced by imipramine, in the forced swim-induced 'behavioral despair' and 'learned helplessness' tests. The investigations supported the use of WS as a mood stabilizer in clinical conditions of anxiety and depression in Ayurveda.

Anticancer

The chemo-preventive effect was demonstrated in a study of WS root extract on induced skin cancer in mice given WS before and during exposure to the skin cancer-causing agent 7,12-dimethylbenz[a]anthracene. A significant decrease in incidence and an average number of skin lesions was demonstrated compared to the control group. Additionally, levels of reduced glutathione, SOD, CAT, and GPX in the exposed tissue returned to near normal values following administration of the extract. The chemo preventive activity is thought to be due in part to the antioxidant/ free radical scavenging activity of the extract at the G2/M phase.

Hypothyroidism

Animal studies reveal ashwagandha has a thyrotropic effect. No changes in T3 levels were observed. *Withania* may also stimulate thyroid activity indirectly, via its effect on cellular antioxidant systems. *Withania* extract significantly decreased lipid peroxidation in the liver homogenate and significantly increased catalase activity, promoting scavenging of free radicals that can cause cellular damage. These results indicate ashwagandha may be a useful botanical in treating hypothyroidism. An aqueous extract of dried *Withania* root was given to mice via gastric intubation at a dose of 1.4 g/kg body weight daily for 20 days. Serum was collected at the end of the 20- day period and analyzed for T3 and T4 concentrations and lipid peroxidation was measured in liver homogenate via antioxidant enzyme activity. Significant increases in serum T4 were observed, indicating the plant has a stimulatory effect at the glandular level[38].

Antimicrobial

Various research on antimicrobial activity has been conducted on *Withania* species. Summarizes the antimicrobial activity of distinct species. Both aqueous as well as alcoholic extracts of the plant (root as well as leaves) were found to possess strong antibacterial activity against a wide range of bacteria, as revealed by in vitro Agar Well Diffusion Method. The methanolic extract was further sub-fractionated using various solvents and the butanolic sub-fraction was found to possess maximum inhibitory activity against a spectrum of bacteria including *Salmonella typhimurium*.

Recent advances in nanoscience and nanotechnology radically changed the way we diagnose, treat, and prevent various diseases in human life. Hence, nanoparticle synthesis from plant extracts tentatively offers a route for large scale production of commercially attractive nanoparticles. Silver nanoparticles (AgNPs) are one among the foremost vital and interesting nanomaterials among several metallic nanoparticles that are involved in biomedical applications. Silver nanoparticles play an important role in nanoscience and nanotechnology, particularly in nanomedicine. Although several noble metals have been used for various purposes. *Withania* is one such genus belonging to the family of Solanaceae, a monotypic family of single genera with around 23 species. Most of these species have not been explored fully despite the enormous bioactivity reports concerning various potentials such as: cardiac and circulatory stimulants; anti-tumor; antipyretic; antiepileptic; anti-inflammatory; antiulcer; antispasmodic; diuretic antihypertensive; cholesterol lowering; antioxidant; antidiabetic; hepato protective; antibacterial and antifungal activities.

The antimicrobial properties of silver are known for thousands of years. It has been castoff to treat a wide variety of infections. But it was found that high doses of silver when administered intravenously could cause convulsions and even death. Thanks to their small size and hence large surface area, they can undergo more efficient binding with the microorganism. Considering the possible application of silver nanoparticles in various biomedical fields, the antimicrobial activity of the synthesized silver nanoparticles has been evaluated by well diffusion method. The clinical isolates of human pathogenic bacterial strains (including both Gram positive and Gram negative). The synergetic effect of silver nanoparticles with *Withania somnifera* has also been studied by combining them with antibiotics against Gram positive and Gram-negative bacteria. Anti-inflammatory activity of the synthesized silver nanoparticles was also evaluated with *Withania Somnifera* root extract and standard drug[39].

LITERATURE REVIEW

2. REVIEW OF LITERATURES

LITERATURES ON GREEN SYNTHESIS OF METAL NANOPARTICLES

Kamyar Shameli., *et al.* (2013) reported the synthesis and characterization of polyethylene glycol mediated Silver Nanoparticles by the Green Method. In summary, we have described a simple and green method to synthesize colloidal silver nanoparticles by using green reducing agents which requires no special physical conditions. The FTIR spectrum suggested the complexation present between PEG and silver nanoparticles, enabling the formation of metallopolymer Ag [PEG] and the stability of the silver nanoparticles was confirmed with zeta potential measurements [40].

ProtimaRauwel. *et al.* (2015) described on the Green Synthesis of Silver Nanoparticles and their Morphologies Studied via TEM (Transmission electron microscopy). During the last decades, many efforts were put into the development of new green synthesis methods. Living organisms have huge potential for production of nanomaterials that can be applied to many fields and more specifically to biomedicine. However, the low synthesis rate and the limited number of size and shape distributions available oriented the investigations to the use of fungi and algae [41].

Youmie Park., *et al.* (2015) studied on a New Paradigm Shift for the Green Synthesis of Antibacterial Silver Nanoparticles Utilizing Plant Extracts. General information regarding the green synthesis of antibacterial silver nanoparticles. The most obvious merits of green synthesis are the increased biocompatibility of the resulting silver nanoparticles and the ease with which the reaction can be carried out. This review summarizes some of the plant extracts that are used to produce antibacterial silver nanoparticles [42].

Thangavel Rajagopal., *et al.* (2015) investigated on Synthesis of silver nanoparticles using Catharanthus roseus root extract and its larvicidal effects. Photosynthesis of silver nanoparticles has attracted considerable attention due to their biocompatibility, low toxicity, cost-effectiveness and being a novel method has an eco-friendly approach. The structure and proportion of the synthesized nanoparticles was defined by exploitation ultraviolet spectrophotometry, X-ray diffraction, Fourier transform infrared spectroscopy, energy dispersive X-ray spectroscopy and scanning electron microscopy methods. Reduction of silver ions occurred when silver nitrate solution was treated with aqueous root extract at 60°C. Synthesized silver nanoparticles (AgNPs) were confirmed by analyzing the excitation of surface Plasmon resonance (SPR) using UV-vis spectrophotometer at 423nm [43].

Subha V., *et al.* (2015) concludedan eco-friendly approach for synthesis of silver nanoparticles using ipomoea pes-caprae root extract and their antimicrobial properties. In this investigation for the first-time roots extract of I. pes-caprae were used to synthesize the silver nanoparticles successfully using the green method. From the root of I. pes-caprae can be used to the synthesis the silver nanoparticles. The preparation time of silver nanoparticles was reduced to 3 hrs. From that of previously established work. Silver nanoparticles (AgNPs) were obtained through green synthesis using Ipomoea pes-caprae root extract for the first time. The active biomolecules present in the roots of I. pes-caprae, are ergoline alkaloids, indolizidine alkaloids, benzenoids and phenolic compounds act as both the stabilization and reduction of silver nanoparticle [44].

AdnanHaider. *et al.* (2015) developed Preparation of Silver Nanoparticles and Their Industrial and Biomedical Applications Silver nanoparticles (Ag-NPs) have diverted the attention of the scientific community and industrialist itself due to their wide range of applications in industry for the preparation of consumer products and highly accepted application in biomedical fields (especially their efficacy against microbes, anti- inflammatory effects, and wound healing ability) [45].

V. kathiravan., et al. (2015) investigated on green Synthesis of Silver Nanoparticle Using Plant Root Extract of Crotonsparsiflorus and their Antimicrobial Activity. The bio-reduction of aqueous Ag+ ions by the root extract of the C. Sparsiflorus plant has been demonstrated. The reduction of the metal ions through leaf extracts leading to the formation of silver nanoparticles of fairly well-defined dimensions. Reduction of silver ion into silver particles during exposure to the plant extracts could be followed by color change. Silver nanoparticle exhibit dark yellowish-brown color in aqueous solution due to the surface Plasmon resonance phenomenon [46].

Govindaswamy Rajkumar *et al.* (2016) remarked the review clearly revealed on green synthesis and characterization of zinc nanoparticle Using Andrographis paniculata leaf extract. The synthesis of Zinc Nanoparticles and it is also known to have the ability to inhibit the growth of various pathogenic microorganisms. The synthesized Zinc Nanoparticles can be used for various applications due to its eco – friendly, non-toxic and compatibility for pharmaceutical and other applications [47].

K. Elumalai., *et al.* (2016) described on preparation and characterization of zno nanoparticles using moringa oleifera extract by green synthesis method. In this communication, preparation of Zinc Oxide nanoparticles using Moringa Oleifera aqueous extract from leaf, flower and bark by green synthesis sol-gel method is done. Zinc Oxide Nanoparticles are analyzed by X-ray diffraction (XRD), Scanning Electron Microscope (SEM), UV-Visible and Fourier Transform Infrared spectroscopy (FTIR). From UV-Visible spectroscopy, higher band gap energy of 4.3eV is obtained in the near visible region at the wavelength of 286.5 nm [48].

Jagpreet Singh., *et al.* (2016) investigated on green synthesis and characterization of silver nanoparticles and their applications: a green nano world. Lant extract mediated biological synthesis of nanoparticles is known as Green Synthesis or Green Nanotechnology and these nanoparticles are known as biogenic nanoparticles. Green synthesis provides benefits over chemical and physical method as it is cost effective, ecofriendly, more stable and there is no need any high energy, pressure, temperature and toxic chemicals [49].

Henry F. Aritonang., *et al,* (2019) conducted the study to synthesis silver nanoparticles using aqueous extract of medicinal plants' (Impatiens balsamina and Lantana camara) Fresh Leaves and Analysis of Antimicrobial Activity. Medicinal plants namely aqueous extracts of fresh leaves of I. balsamina and L. camara, can be used as bio reduction agents to produce Ag nanoparticles. The formation of Ag nanoparticles in the extract was observed by the color change of I. balsamina extract into brownish yellow while of L. camara extract into grayish brown. Color changes that occur indicate that Ag particles have formed. The Ag particles produced had an increased size due to the increased concentrationofAgNO3 solution, but the average size is still in nanometer. Ag nanoparticles contained in the extract were able to inhibit the growth of S. aureus and E. coli bacteria, and the best antibacterial activity was exhibited by the L. camara extract containing Ag nanoparticles [50].

N. A. Tamilselvi., *et al.* (2019) studied on health benefits, therapeutic and pharmacological properties of Moringa. Moringa yields at least four different edible parts viz., pods, leaves, flowers and seeds. Moringa leaves are the excellent source of protein, β-carotene, vitamins, A, B, C and E, riboflavin, nicotinic acid, folic acid, pyridoxine, amino acids, minerals and various phenolic compounds, phytochemicals and omega 3 and 6 fatty acids. The leaves of moringa are rich in palmitic and linolenic acids whereas the seeds are predominated by oleic acid. Perhaps using the multi-mix approach of food product development more food products could be developed especially for programs on malnutrition [51].

Harish Kumar K., *et al,* (2018) described on metallic nano particle and investigated the existence of metallic nano particles in solution. In 1908, Mie gave a quantitative explanation of their color. This review summarizes the properties, advantages, disadvantages and characteristics of metal nanomaterials. This review also highlights on how metallic nanomaterial's work as a catalyst and why is it necessary for stabilization. It provides the readers, detailed information on the synthesis by various methods, characterization, with particular focus on therapeutic application along with potential side effects and their future perspectives [52].

Summon Das., *et al.* (2018) investigated on green synthesis of silver nanoparticle and zinc oxide nanoparticles from different plant extract and their antimicrobial activity against multi drug resistant bacteria. The metallic nanoparticle like Ag and ZnO can be produced by green synthesis method and synthesized nanoparticle is applied to prevent MDR organism [53].

Jagpreet Singh., *et al.* (2018) investigated on 'Green' synthesis of metals and their oxide nanoparticles: applications for environmental remediation in materials science, "green" synthesis has gained extensive attention as a reliable, sustainable, and eco-friendly protocol for synthesizing a wide range of materials/nanomaterials including metal/metal oxides nanomaterials, hybrid materials, and bioinspired materials. Finally, we covered applications of such synthesized products to environmental remediation in terms of antimicrobial activity, catalytic activity, removal of pollutants dyes, and heavy metal ion sensing [54].

Mukti Sharma, *et al.* (2018). The present communication warrants the presence of significant wound healing bio-efficacy of aq. alc. extract of the seed (49.78%) of the plant *Madhuca longifolia*. In vivo wound healing parameters (wound area, wound closure, epithelization period, skin breaking strength and hydroxyproline content) have been examined in *Swiss albino* mice models. The bio-fabricated ($M_lf@AgNp_s$) nano-biomaterials using the plant *M. longifolia* have lustrous prospects for the development of complimentary herbal nanomedicine scaling-up the wound healing bio-efficacy [55].

R. Vijayaraj., *et al.* (2018) Conducted the study on *in vitro* anti-inflammatory activity of silver nanoparticle synthesized Avicennia marina (Forssk.) Vierh. A green synthetic approach. In the present study, 15 phytochemical constituents have been identified from ethanol leaf extract of A. marina by GCMS analysis. The phytoconstituent analysis revealed presence of alkaloids, flavonoids, saponin, and phytosterols. Hence, this study gives an idea that the compound of A. marina can be used as a lead compound for designing a potent anti-inflammatory drug which can be used to cure inflammation [56].

Lekshmanan G., *et al.* (2018) described on plant mediated synthesis of silver nanoparticles using fruit extract of *cleome viscosa* assessment of their anti-bacterial and anti-cancer activity.it has vast potential and effects in future world [57].

Mohana Sriramulu., *et al.* (2017) study was conducted and concluded on photo catalytic, antioxidant, antibacterial and anti-inflammatory activity of silver nanoparticles synthesized using forest and edible mushroom. Silver nanoparticles are successfully synthesized by cost effective, eco-friendly method using Ganoderma lucidum and Agaricus bisporus aqueous extracts. The zone of inhibition proves silver nanoparticles has good antibacterial activity against E. coli and S. aureus. 97% direct blue 71 dye degradation was achieved within 60 min using FRT synthesized silver nanoparticles. Antimicrobial activity and photo catalytic activity determine that silver nanoparticles have outstanding applications in waste water treatment [58].

Nivedita patel., *et al.* (2014) described onphytochemical analysis and antibacterial activity of moringa Oleifera. The aim of the present study was to find out antibacterial property of Moringa oleifera, family Moringaceae. Moringa oleifera is a very useful tree in tropical countries. In ayurvedic all parts of the tree are used in different healing procedures for different diseases. The ethanolic and aqueous extract were active against all strains but the ethanol leaf extract showed maximum activity against Streptococcus mutant and aqueous extract shows maximum activity against Proteus vulgaris [59].

Muhammad Rafique., *et al.* (2017) described A review on green synthesis of silver nanoparticles and their applications. Development of reliable and eco-accommodating methods for the synthesis of nanoparticles is a vital step in the field of nanotechnology. Silver nanoparticles are important because of their exceptional chemical, physical, and biological properties, and hence applications. In the last decade, numerous efforts were made to develop green methods of synthesis to avoid the hazardous byproducts. This review describes the methods of green synthesis for Ag-NPs and their numerous applications. It also describes the comparison of efficient synthesis methods via green routes over physical and chemical methods, which provide strong evidence for the selection of suitable method for the synthesis of Ag-NPs [60].

Anandalakshmi K., *et al.* (2017) described on Green Synthesis and Characterization of Silver Nanoparticles Using Vitex negundo (Karu Nochchi) Leaf Extract and its Antibacterial Activity.AgNPs using Vitex negundo leaf extract with different concentrations (1-5 mL) were synthesized by green synthesis method. The formation of Ag NPs was confirmed by UV-visible absorption spectroscopic analysis. Zeta potential measurement of the synthesized Ag NPs shows incipient instability. The synthesized Ag NPs exhibits the best antibacterial activity on gram-positive and gram-negative bacteria [61].

S. Saranya., *et al.* (2017) investigated on Plant extracts from Musa ornate and Zea mays were used for the green synthesis of Copper (Cu) and Zinc oxide (ZnO) nanoparticles (NPs) from copper chloride and zinc sulphate solution respectively. Green synthesized metallic nanoparticles were characterized by UV– visible spectrophotometer, X-ray diffractometer (XRD), Transmission Electron Microscope (TEM), Atomic Force Microscope (AFM) and Zeta potential particle size analyzer. Antimicrobial activities of the metallic nanoparticles were performed by well diffusion method against Escherichia coli, Staphylococcus aureus, Streptococcus agalactiae and Salmonella enterica [62].

Anu Mary Ealias., *et al.* (2017) described on the classification, characterization, synthesis of nanoparticles and their application. As per ISO and ASTM standards, nanoparticles are particles of sizes ranging from 1 to 100nm with one or more dimensions. The nanoparticles are generally classified into the organic, inorganic and carbon-based particles in nanometric scale that has improved properties compared to larger sizes of respective materials. The nanoparticles show enhanced properties such as high reactivity, strength, surface area, sensitivity, stability, etc. because of their small size. The nanoparticles are synthesized by various methods for research and commercial uses that are classified into three main types namely physical, chemical and mechanical processes that has seen a vast improvement over time [63].

PLANT PROFILE

3. PLANT PROFILE

WITHANIA SOMNIFERA[23,24]

Taxonomic Classification

Kingdom	- Plantae
Subkingdom	- Tracheobionta, vascular plants;
Super Division	- Spermatophyta, seeds plants;
Division	- Angiospermae
Class	- Dicotyledons
Subclass	- Asteridae
Order	- Tubiflorae
Family	- Solanaceae
Genus	-*Withania*
Species	- Somnifera Dunal

Botanical Description

Synonyms Latin - physalias somnifera L, *Withania* kansensis Kuang

Sanskrit - Ashwagandha, Turangi-gandha

Hindi - Punir, Asgandh

Tamil - Amukkura, Amkulang, Ashwagandhi

Malayalam - Amukkuram

Telugu - Pulivendram, Panneru

Ayurvedic - Akshiva, Haritashaaka, Raktaka, Tikshnagandhaa

English - Winter cherry

Geographical Source

This species is a short, tender perennial shrub growing 35–75 cm (14–30 in) tall. Tomentose branches extend radially from a central stem. Leaves are dull green, elliptic, usually up to 10–12 cm (3.9–4.7 in) long. The flowers are small, green, and bell-shaped. The ripe fruit is orange-red. The species name *"somnifera"* means "sleep-inducing" in Latin The name "ashwagandha" is a combination of the Sanskrit words *'ashva'*, meaning horse, and *'gandha'*, meaning smell, reflecting that the root has a strong horse-like odor. *Withania Somnifera* is cultivated in many of the drier regions of India. It is also found in Nepal, Sri Lanka, China, and Yemen. It prefers dry stony soil with the sun to partial shade. To propagate it can be grown from seed in the early spring, or greenwood cuttings in the later spring.

Morphology

Withania Somnifera is a small, woody shrub in the Solanaceae family that grows about two feet in height. It can be found growing in Africa, the Mediterranean, and India. An erect, evergreen, tomentose shrub, 30-150 cm high, is found throughout the drier parts of India in waste places and on bunds. Roots are stout fleshy, whitish brown; leaves simple ovate, glabrous, those in the floral region smaller and opposite; flowers inconspicuous, greenish or lurid-yellow, in axillary, umbellate cymes; berries small, globose, orange-red when mature, enclosed in the persistent calyx; seeds yellow, reniform. The roots are the main portions of the plant used therapeutically. The bright red fruit is harvested in the late fall and seeds are dried for planting in the following spring.

Traditional Uses

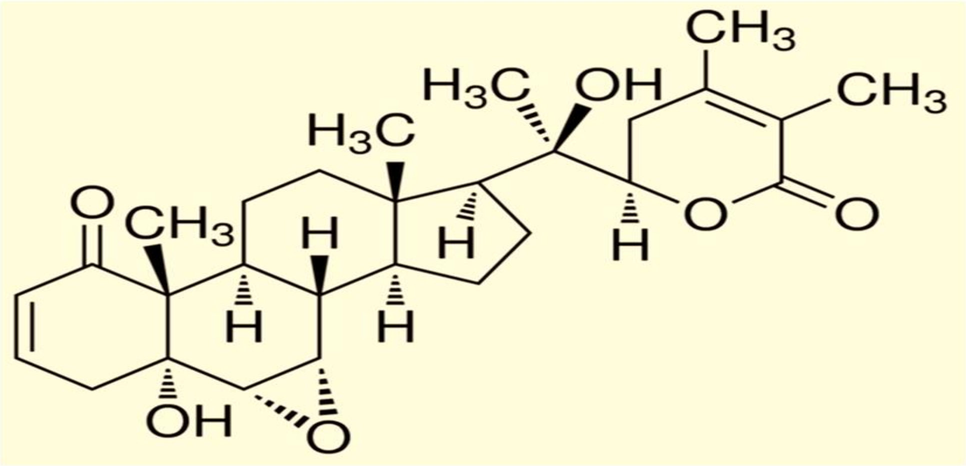
Traditionally, Anxiolytic-Anti-depressive, Antifungal, Anti-malarial, Antibacterial, Hypoglycemic effect, Anticancer activity, Anti-inflammatory, Antitumor, Anti-stress, Antioxidant, Immunomodulatory, Antidiabetic.In Sanskrit, ashwagandha means “smell of the horse” due to its unique smell. (It doesn't smell like a horse per se, but it has a pungent barn-like, earthy fragrance. Gum is bland and mucilaginous. Seeds are acrid and stimulant. The bark is an emmenagogue and even abortifacient, antifungal, antibacterial. Flowers are Tumor, inflammation, hysteria, cholesterol-lowering. The plant is also a cardiac circulatory tonic and antiseptic. Root juice is employed in Diabetes, hypertension, stress, arthritic diseases, and antiepileptic. Used for nervous debility, chronic stress, asthma, deep-seated inflammation, and as Antiparkinsonian properties.

Chemical Constituents Present in Roots[25]

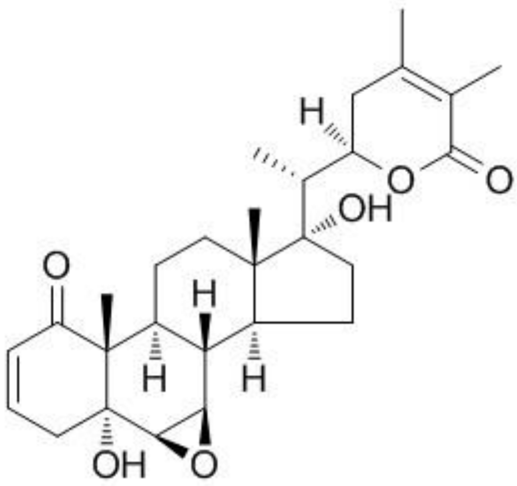
Chemical constituents of *Withania Somnifera* are always of interest to researchers. The biologically active chemical constituents are alkaloids (ashwagandhine, cuscohygrine, anahygrine, tropine, etc.), steroidal compounds, including ergostane type steroidal lactones, withaferin A, withanolides A-y, withasomniferin-A, withasomidienone, withasomniferols A-C, withanone, etc. Other constituents include saponins containing an additional acyl group (sitoindoside VII and VIII), and withanolides with glucose at carbon 27 (sitoindoside IX and X). Apart from these contents plants also contain chemical constituents like withaniol, acylsteryl glucosides, starch, reducing sugar, hantreacotane, ducitol, a variety of amino acids including aspartic acid, proline, tyrosine, alanine, glycine, glutamic acid, cystine, tryptophan, and high amount of iron.

Root bark: Withasomnine and Withanolide A, phytosterols like Pseudotropine, isopelletierine, 3α-tigloyloxtropine tropine, *dl*-isopelletierine-3-tropyltigloate, cuscohygrine, anaferine, hygrine.

Chemical Structure of Phyto Constituents:



Withanolide A



Withanone

PHYSICAL APPEARANCE

Root extract is having:

Colour - pale yellowish

Odor - pungent (horses' urine)

pH - 8.5

POLYMER PROFILE

4. POLYMER PROFILE

SILVER NITRATE [4,17,20]

Silver Nitrate

Silver nitrate is an inorganic silver compound that is used to prepare many types of silver derivatives.

ChemicalFormula

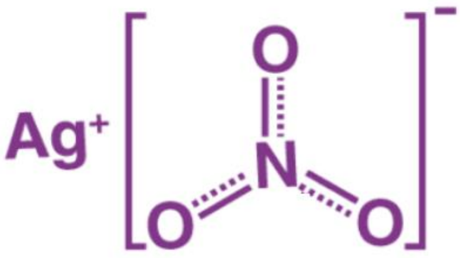
$AgNO_3$,

Molar Mass

169.87 g/mol.

Chemical Structure

It consists of the silver cation (Ag^+) and the nitrate ion (NO_3), in which the central nitrogen atom is covalently bonded to three oxygen atoms with a net charge of -1.It consists of an ionic bond between the silver cation (Ag^+) and the nitrate anion (NO_3^-). Due to the ionic nature of this compound, it readily dissolves in water and dissociates into its constituent ions.



Preparation

Silver nitrate is prepared industrially by reacting elemental silver with either dilute or concentrated nitric acid to give silver nitrate along with nitrogen oxides (NO or NO_2) as a byproduct.

$3\ Ag + 4\ HNO_3\ (dilute) \rightarrow 3\ AgNO_3 + 2\ H_2O + NO$

$Ag + 2\ HNO_3\ (concentrated) \rightarrow AgNO_3 + H_2O + NO_2$

Physical Properties

Silver nitrate is found as a white odorless solid.

Density - 4.35 g/Ml

Melting point - 210 °C

Boiling point - 440 °C

Chemical Properties

Silver nitrate is water-soluble and non-hygroscopic. Unlike many other silver salts, it is not sensitive to light. It is an oxidizing agent and is quite reactive as the nitrate ion can be easily replaced by other groups. Thus, it is a useful starting material for making many different silver compounds including silver halides, silver oxide, etc. Silver nitrate is fairly stable to light and heat, but decomposes when heated to higher temperatures to give metallic silver along with toxic NO_2 gas:

$2\ AgNO_3 \rightarrow 2\ Ag + O_2 + 2\ NO_2$

Uses

Silver nitrate has several medical uses as it has antiseptic properties. Thus, it is used for treating some infections, warts, and ulcers and is also used as a cauterizing agent and a disinfectant. Its other applications include the preparation of photographic films and explosives, the manufacture of many silver compounds, analytical reagents, biological staining agents, and organic synthesis.

Health effects / Safety hazards

Silver nitrate is toxic and corrosive and must be handled with care. It is used in very dilute solutions for medical applications. Skin or eye contact with small amounts of silver nitrate or itsdilute solutions will cause greyish-black staining of the tissues (called argyria), while exposure to higher concentrations can cause burns.

AIM AND OBJECTIVE

5. AIM AND OBJECTIVE

Objective Of theResearch Work:

Green synthesis of nanoparticles involves the use of plant or different plant parts for the bioreduction of metal ions into their elemental form**.** Green synthesis is defined as the use of environmentally compatible materials such as bacteria, fungi, and plants in the synthesis of nanoparticles. These attractive green strategies are free of the shortfalls associated with conventional synthetic strategies, i.e., they are eco-friendly alternatively, synthesis from biologically derived extracts offers several advantages such as rapid synthesis, high yields, and importantly, the lack of costly downstream processing required to produce the particles.

The particles produced by green synthesis differ from those using physicochemical approaches. Green synthesis, a bottom-up approach, is similar to the chemical reduction where an expensive chemical reducing agent is replaced by extract of a natural product such as leaves of trees/crops or fruits for the synthesis of metal or metal oxide nanoparticles. Hence, nanoparticle synthesis from plant extracts tentatively offers a route for the large-scale production of commercially attractive nanoparticles.

Nanotechnology deals with materials thatexhibit remarkable physical, chemical, and biological properties because of their nanoscale size. Recent advances in nanoscience and nanotechnology radically changed the way we diagnose, treat, and prevent various diseases in all aspects of human life.

Silver nanoparticles (AgNPs) are one of the most vital and fascinating nanomaterials among several metallic nanoparticles that are involved in biomedical applications. Silver nanoparticles play an important role in nanoscience and nanotechnology, particularly in nanomedicine. Although several noble metals have been used for various purposes silver nanoparticles have been studied extensively.

Withania is one such genus belonging to the family of Solanaceae, a monotypic family of single genera with around 23 species. Most of these species have not been explored fully despite the enormous bioactivity reports concerning various potentials such as Anxiolytic-Anti-depressive, Antifungal, Anti-malarial, Antibacterial, Hypoglycemic effect, Anticancer activity, Anti-inflammatory, Antitumor, Anti-stress, Antioxidant, Immunomodulatory, and Antidiabetic. The root bark of *Withania Somnifera* contains two additional alkaloids (total alkaloids, 0.31%), viz. withanolide-A and Withanone, belonging to the sympathomimetic group of bases. In addition, traces of essential oil with a pungent smell, phytosterol, waxes, and resins are found in the *Withania Somnifera* plant, and it contains a rich and rare combination of steroidal lactones, saponins, isopelletierine, anaferine, cuseohygrine, anhydride.

In the present study, extracellular production of silver nanoparticles was carried out victoriously. This offers a rapid synthesis of silver nanoparticles ecologically and makes them a successful alternative for more popular chemical and physical methods of synthesis. The antimicrobial properties of silver have been known for thousands of years. Recently, silver nanoparticles have gained more attention because of their small size and large surface area, and they can undergo more efficient binding with the microorganism.It has been used to treat a wide variety of diseases. But it was found that high doses of silver when administered intravenously could cause convulsions and even death. Thus, the dose of silver used in medical applications can be minimized which in turn can minimize their toxicity. In this study, the synthesized nanoparticles can be given orally, which reduces the toxicity than administered intravenously. The antimicrobial activity of the synthesized silver nanoparticles has been evaluated by the agar well diffusion method. The synergetic effect of silver nanoparticles with the phytoconstituents has also been studied by combining them with antibiotics against various strains of Gram-positive and Gram-negative bacteria. Along with the antibacterial study the anti-inflammatory activity of the *Withania Somnifera*has also been seen.

The main objective of the present study was to fabricate silver nanoparticles of *Withania Somnifera* root extract by green synthesis because of their environmental and cost-effective properties. The plant possesses a wide variety of phytochemical constituents, which are responsible for its medicinal properties. These phytochemicals present in the plants contribute to reducing silver nitrate into silver ions. The synthesized silver nanoparticle was characterized using various microscopic and spectroscopic techniques and its investigation for anti-bacterial and anti-inflammatory activity.

In the present study, the root extract of *Withania Somnifera* aimed to formulate as silver nanoparticles. Bioactive secondary metabolites such as phenols, flavonoids, alkaloids, and terpenoids are present in medicinal plants. The roots of *Withania Somnifera*are used for treating many other diseases. This study also includes checking whether the extractor formulated silver nanoparticles is having anti-bacterial and anti-inflammatory activity.

The following objectives are outlined to achieve the aim and need of the study:

- To select the appropriate plant part and its extraction method.
- To perform Pre-formulation studies.
- To develop the preparation of silver nanoparticles.
- To perform different evaluation parameters for the bio fabricated silver nanoparticles.
- To perform the Antibacterial activity and *in-vitro* anti-inflammatory activity of silver nanoparticle of *Withania Somnifera*.

PLAN OF WORK

6. PLAN OF WORK

The present works were carried out to bio fabricate and characterize the silver nanoparticle of *Withania Somnifera* root extract by using silver nitrate and investigating their anti-microbial and anti-inflammatory activity.

The following experimental protocol was therefore designed to allow a systemic approach to the study:

- Collection of literature related to green synthesis, silver nanoparticles, and the *Withania Somnifera* plant.
- Selection of plant part and its extraction method.
- Preparation of silver nanoparticles.
- Preliminary analysis (color change, UV-analysis).
- Evaluation of silver nanoparticles for the following physicochemical parameters:
    - UV Analysis.
    - FTIR Studies.
    - Particle Size Analysis.
    - Zeta Potential Analysis.
    - X-Ray Diffraction Studies.
    - SEM (Scanning Electron Microscopy).
    - *In vitro* Anti- microbial study,

      Agar Diffusion Method.
    - *In vitro* Anti-inflammatory study,

      Denaturation of Protein Albumin.

MATERIALS AND INSTRUMENTS

7. MATERIALS AND INSTRUMENTS

7.1 MATERIALS

7.1.1 List of Materials Used:

Thematerials shown below were used for the biofabrication of silver nanoparticles. The entire materials used were of best quality.

Table No. 2: List of Materials Used forThe Research Work

Sl.no	Name	Company name
1	*Withania Somnifera*	Local Area (Kerala)
2	Double Distilled water	Leo Scientific, Erode
3	Silver nitrate	Rankem Pvt.Ltd, Hyderabad
4	Nutrient agar	Himedia, Mumbai
5	Amoxyllin	LLI Health Care, New Delhi
6	Bovine Serum Albumin	Sigma Aldrich, Coimbatore
7	Potassium Dihydrogen Phosphate	Himedia, Mumbai
8	Aceclofenac	Vivid Biotech, Mumbai
9	Distilled Water	Leo Scientific, Erode

7.2 INSTRUMENTS

7.1.2 List of Instruments Used:

The following instruments were used for the research work.

Table No. 3: Instruments Used forThe Research Work

Sl.no	NAME	COMPANY
1	Electronic balance	SHIMADZU, Japan
2	UV-visible light spectroscopy	UV 1800 SHIMADZU, Japan
3	IR- Spectrophotometer	FTIR-8400 S SHIMADZU, Japan
4	Scanning Electron Microscopy (SEM)	JOEL JSM-T330A, Japan
5	X-Ray Diffractometer (XRD)	LC-10AT, Shimadzu, Japan
6	Particle Size Analyzer	MICROTEC BLUE WAVE, Germany
7	Magnetic Stirrer	REMI, VASAIN (India)
8	Melvern Zetasizer	MALVERN NANO ZS-90, UK.
9	Cold Centrifuge	REMI, Mumbai.
10	Refrigerator	GODREJ
11	PH-meter (Digital)	LI 613, ELICO
12	Mixer Grinder	PREETHI ZODIA MIXER 750W
13	Analytical Balance	SHIMADZU, Japan

EXPERIMENTAL PROCEDURE

8. EXPERIMENTAL PROCEDURE

8.1.Plant Material Collection and Preparation of Extract

- The roots of the *Withania Somnifera* plant were collected from in and around areas of Palakkad District and good grade roots were selected from them.
- The roots selected were cut into thin slices.
- Freshly collected roots were washed thoroughly by using distilled water to remove the soil particlesand other impurities present in the root part.
- The outer layer of the root was removed and allowed to dry in shade for 15 days.
- After the complete removal of water, the root was sliced into small pieces and grinded to a coarse powder using a mixer grinder.
- About 80gm of grinded powder was taken and boiled with 800 ml of double-distilled water (DDW) for about 1 hour.
- After cooling to room temperature, the mixture was filtered using the Whatman filter paper no.1, and the root extract of *Withania Somnifera* was thus prepared.
- This was continued several times for obtaining the required quantity of the root extract.
- The prepared root extract can be stored in the refrigerator (4°C) for future uses [51].

8.2. Procurement of Ratio for Formulation of Silver Nanoparticle

To find out the optimum ratio of silver nanoparticle formulation. The different ratios of *Withania Somnifera* root extract:silver nitrate solutionwasF1 (1:1), F2 (1:3), F3 (1:6), and F4 (1:9).The selection of the appropriate ratio is done by taking the color of silver nitrate and *Withania Somnifera* root extract as control. Among the different ratios of the formulation showing an acceptable color change is selected and it is the first indication of the formation of the *Withania Somnifera* root extract Nanoparticle.UV-visible spectroscopy was conducted in a range of 200-800nm and the formulation showing absorbance within the limit of silver nanoparticles is selected for further study.

8.3.Preformulation Study [53]

Preformulation testing is an investigation of the physical and chemical properties of a drug substance alone and when combined with a polymer. The overall objective of Preformulation testing is to generate useful information for the investigator in developing a stable dosage form obviously, the type of information needed will be depended on the formation to be developed. The use of the preformulation parameter maximizes the chances of formulating an acceptable and stable product.

8.3.1.Colour, Odour, Appearance

The extract was evaluated for its color, odor, taste, and appearance and the results have been noted.

8.3.2.Melting Point Determination

The melting point is the main indication of the purity of the sample since the presence of a relatively small amount of impurity can be detected by a lowering as well as widening in the melting point range. Melting point determination of the obtained *Withania Somnifera*sample was done by the open capillary method. Before performing the analysis, the equipment was calibrated and immediately after this the *Withania Somnifera*sample was compacted into capillary tubes each 6 mm long and 1mm diameter. The capillaries were introduced vertically into the equipment, with heating 10˚C per minute. The reading was performed three times. The melting point of the drug was noted and compared with the melting point of the reference sample cited in Indian pharmacopeia.

8.4.Synthesis of Silver Nanoparticles of *Withania Somnifera* Root Extract

1mM of silver nitrate (AgNO3) was prepared in a 1000ml beaker (0.1698 g AgNO3 is added to 1000ml of distilled water).This beaker is placed on a magnetic stirrer for complete mixing of the silver nitrate solution.The 100 ml root extract of *Withania Somnifera* aqueous root extract was added drop by drop todifferent ratios of silver nitrate solution as 100ml(F1), 300ml (F2), and 600 ml (F3), 900 ml (F4) ratio by using a dropper. The reaction was carried out in a beaker kept on stirring at a temperature of 60◦C for about 8 hours.

The change in the color of the solution of different ratios was noted because the color of the solution indicates the formation of *Withania Somnifera* root extract Nanoparticles. Then the formulation was kept under dark conditions for 24 hours and the solution was centrifuged at 4,000 rpm, 20◦C for 15minutes.Then the supernatant was removed from the tube and the product was taken which was placed for drying under dark conditions.[54]

Further, the synthesized samples were used for characterization and evaluations.

8.5. EVALUATION

To confirm the presence of silver nanoparticles in the formulation, UV-VISIBLE spectrophotometer analysis, and visual observations were performed along with the following.

8.5.1. UV- VISIBLE Spectrophotometer

To determine the time point of the maximum production of silver nanoparticles,the absorption spectra of the AgNO3, *Withania Somnifera* root extract, and F1, F2, F3, and F4 formulations were taken in between 200 to 800nm using the UV–Visible spectrophotometer. The deionized water was used as the blank.

The best formulation can be selected from the absorption spectrum of the different formulations by taking the characteristic peak of silver nanoparticles.

8.5.2. FOURIER TRANSFORM INFRAREDSPECTROSCOPY (FTIR)

FTIR analysis was carried out to identify the possible biomolecules responsible for the reduction of the Ag^+ ions and capping of the bio-reduced silver nanoparticles synthesized. To determine the involvement of functional groups in the formation of *Withania Somnifera* root extract nanoparticles FTIR was conducted in the range of 4500 to 500 cm. The FTIR of Silver nitrate, *Withania Somnifera* root extract, and the synthesized *Withania Somnifera* root extract nanoparticles were carried out. The synthesized nanoparticles of *Withania Somnifera* root extract also contains biomolecules that are not capped on nanoparticles; they are removed by dissolving in DDW and centrifuged at 500rpm for about 10 min, the procedure is repeated 3 times, and the final pellet obtained is dried at 60°C in a hot air oven and used for the characterization.

8.5.3.Particle Size Analysis

The particle size analysis of*Withania Somnifera*and different formulations of silver nanoparticles was carried out using a Microtrac Blue wave-particle size analyzer. Before measurement of the samples, they have to be diluted with de-ionized water to obtain a suitable concentration for measurement. The results obtained for particle size distributions were used to confirm the formation of nano-sized particles[17].

8.5.4. X-Ray Diffraction Analysis (XRD)

The X-ray diffraction pattern indicated the crystalline structure of silver nanoparticles. The XRD spectrum confirmed the presence of silver nanoparticles and it was compared with the *Withania Somnifera* root powder. The phase variety and grain size of synthesized silver nanoparticles were determined by X-ray diffraction spectroscopy (Philips PAN analytical). The synthesized silver nanoparticles were studied with CUKα radiation at a voltage of 40 kV and a current of 30 MA with a scan rate of –5.0 deg/min. The diffracted intensities were recorded from 2Ɵ range from 10°to 80°angle. Crystallographic structures of nanoparticles can also be calculated using the peaks of XRD [55].

8.5.5. SEMAnalysis

The silver nanoparticles were also characterized by scanning electron microscopy (SEM). The SEM analysis of the*Withania Somnifera* root powder and the synthesized silver nanoparticles was carried out. The direct electron microscopic visualization allows measuring the size and shape and morphology of bio-capped silver nanoparticles formed. The sample was lightly sprinkled on a double side adhesive tape stuck to an aluminum stub and the stubs were coated with platinum of thickness to about 10A° under an argon atmosphere using a copper sputter module in a high vacuum evaporator operated at a voltage of 10.00Kv, magnification 50.00KX, working distance of 8.5mm. The stubs containing the coated samples were placed in scanning electron microscopy and analyzed the surface morphology[56].

8.5.6. Zeta Potential

Synthesized Silver nanoparticles were dispersed in deionized water followed by ultra-sonication. Then the solution was filtered and centrifuged for 15 minutes at 25°C with 5000 rpm and the supernatant was collected. The supernatant was diluted 4 to 5 times and then the particle distribution in liquid was studied in a computer-controlled particle size analyzer (ZETA sizer Nano series, Malvern instrument Nano Zs)[11].

8.5.7. Anti-BacterialActivity

It is evident from the literature that silver nanoparticles exhibit pronounced anti-bacterial activity.Anti-bacterial activity screening was performed for the synthesized compound (silver nanoparticle). Four bacterial strains were used, two weregram-positive and the other two weregram-negative. Various concentration of silver nanoparticle was used to test anti-bacterial activity. The standard used during the screening was Amoxyllin.

Requirements:

Nutrient agar, nutrient broth, Petri dishes, spatula, test compounds, standard drug, solvent, whattmann filter paper.

The following strains have been used for the study:

Gram-PositiveBacteria

Staphylococcus aureus

Bacillus subsites

Gram-NegativeBacteria

Escherichia coli

Klebseilla pneumonia

Standard: Amoxyllin

Solvent: Distilled water

Disc Diffusion Method

The medium was prepared by dissolving the specified quantity of the dehydrated medium in purified water by heating on a water bath and was dispensed in a 100ml volume conical flask. The conical flask was closed with a cotton plug and was sterilized by autoclaving at 121°C (15lb) for 45 minutes. A total of25ml of agar medium was poured into a sterile vessel. The Petri dishes were specially selected with a flat bottom and were placed on a level surface to ensure that the layer of medium is in uniform thickness. Each petri dish was inoculated with 0.2ml of different bacterial strains mixed well with the nutrient agar medium and then allowed to solidify.

The antibacterial activity of the *Withania Somnifera*root extract and formulated silver nanoparticles was determined using the disc diffusion method. Four different bacterial strains

Staphylococcus aureus, Bacillus substilis, Escherichia coli& Klebseilla pneumonia were inoculated into the nutrient broth and kept in the rotatory shaker for incubation at 37°C for 24 hrs. 100 µl of the overnight incubated bacterial cultures after a series of dilutions were uniformly spread on the surface of the freshly prepared agar medium with the help of a sterilized glass rod. Subsequently, the sterilized circular Whatman filter papers with a diameter of 25 mm which were impregnated with the prepared silver nanoparticles of different concentrations were gently put down on the surface of inoculated nutrient agar plates usingsterilized tweezers. Eventually, the zone of inhibition in millimeters was measured after incubation at 37°C for 24 hrs [44].

8.5.8.*In-Vitro* Anti-Inflammatory Activity

Denaturation of protein albumin:

The formulated silver nanoparticle was subjected to*In-vitro*Anti-Inflammatory activity by protein denaturation method. The reference drug used in this method was Aceclofenc,a Non-steroidal Anti-Inflammatory drug. The different concentration of *Withania Somnifera* root and silver nanoparticle was prepared in the dose of 20 µg/ml, 40 µg/ml, 80 µg/ml, and 100 µg/ml, and the standard drug of Aceclofenac was prepared in the dose of 20 µg/ml, 40 µg/ml, 80 µg/ml and 100 µg/ml respectively. The reaction mixture consist of different concentrations of silver nanoparticle and the standard drug were mixed with bovine albumin serum (2 ml) and make up the volume with pH 6.4 phosphate-buffered saline and subjected to incubation for 15 min at 27ºC. After incubation, denaturation was produced by boiling the reaction mixture at 70ºC for 10 min. The reaction mixture was cooled and measured absorbance at 660nm by using distilled water as a blank. The experiment was carried out in triplicate. By applying the following formula, the percentage inhibition for denaturation of protein was calculated[45].

$$\% \, Inhibition = \frac{A_t - A_c}{A_t} \times 100$$

Were,

A_t = Absorbance of Test, A_c = Absorbance of Control

RESULT AND DISCUSSION

9. RESULT AND DISCUSSION

9.1. PREFORMULATION STUDIES

9.1.1. CHARACTERIZATION OF ROOT:

The root of *Withania Somnifera* was evaluated by physical characters and determining the melting point.

9.1.2 PHYSICAL OBSERVATION STUDIES:

Colour - Pale yellowish

Odour -Characteristic

Taste -Bitter

PH - 8.5

9.1.3. SOLUBILITY STUDIES:

Withania Somnifera is soluble in hot water, quite soluble in alcohol and insoluble in cold water.

9.1.4 MELTING POINT DETERMINATION:

The melting point of *Withania Somnifera* was found to be 189° C.

9.2 PROCUREMENT OF RATIO FOR THE FORMULATION DEVELOPMENT OF SILVER NANOPARTICLE

9.2.1. Visual Identification of Silver Nanoparticles of *Withania Somnifera*Root Extract:

The primary indication is by the visual proof, the formation of silver nanoparticles is identified by the colour change of silver nitrate solution from colourless to dark brown colour. The addition of *Withania Somnifera* root extract to silver nitrate solution produced an instantaneous colour change from an initial pale-yellow solution to a dark brown colour.Excitation of surface plasmon resonance and reduction of silver ions into silver nanoparticles are the two major causes for the change in the colour of the solution, this is known as the Plasmon resonance phenomenon. Temperature, time and stirring accelerated the reaction for the colour change. The metal nanoparticles have free electrons, which give the Surface Plasma Resonance absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with a light wave.

To find out which is the optimum ratio for the evaluation of silver nanoparticles of *Withania Somnifera* root extract the colour of root extract and Silver Nitrate (AgNO3) was taken as the control and the change in the colour was observed.

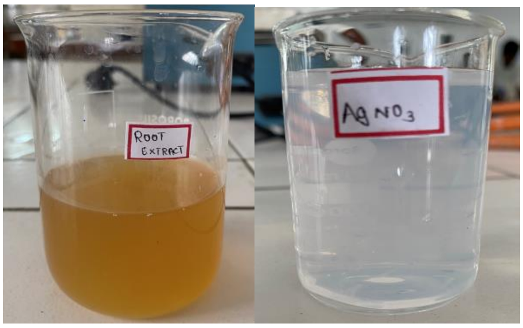


Figure no. 5 Colour of *Withania Somnifera root extract* and Silver Nitrate

The various ratios of *Withania Somnifera* root extract: silver nitrate solution was done as F1, F2, F3 and F4. Among these formulations, the F4 (1:9) only showed an acceptable colour change and it is the primary indication for choosing F4 formulation for further studies.

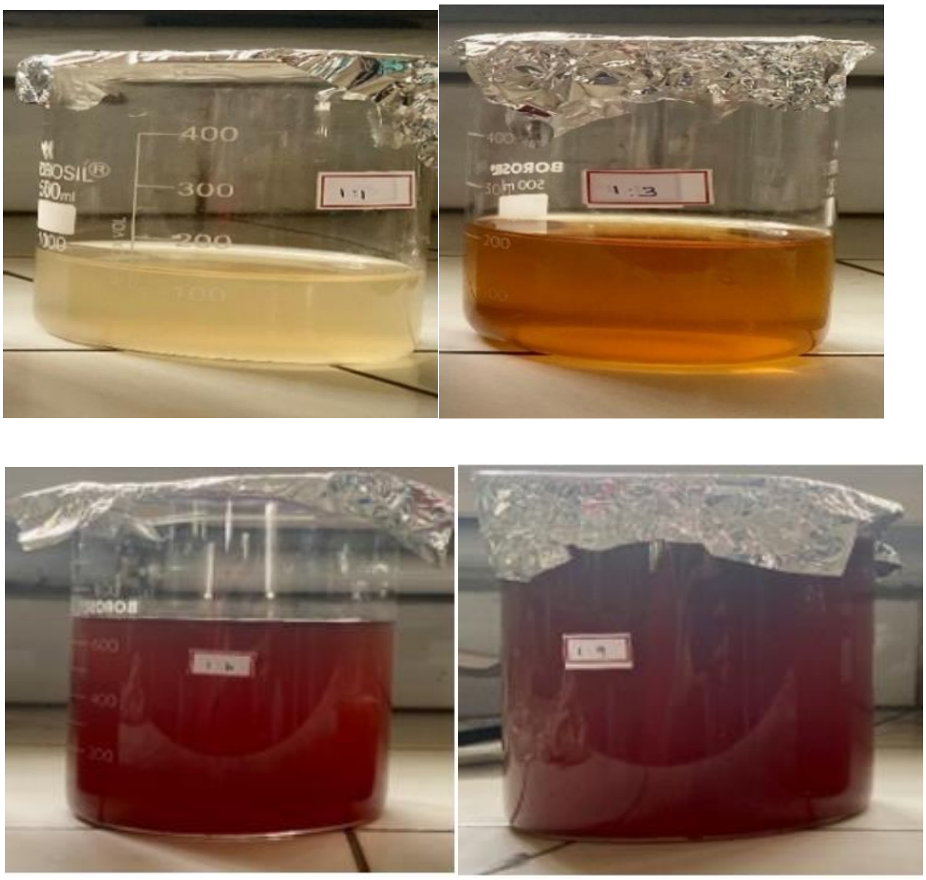


Figure no.6: Colour change for the selection of appropriate ratio after the development of silver nanoparticles.

9.2.2 ULTRAVIOLENT-VISIBLE (UV) ABSORPTION SPECTRA ANALYSIS:

9.2.3 UV Spectrum of *Withania Somnifera* Root Extract

The UV spectra of *Withania Somnifera* root extract were analyzed spectrophotometrically in between 200-800 nm.

The UV spectrum of *Withania Somnifera* root extract is presented in **Table (4)** and **Figure (7).** The UV absorption peaks showed at 725nm,710nm,655nm and 550nm.

Table no 4: UV Absorption peaks of *Withania Somnifera*

Sl.no	Peaks (nm)	Absorbance
1	725.00	0.510
2	710.00	0.516
3	655.00	0.530
4	550.00	0.683

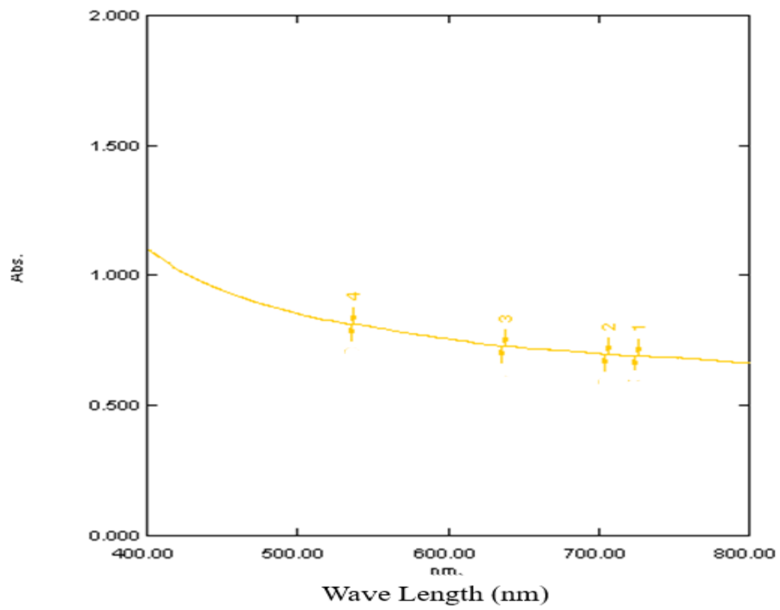


Figure no. 7: UV spectrum of *Withania Somnifera* root extract

9.2.4 UV Spectrum of Silver Nitrate

The UV spectra of Silver Nitrate were analyzed spectrophotometrically in between 200-800 nm.

The UV spectrum of Silver Nitrate is presented in **Table (5)** and **Figure (8).** The UV absorption peaks showed at 260nm and 255nm.

Table no 5: UV Absorption peaks of Silver Nitrate

Sl. no	Peaks (nm)	Absorbance
1	260	0.020
2	255	0.037

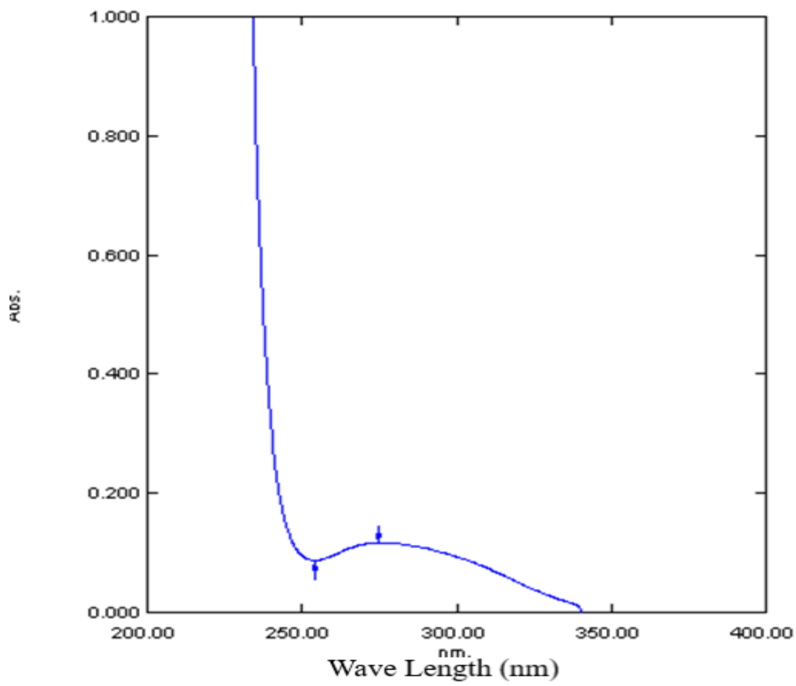


Figure no.8: UV spectrum of silver nitrate

9.2.5 UV Spectrum of Formulated Silver Nanoparticles of *Withania Somnifera* F1, F2, F3 and F4.

Table no 6: Absorption peaks of F1, F2, F3 and F4

SL NO	Peaks (nm)	Absorbance
F1	265.00	0.041
F2	340.00	0.149
F3	345.00	0.255
F4	**431.00**	**0.340**

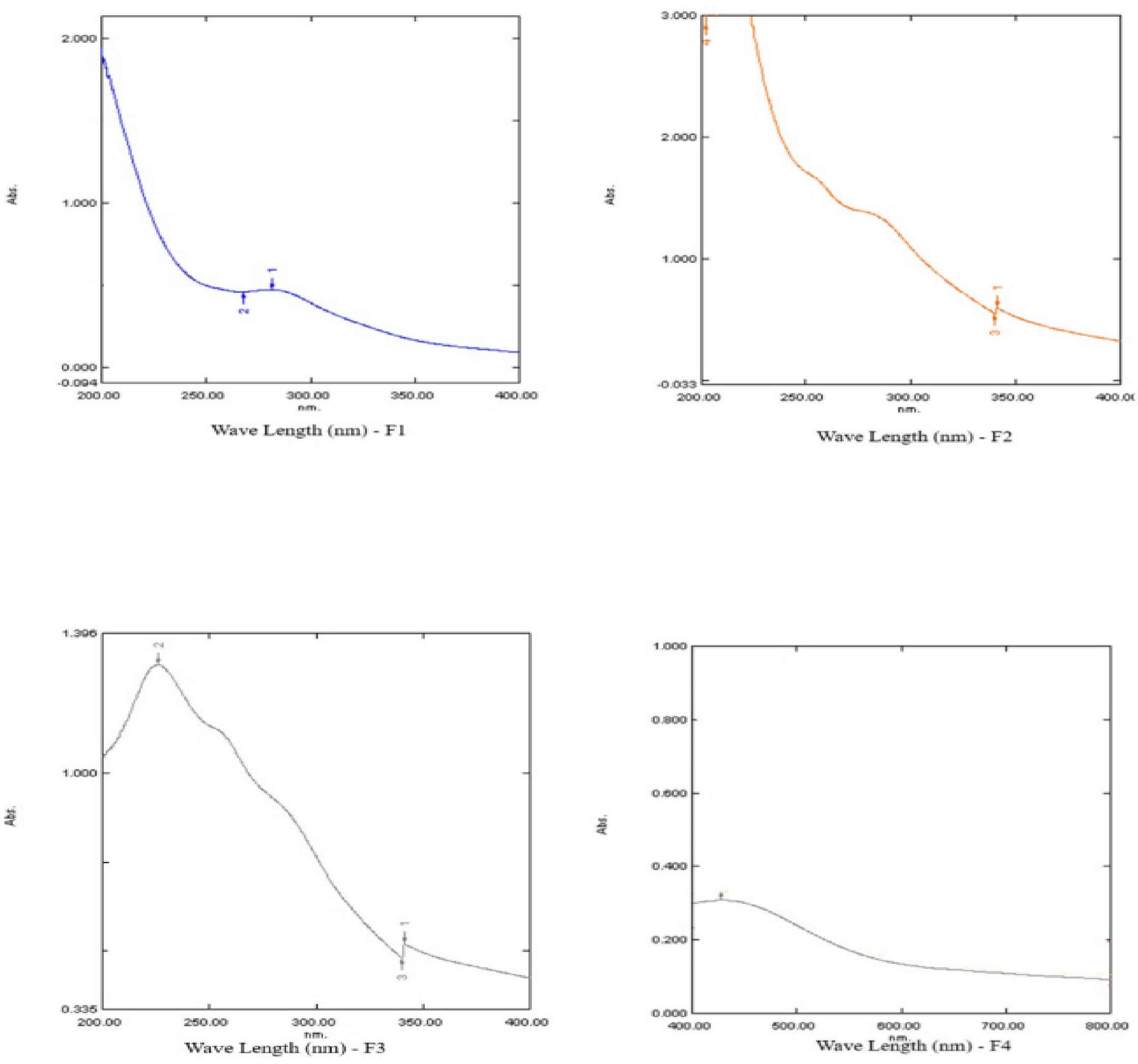


Figure no.9: UV spectrum of F1, F2, F3 and F4

UV Spectrum of *Withania Somnifera* root extract, Silver Nitrate and *Withania Somnifera* silver nanoparticles are presented in (**Figure no: 7,8,9**). The peaks observed for synthesized *Withania Somnifera* silver nanoparticles are 265nm,340nm,345nm and 431nm for F1, F2, F3 and F4 formulations.

Many studies reported that the characteristic peak of silver nanoparticles shows in the range of 400-450nm which was absent in the *Withania Somnifera* root extract and Silver Nitrate, whereas a peak at **431nm** has successfully appeared in F4 formulation of *Withania Somnifera root* Silver Nanoparticles.

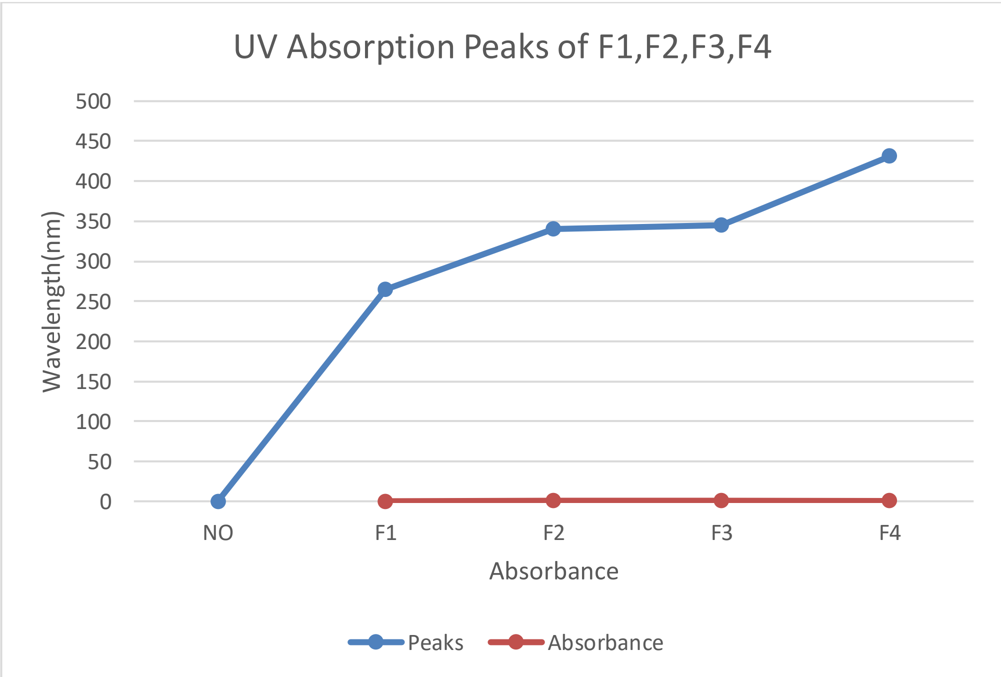


Figure no. 10: UV Absorption peaks of F1, F2, F3 and F4

The peak at 431nm is observed in the F4 Formulation which is very close to the reference peak so we confirmed that *Withania Somnifera* root extracts contain Silver Nanoparticles which led us to further investigation on the synthesis of silver nanoparticles from *Withania Somnifera* root extracts.

9.2.6 Colour Change of the Solution After the Addition of Extract at Different Time Intervals of F4 formulation.

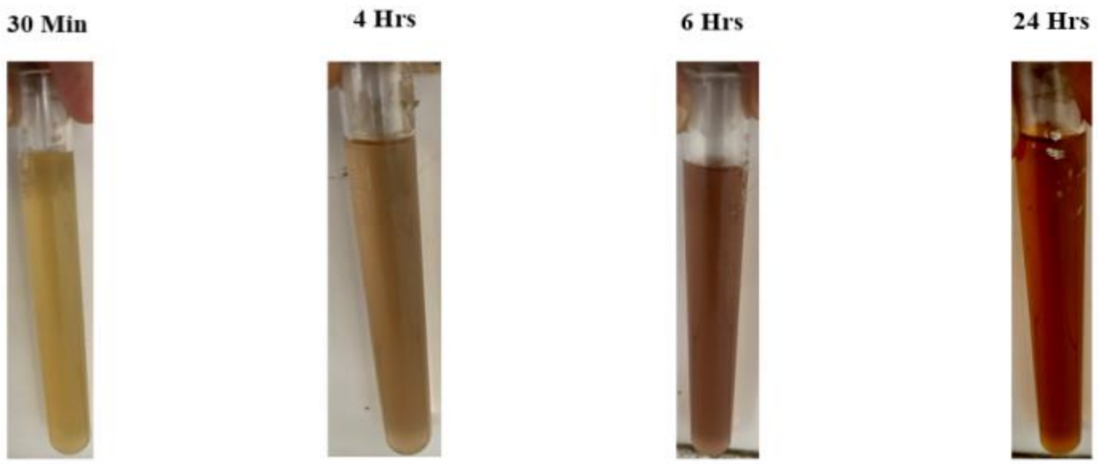


Figure no.11: Colour change of the solution after addition of extract at different time intervals (F4).

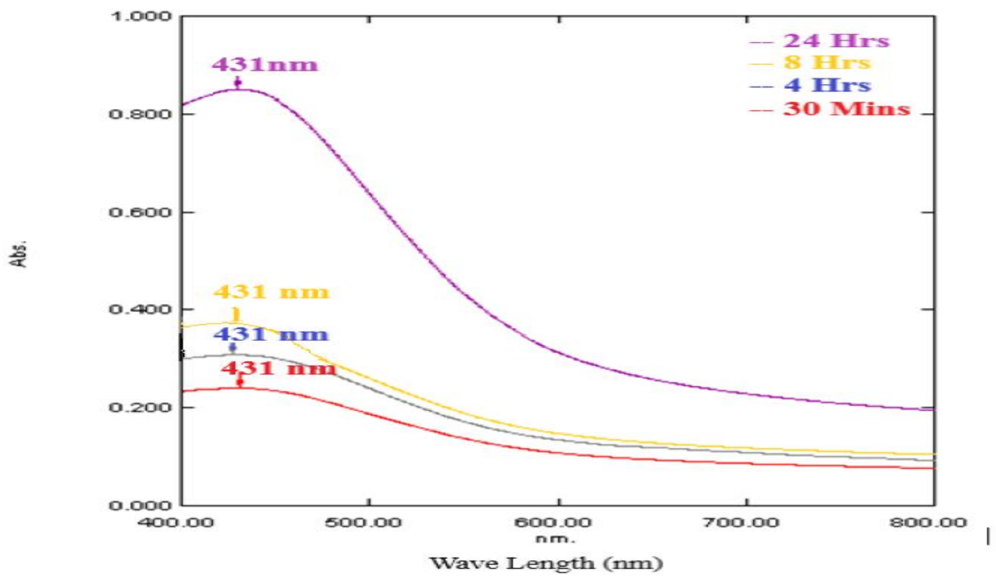


Figure no.12: UV-VISIBLE absorption spectra of synthesized *Withania Somnifera* silver nanoparticles (F4)

The characteristic colour variation is due to the metal ions and the plant extracts. By stability, we mean that there is observed colour variation in the optical properties of the nanoparticle solution with time but shows the same Absorption peak. **Figures no. 11** and **12** display the colour change and UV visible spectra of the F4 formulation at different time intervals.

9.3 FOURIER TRANSFORM INFRARED SPECTROSCOPY:

9.3.1 FT-IR of the *Withania Somnifera Root and polymer*

9.3.2 FT-IR of *Withania Somnifera Root:*

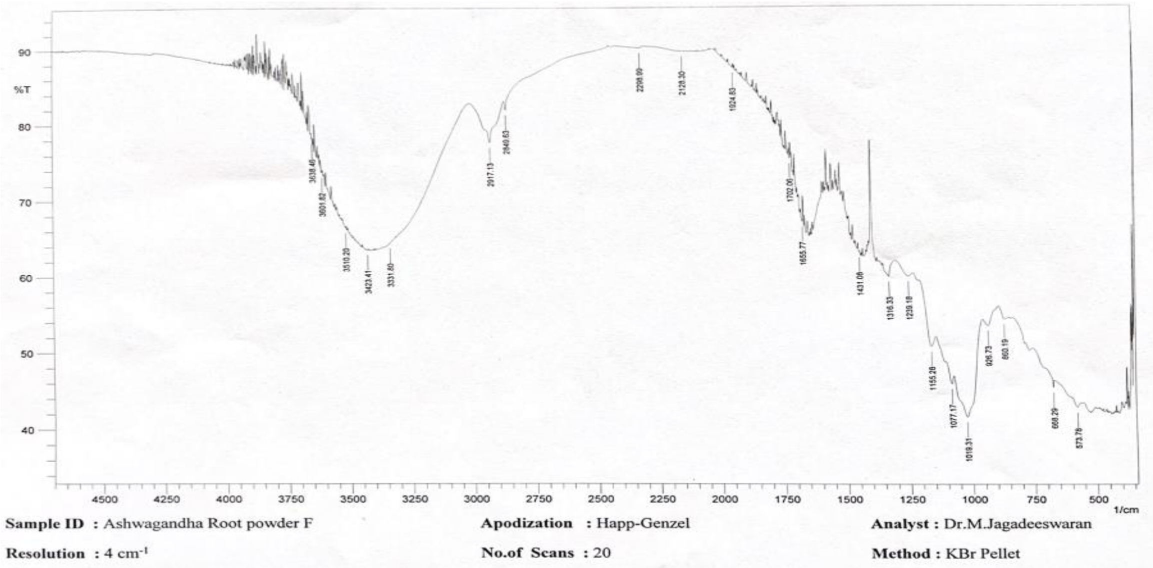


Figure no. 13: FT-IR spectra of *Withania Somnifera*

9.3.3 FT-IR of the Polymer:

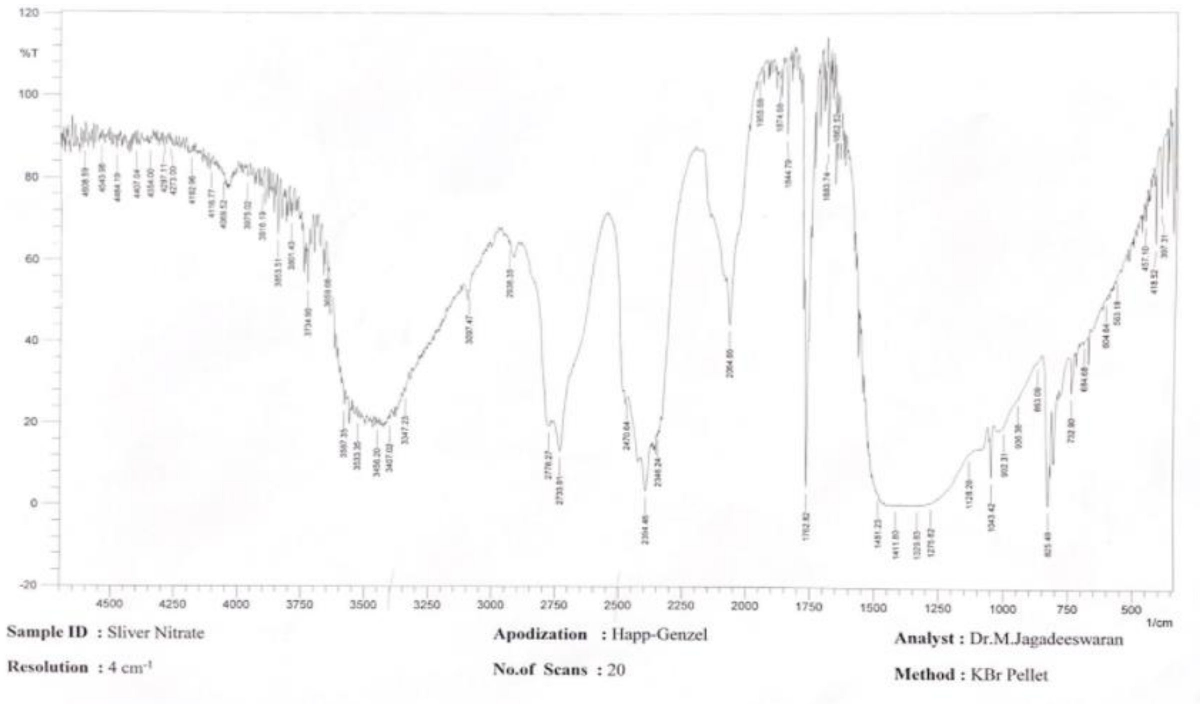


Figure no.14: FT-IR spectra of silver nitrate

Table no.07: Interpretation of IR Spectrum of Silver Nitrate and *Withania Somnifera*

Transition	IR Range (cm^{-1})	Absorption wave number (cm^{-1})	
		Silver Nitrate	*Withania Somnifera*
O-H Stretching Alcohols, phenols	3500-3200	3238.26,3407.02,3456.20	3423.41,3422.4
O-H Stretching Carboxylic acid	3300-2500	3097.47,3149.54,2938.35 ,2948.96,2778.27,2733.91, 2470.64	2917.13
C-H Stretching Alkane	3000-2850	2938.35,2733.91,2778.27, 2346.24	2849.63
HC≡CH Stretching Alkynes	2260-2100	2064.66,2108.05	2298.99,2128.30
C═O Stretching Carbonyl	1760-1665	1683.74, 1662.52	1702.06
-C═C- Stretching Alkenes	1680-1640	1662.52	1655.77
C═C Stretching Heterocyclic aromatic	1550-1475	1481.23	1431.08
C-O Stretching Alcohol, Carboxylic acid	1320-1000	1275.82,1081.99,1043.42	1316.33,1239.18, 1077.17
═C-H Bending Alkenes	1000-650	863.09,825.48,732.90, 684.68	926.73,880.19,668.29

9.3.4. FT-IR Spectroscopy Analysis of Different Formulations of *Withania Somnifera* Root Extract Silver Nanoparticles.

9.3.5. FT-IR of F1

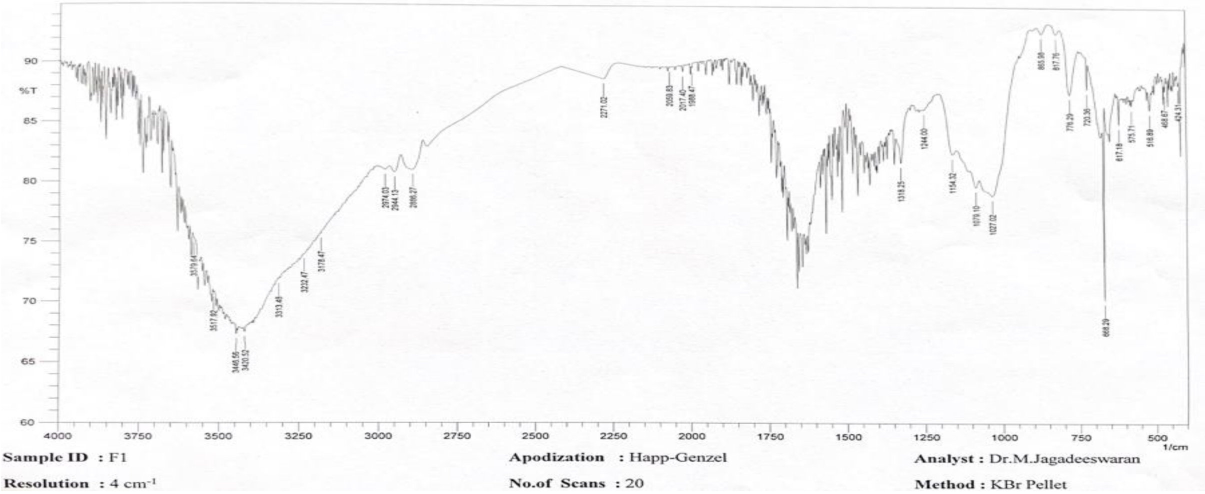


Figure no. 15: FT-IR of F1

9.3.6. FT-IR of F2

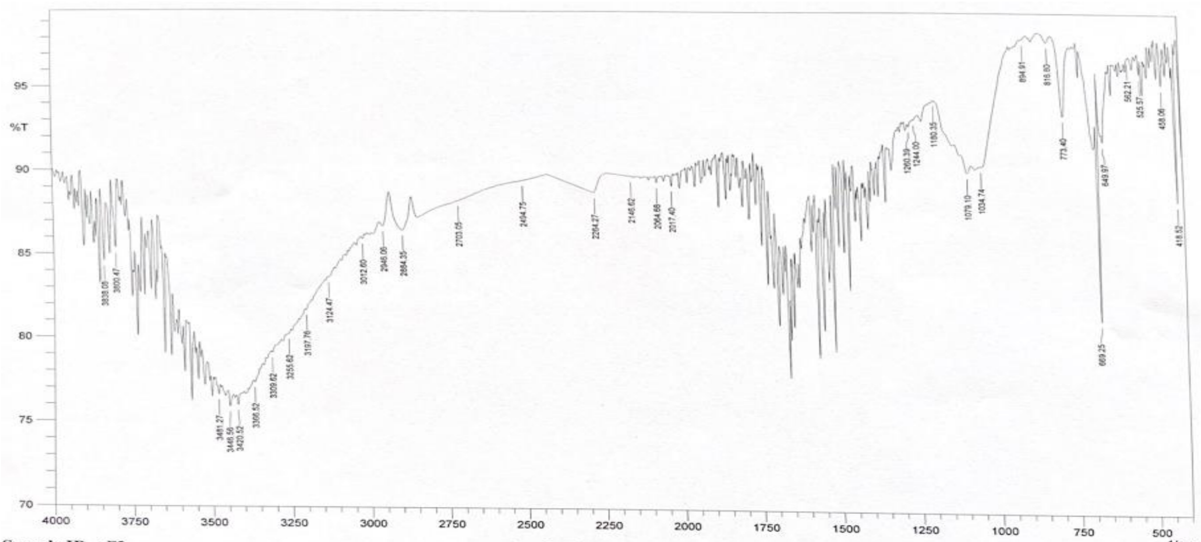


Figure no. 16: FT-IR of F2

9.3.7. FT-IR of F3

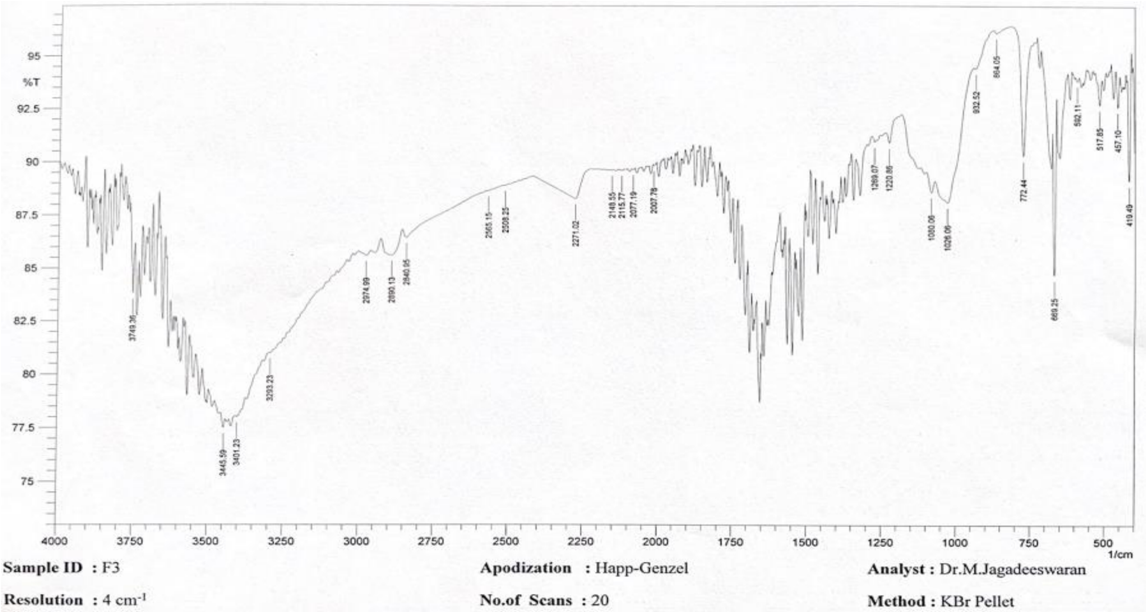


Figure no. 17: FT-IR of F3

9.3.8. FT-IR of F4

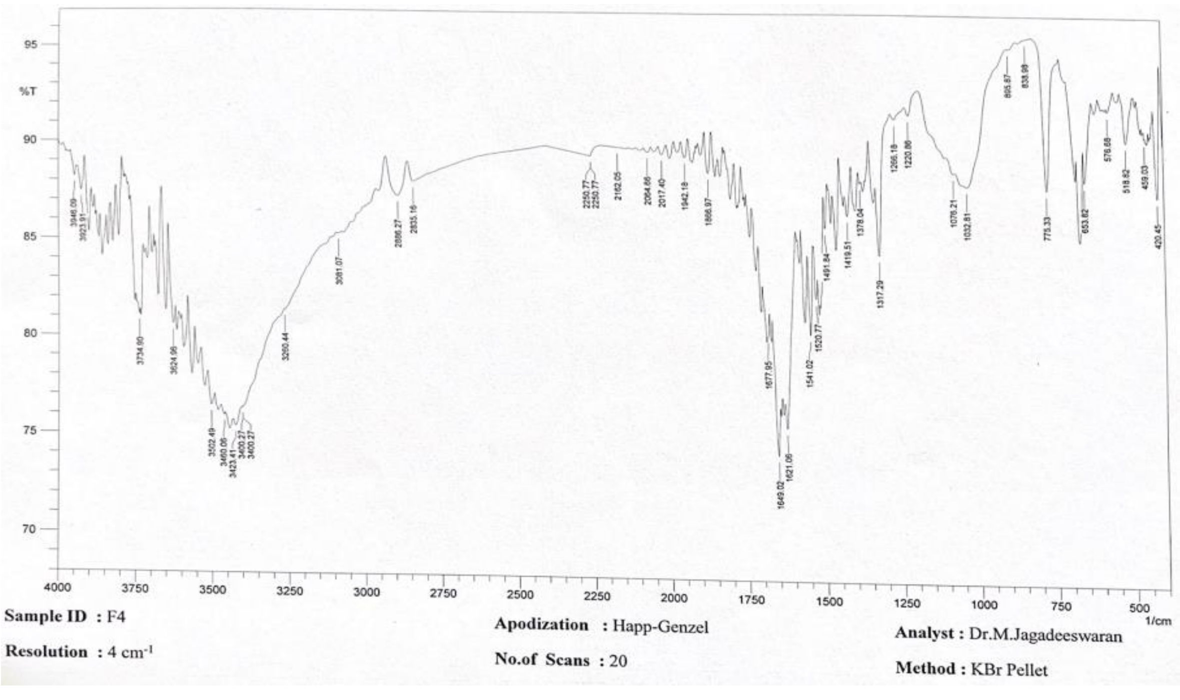


Figure no. 18: FT-IR of F4

Table no.08: Interpretation of IR Spectrum of synthesized Formulations of *Withania Somnifera*Silver Nanoparticle

Transition	IR Range (cm^{-1})	Absorption wave number (cm^{-1}) of Synthesized Silver Nanoparticles			
		F1	F2	F3	F4
O-H Stretching Alcohols,phenols	3500-3200	3446.56,3313.48,3232.47	3481.27	3445.59,3293.23	3366.52,3260.44
O-H Stretching Carboxylic acid	3300-2500	3232.47,3178.47, 2974.03	3255.62,3012.60,2946.06	3293.23,2974.99,2890.13,2565.15	3260.44,3081.07,2886.27,2835.16
C-H Stretching Alkane	3000-2850	2974.03,2886.27	2946.06,2884.35	2974.99,2565.15	2886.27
HC≡CH Stretching Alkynes	2260-2100	-	2146.62	2148.55,2115.77	2250.77,2250.77,2162.05
C═O Stretching Carbonyl	1760-1665	-	-	-	1677.95,1649.02
-C═C- Stretching Alkenes	1680-1640	-	-	-	1621.06
C═C Stretching Heterocyclic aromatic	1550-1475	-	-	-	1541.02,1520.77,1491.84
C-O Stretching Alcohol, Carboxylic acid	1320-1000	1318.25,1244.00	1260.39,1244.00,1180.35,1079.10,1034.74	1269.07,1220.86,1080.06,1026.06	1317.29,1266.18,1220.86,1076.21,1032.81
═C-H Bending Alkenes	1000-650	865.98,817.76,776.29,	894.91,816.80,773.40,669.25	932.52,864.05,772.44,669.25	895.87,838.98,775.33,653.82

FTIR spectroscopy was done to identify the possible interactions between drug and the polymers and for the stabilization of the metal nanoparticles synthesized. FT-IR spectroscopy was done for the Withania *Somnifera*root and silver nitrate. The Silver Nitrate spectrum, which has several peaks at 3423.1,3407.02,1481.23,1275.82., etc were observed and that was matched with the reference spectra from IP. The Withania *Somnifera*root spectrum was observed in 3423.41,2917.13,1702.06., etc.

The characteristic peaks for *Withania Somnifera* can be observed. Similar peaks were seen in mixture of *Withania Somnifera* andsilver nanoparticles. There was no dissimilar shift /disappearance/appearance of peaks combined spectra that indicated good drug -polymer compatibility and no chemical interaction between drug and polymer. Hence, the polymer was suitable for the development of nanoparticles. The values are represented in **Table no.7** and **Table no.8.**

The proteins, polysaccharides and enzymes present in the extract contain -OH group which undergoes stretching vibrations and produces a peak at 3423.41 in *Withania Somnifera*, which is shifted to 3260.44 in synthesized nanoparticles indicating their role in the formation of silver nanoparticles.

The FTIR spectrum of silver nanoparticles showed the band between 3500-3200 cm^{-1} corresponds to O-H stretching H bonded alcohols and phenols. The peaks found around 1500-1550 $^{cm-1}$ showed a stretch for the C-H bond, peaks around 1450-1500 $^{cm-1}$ showed the bond stretch for N-H.

9.4. Particle Size and Polydispersity Index:

The particle size distribution is the most important characteristic of silver nanoparticle formulation. The particle size distribution and Polydispersity index of Withania *Somnifera* root powderand synthesized *Withania Somnifera* root extract silver nanoparticle formulations was carried out.

The particle size was measured by a Malvern particle size analyzer. The nanoparticles mean particle size ranges from 1-100d.nm. Polydispersity index gives a degree of particle size distribution and promotes the physical stability of silver nanoparticles. The ranges of Polydispersity index of the formulations from F1 to F4 from 0.171 to 0.0440 respectively.

The **Table no. 09** shows the Particle size and polydispersity index of *Withania Somnifera*root powder and synthesized *Withania Somnifera* root extract silver nanoparticle formulations.

9.4.1. Particle size and polydispersity index of *Withania Somnifera*root powder and synthesized *Withania Somnifera* root extract silver nanoparticle formulations.

Table no.09: Particle size and polydispersity index

S. No	Formulations	Average Particle size (d. nm)	Polydispersity index
1	*Withania Somnifera* Root powder	218.4	0.171
2	F1	70.4	0.041
3	F2	66.85	0.043
4	F3	65.14	0.0442
5	F4	64.14	0.044

9.4.2 Particle size distribution and polydispersity index of *Withania Somnifera* root powder.

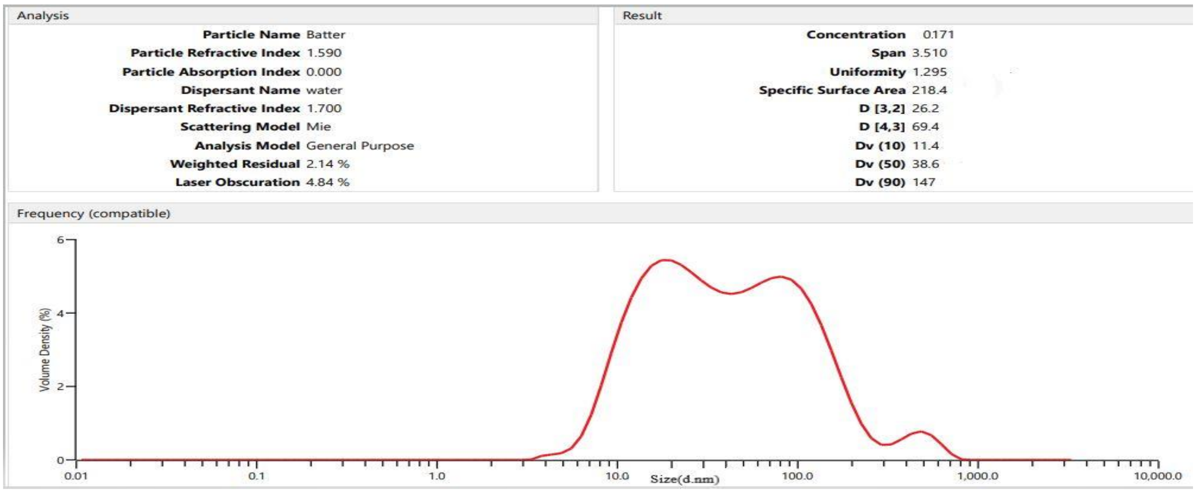


Figure no. 19: Particle size distribution and polydispersity index of *Withania Somnifera* root powder.

9.4.3. Particle size distribution and polydispersity index of F1.

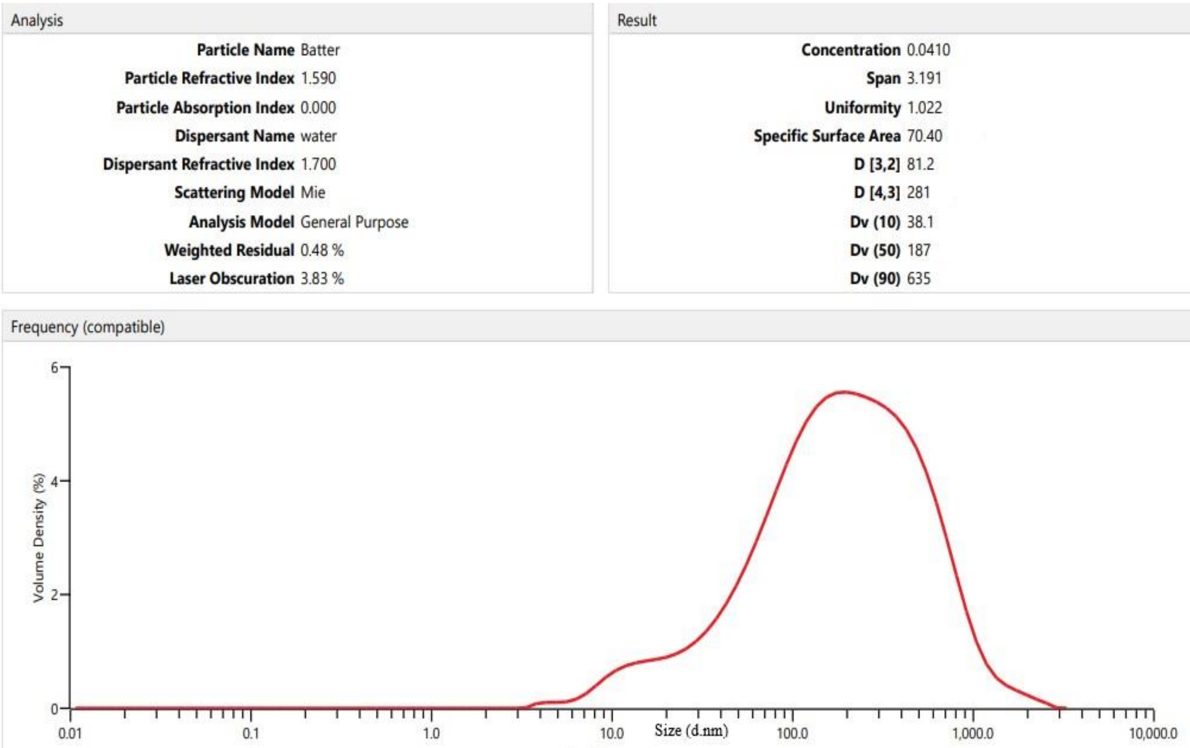


Figure no.20: Particle size distribution and polydispersity of F1

9.4.4 Particle size distribution and polydispersity index of F2.

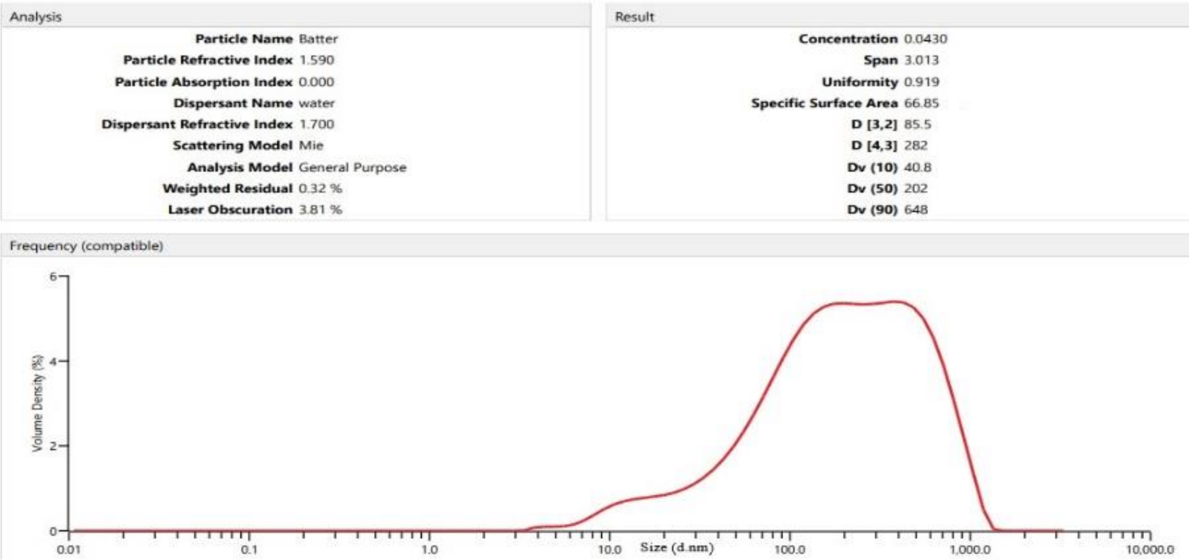


Figure no.21: Particle size distribution and polydispersity of F2

9.4.5 Particle size distribution and polydispersity index of F3.

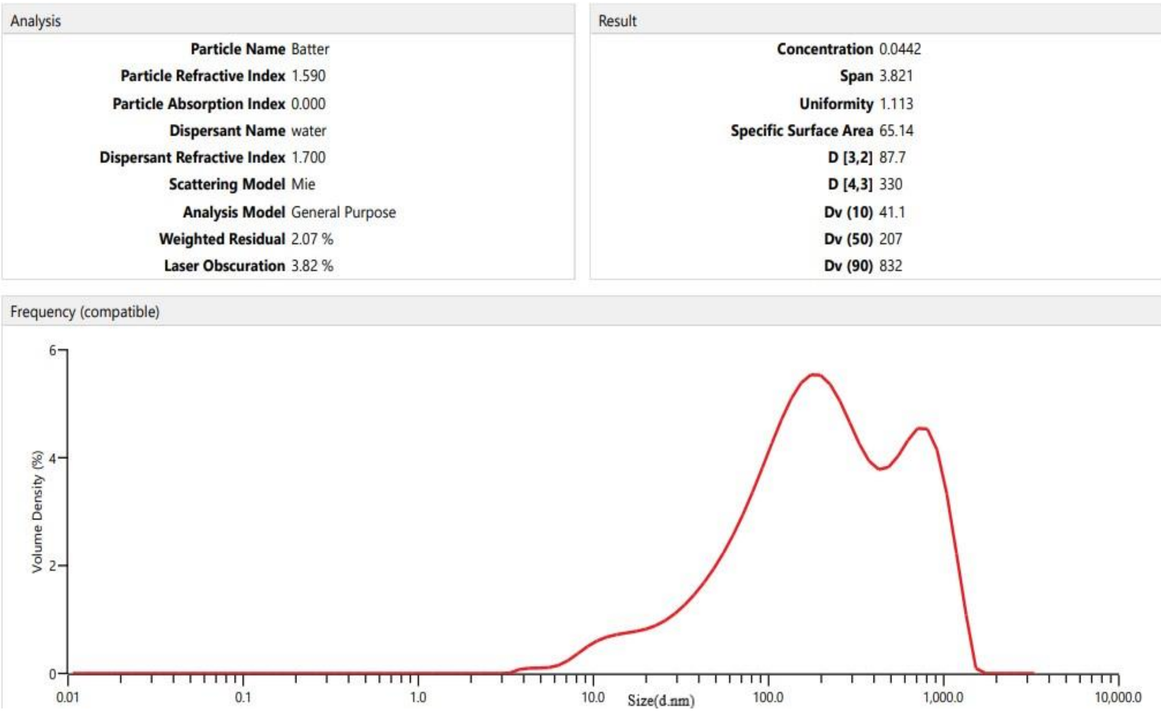


Figure no.22: Particle size distribution and polydispersity of F3

9.4.6 Particle size distribution and polydispersity index of F4.

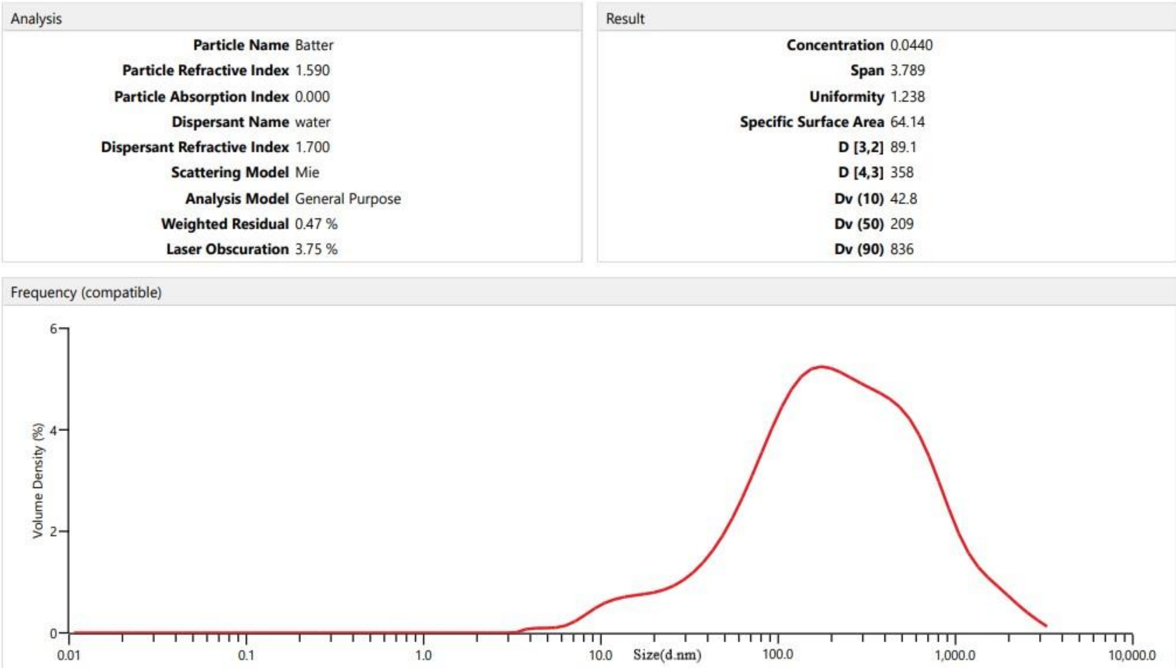


Figure no.23: Particle size distribution and polydispersity of F4

By comparing the particle size of *Withania Somnifera* root powder and synthesized *Withania Somnifera* root extract silver nanoparticles we confirmed the formation of silver nanoparticles.

The mean particle size of nanoparticles ranges from 1-100 d.nm. The particle size of *Withania Somnifera* root powder was found to be 218.4nm with a PDI of 0. 171. The particle size of synthesized *Withania Somnifera* root extract silver nanoparticles F1 to F4 formulations are F1(70.40 d.nm), F2(66.85 d.nm), F3 (65.14 d.nm), F4(64.1 d.nm) respectively.

Among the formulations F1 to F4, the F4 formulation showed the lowest particle size range of nanoparticles at (64.14nm). It indicates that the F4 formulation have nano size and good uniformity in particle size distribution (0.0440). So, the F4 formulation of synthesized *Withania Somnifera* root extract silver nanoparticle is selected as the best formulation while comparing with F1, F2, and F3 respectively.

9.5. ZETA POTENTIAL:

The determination of the zeta potential parameter (properly related to the double electric layer on the surface of colloidal particles) of a nanoparticle is essential as it provides an indication of the physical stability of nanoparticles. Extremely positive or negative zeta potential values cause larger repulsive forces, whereas repulsion between particles with similar electric charge prevents aggregation of the particles and thus ensure easy redispersion. In the case of combined electrostatic and steric stabilization, a minimum zeta potential of ± 20mV is desirable.

In this study, the zeta potential of *Withania Somnifera*root powder and synthesizedsilvernanoparticles of *Withania Somnifera*root extract were carried out. The zeta potential of *Withania Somnifera*root powder was found to be 130.5mV. The zeta potential values of synthesizedsilvernanoparticles of *Withania Somnifera*root extract formulations, F1–F4 was found to be in the range of F1(-20.3 mV), F2(-18.7 mV), F3 (-16.8 mV), F4(-14.9 mV) respectively, which indicates good physical stability of nanoparticles formed. The zeta potential graphs are presented in **Figures: (24- 28).**

The charge was negative due to the capping of silver nanoparticles on the surface of the *Withania Somnifera* root extract; so, negative zeta potential suggests that the interface is negatively charged due to double layer repulsion between the droplets attributed to nanoparticles. In general, the zeta potential value of ±20 mV is sufficient for the stability of nanoparticles. However, the high zeta potential proposes that the nanoparticles were adequately stabilized. It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed. The obtained values for the formulations indicate stable nanoparticles. Hence, the negative value confirms the repulsion among the particles and proves that they are very stable.

Moreover, the negative charge also proves the stability and thus preventing them from agglomeration. However, the F4 (-14.9 mV) formulation has a high zeta potential compared to the other formulations F1, F2, F3 respectively. So that proposes the F4 formulation of nanoparticles is the best formulation and it shows good physical stability of silver nanoparticles.

Table no 10: Zeta potential of synthesized nanoparticles

S.NO	Formulations	Zeta potential (mV)
1	F1	-20.3
2	F2	-18.7
3	F3	-16.8
4	F4	-14.9

9.5.1. Zeta Potential of *Withania Somnifera* root powder

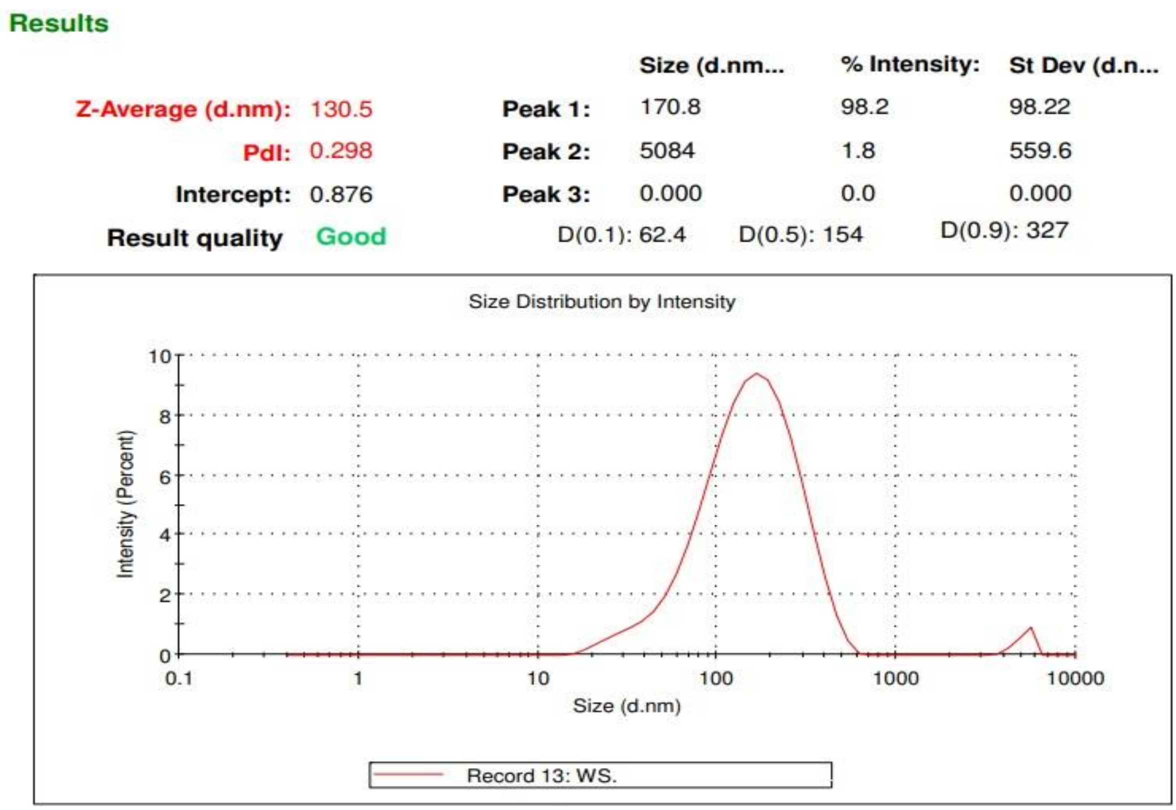


Figure no. 24: Zeta potential of *Withania Somnifera* root powder

9.5.2. Zeta Potential of synthesized nanoparticle F1

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-20.3	Peak 1:	-20.5	98.0	5.55
Zeta Deviation (mV):	5.97	Peak 2:	-1.25	2.0	2.33
Conductivity (mS/cm):	0.121	Peak 3:	0.00	0.0	0.00
Result quality	Good				

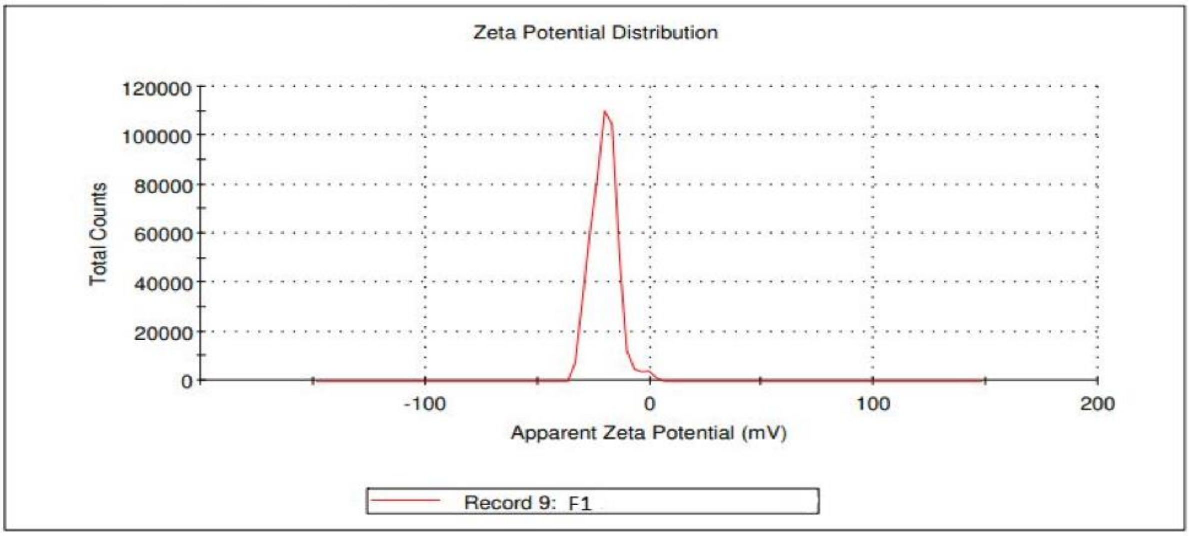


Figure no. 25: Zeta potential of synthesized nanoparticle F1

9.5.3. Zeta Potential of synthesized nanoparticle F2

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-18.7	Peak 1:	-18.7	100.0	5.37
Zeta Deviation (mV):	5.37	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.119	Peak 3:	0.00	0.0	0.00
Result quality	Good				

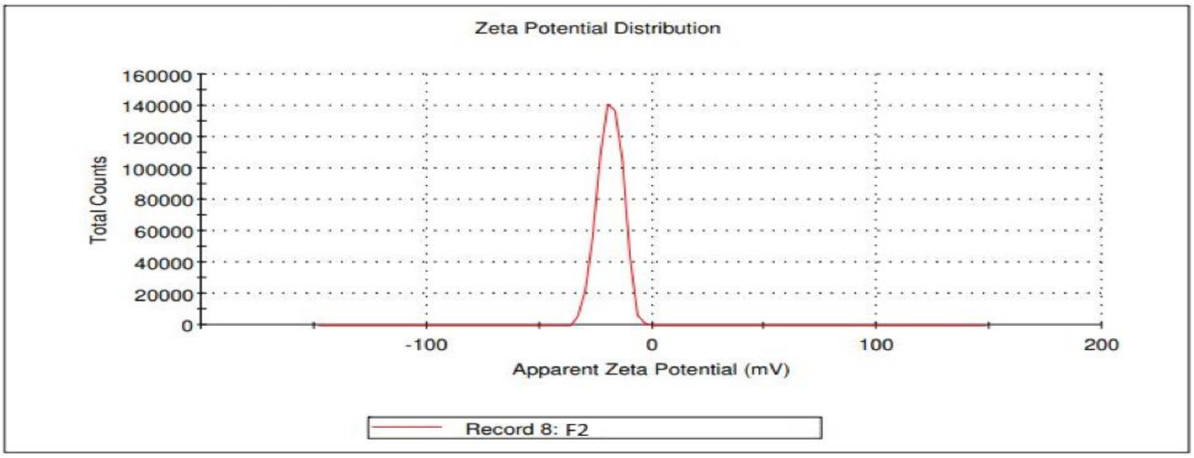


Figure no. 26: Zeta potential of synthesized nanoparticle F2

9.5.4. Zeta Potential of synthesized nanoparticle F3

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-16.8	Peak 1:	-16.8	100.0	8.22
Zeta Deviation (mV):	8.22	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.0681	Peak 3:	0.00	0.0	0.00
Result quality	Good				

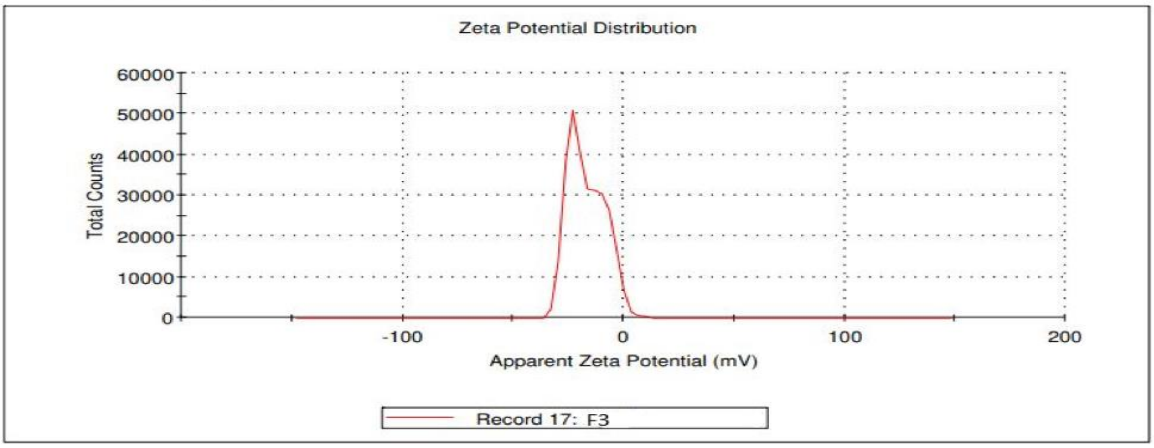


Figure no. 27: Zeta potential of synthesized nanoparticle F3

9.5.5. Zeta Potential of synthesized nanoparticle F4

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-14.9	Peak 1:	-19.2	67.9	5.34
Zeta Deviation (mV):	8.82	Peak 2:	-4.57	32.1	4.76
Conductivity (mS/cm):	0.0684	Peak 3:	0.00	0.0	0.00
Result quality	Good				

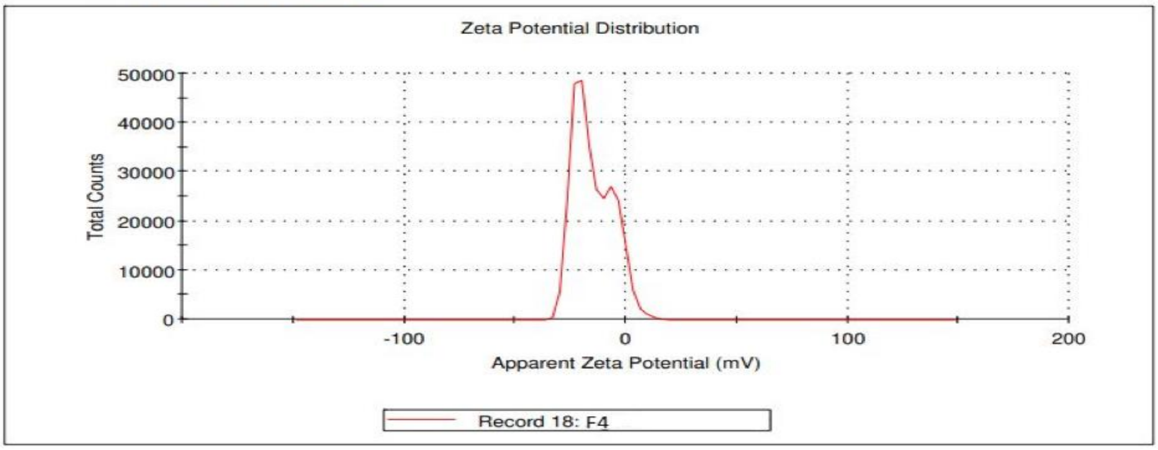


Figure no. 28: Zeta potential of synthesized nanoparticle F4

9.6. SEM ANALYSIS:

The successfully formulated nanoparticles of *Withania Somnifera* root extract and *Withania Somnifera* root powder was examined by scanning electron microscopy. The scanning electron microscopy was carried out to study the surface morphological changes in the formulated nanoparticles.

The size and shapes of the silver nanoparticles are affected by pH, temperature, incubation time and reductant concentration and method of preparation.

The SEM image of *Withania Somnifera* root powder was found abundant and with larger particle size when compared with that of the formulated nanoparticles. The shape of the *Withania somnifera* root powder showed an irregular shape whereas synthesized silver nanoparticles showed small rectangular shaped nanoparticles due to changes in temperature and pH. Thus, the SEM results of synthesized silver nanoparticles of the *Withania Somnifera* root extract showed nanosized and better surface morphology.

The SEM provided further insight into the morphology and size details of the silver nanoparticles that is the *Withania Somnifera* root extract as reducing and overlaying agent in the synthesis of silver nanoparticles of *Withania Somnifera*. Thus, F1, F2, F3 and F4 produced better surface characteristics.

The surface structure of nanoparticles in the SEM appeared good in shape. F4 formulation showed a small rectangular needle-shaped structure when compared with F1, F2 & F3 formulations of synthesized silver nanoparticles of *Withania Somnifera*. The micrograph was in agreement with those measured by particle size distribution. The SEM image of *Withania Somnifera* root extract and *Withania Somnifera* root powder is shown in (**Figure no:29 & 30**).

9.6.1. SEM image of *Withania Somnifera* root powder

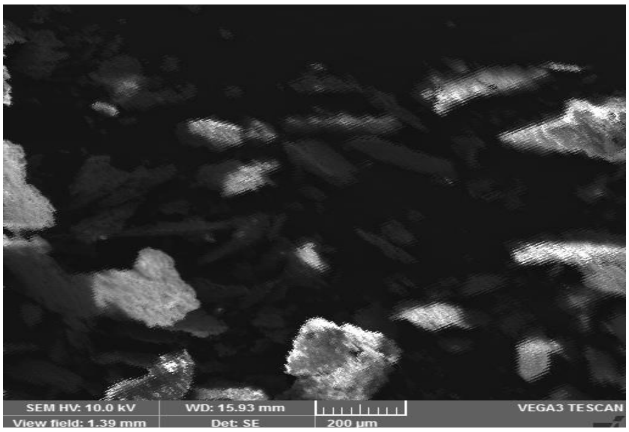


Figure no.29: SEM image of *Withania Somnifera*

9.6.2. SEM image of synthesized silver nanoparticles of *Withania Somnifera* root extract F1, F2, F3, F4.

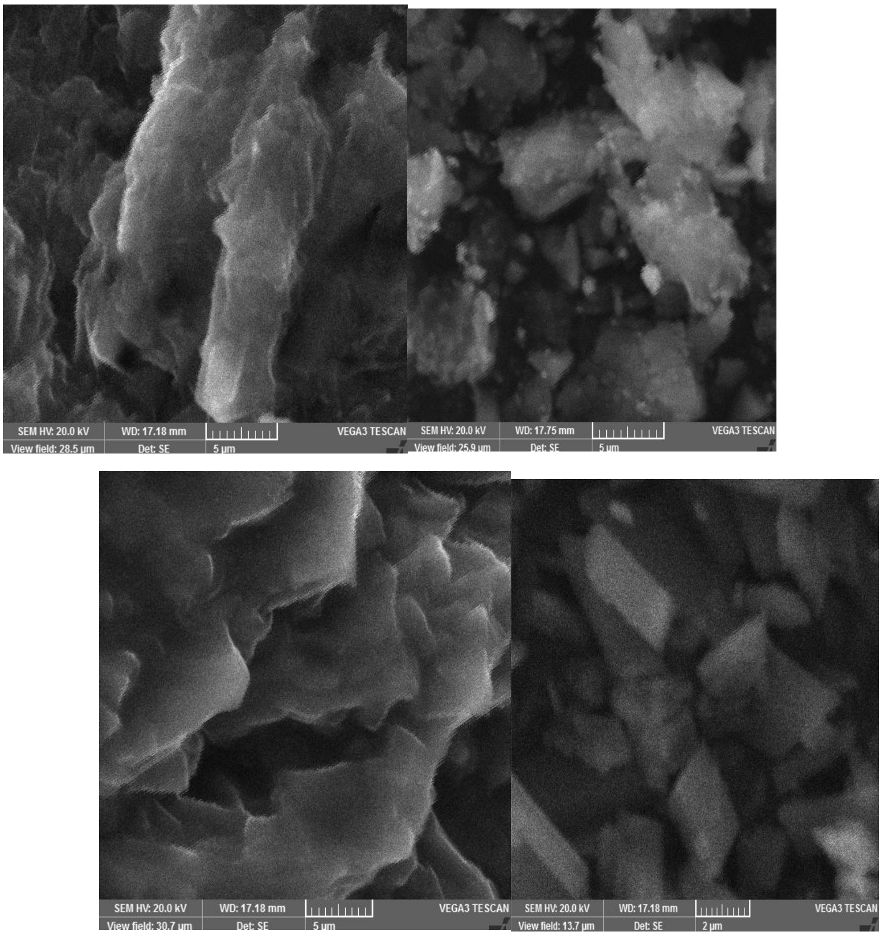


Figure no.30: SEM images of synthesized silver nanoparticles of *Withania Somnifera* root extract F1, F2, F3, F4

9.7. CRYSTALLOGRAPHY BY POWDER X-RAY DIFFRACTION [XRD]:

The nanoparticles synthesized in this method are characterized using powder XRD to confirm the particles as silver and to know the structural information. The XRD of the *WithaniaSomnifera* root powder and silver nanoparticles of *Withania Somnifera* was done.**Figure no.31** showed the XRD of *Withania Somnifera*root powderand **Figures: 32-35**showed the XRD of *Withania Somnifera*formulated silver nanoparticles.

The *Withania Somnifera* root powder was confirmed by the XRD pattern displayed in **Figure no.31**. The XRD diffraction peaks of *Withania Somnifera* root powder at 2θ values are 30.16^0, 36.02^0,4 7.02^0, 52.75^0, 61.72^0, 67.97^0, 72.66^0, 78.12^0.

The amorphous nature of formulated silver nanoparticles of *Withania Somnifera* was confirmed by the XRD pattern displayed in **Figures 32-34**. The silver nanoparticles of *Withania Somnifera* showed diffraction peaks at 2θ values of 27.7^0, 32.1^0,39.8^0,43.5^0, This indicates that the prepared silver nanoparticles are faced centered cubic in nature.

The diffraction peaks synthesized silver nanoparticles when compared with the *Withania Somnifera* root powder showed a maximum reduction of peaks at 2θ angle. Among the formulations F1, F2, F3 and F4, The F4 formulation showed reduced diffraction peaks when compared with that of other formulations.

In addition, two unassigned peaks appeared at 31.39^0 and 46.16^0. These peaks were weaker than those of silver. This may be due to the bioorganic compounds occurring on the surface of the silver nanoparticles. Unpredicted crystalline structures (31.39^0 and 46.16^0) are also present due to the organic compounds in the leaf extract.

The appearance of these peaks is due to the presence of the phytochemical compounds present in the leaf extracts. So, in the study, the appearance of stronger planes appeared in the formulations F1, F2, F3 and F4 indicating that silver is a major constituent in the biosynthesis of silver nanoparticles using *Withania Somnifera* root extract.

9.7.1. XRD of *Withania Somnifera* root

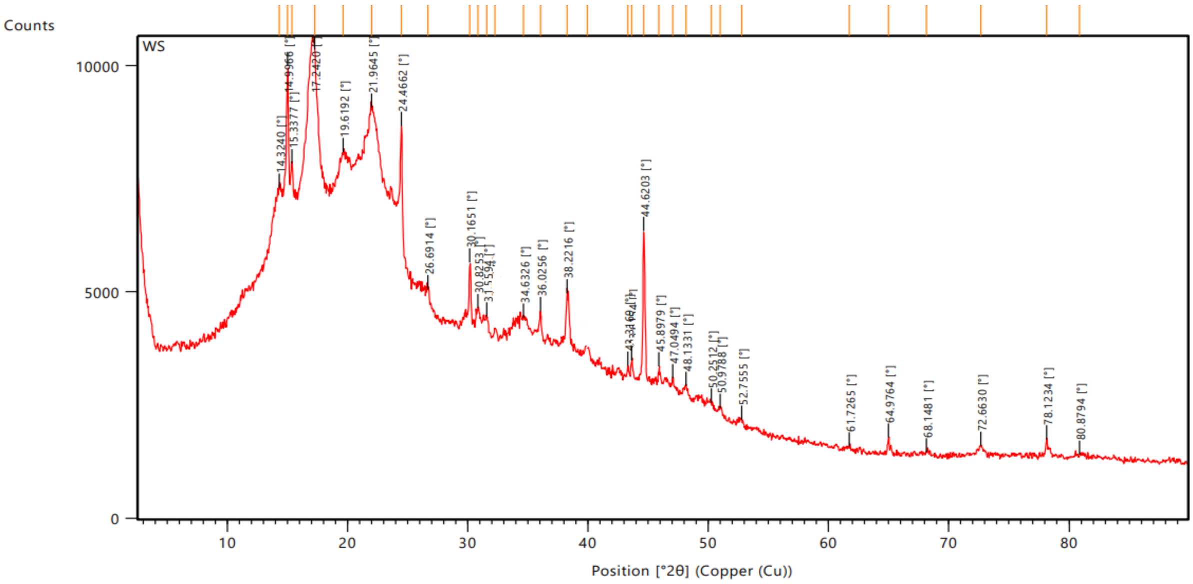


Figure no: 31 Shows XRD of *Withania Somnifera* root

9.7.2. XRD of synthesized nanoparticles F1

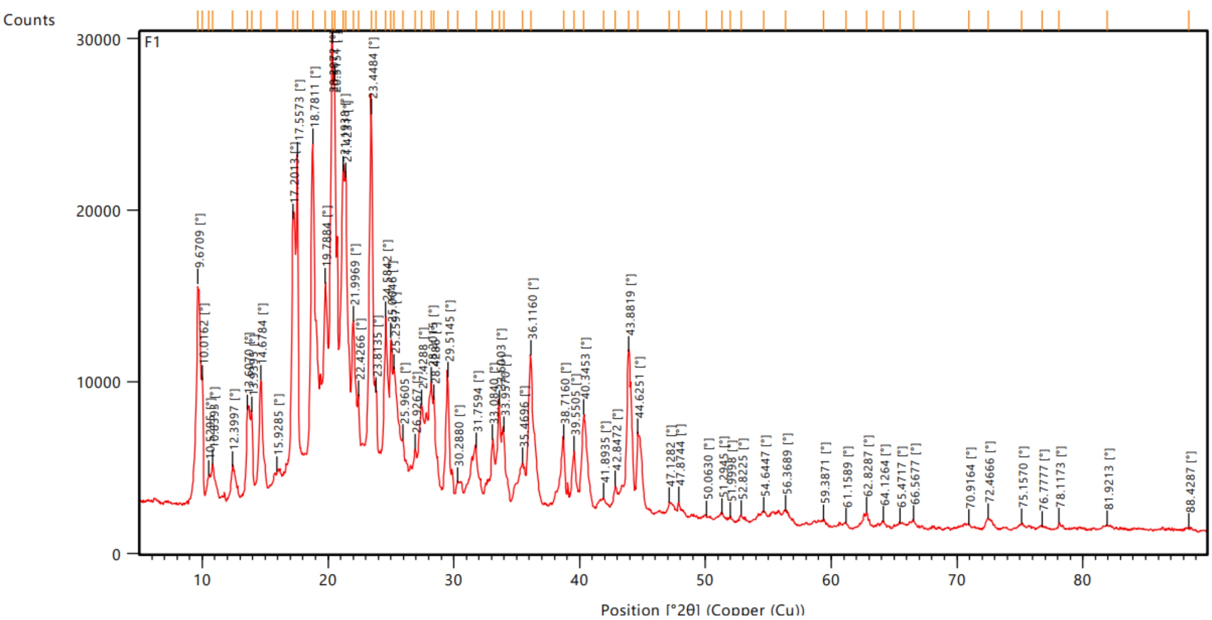


Figure no.32: Shows XRD of synthesized nanoparticles F1

9.7.3. XRD of synthesized nanoparticles F2

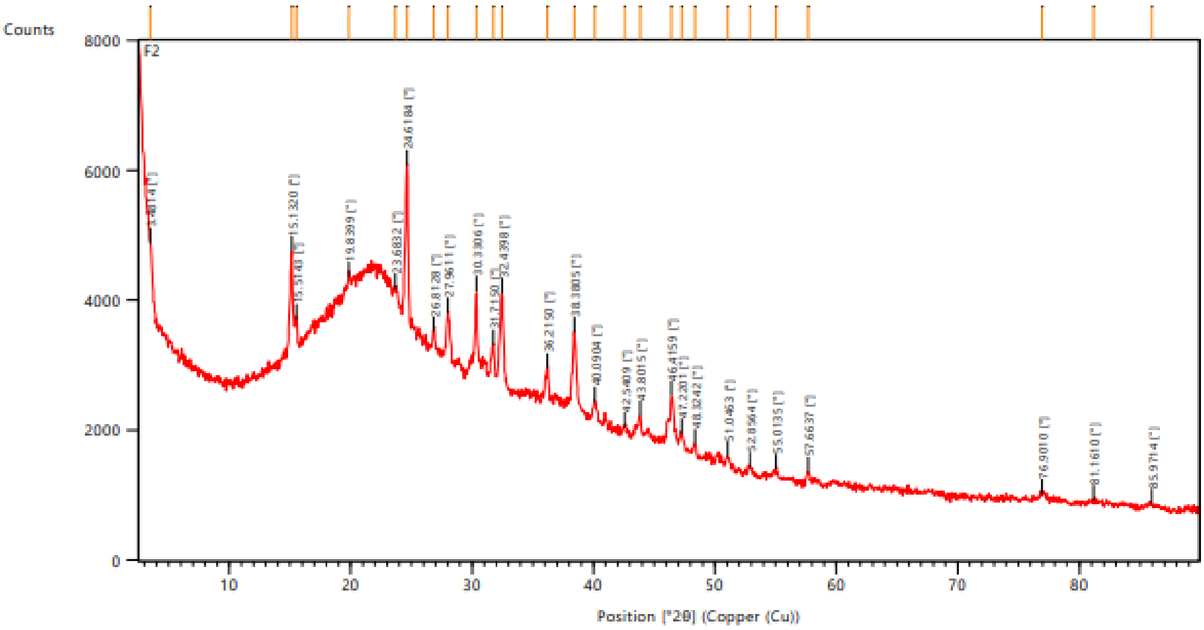


Figure no.33: Shows XRD of synthesized nanoparticles F2

9.7.4. XRD of synthesized nanoparticles F3

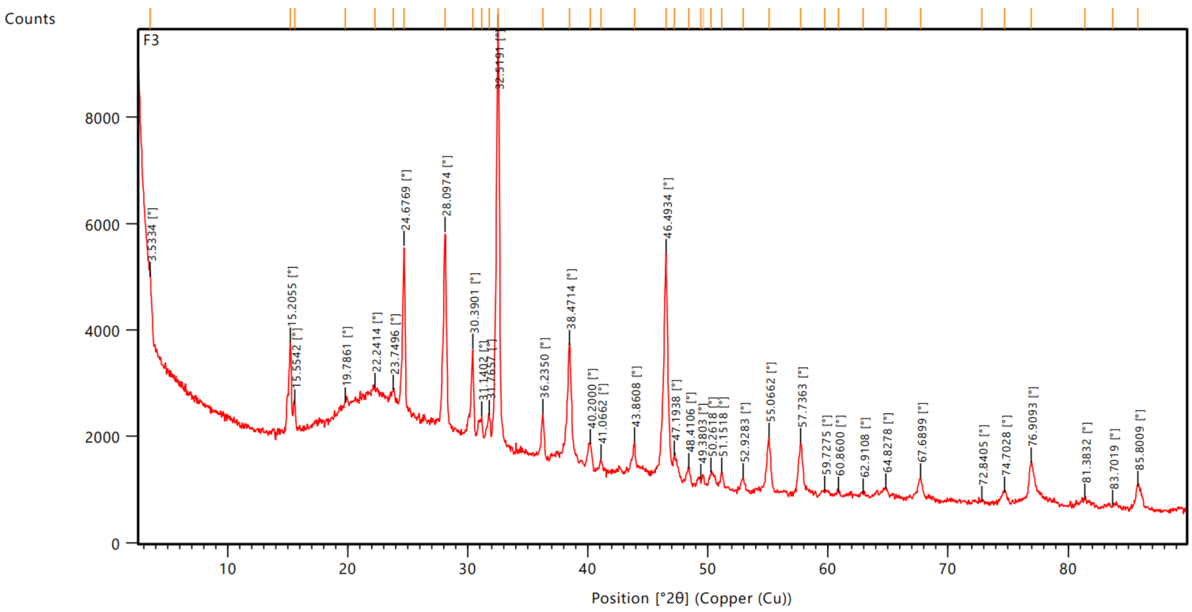


Figure no.34: Shows XRD of synthesized nanoparticles F3

9.7.5. XRD of synthesized nanoparticles F4

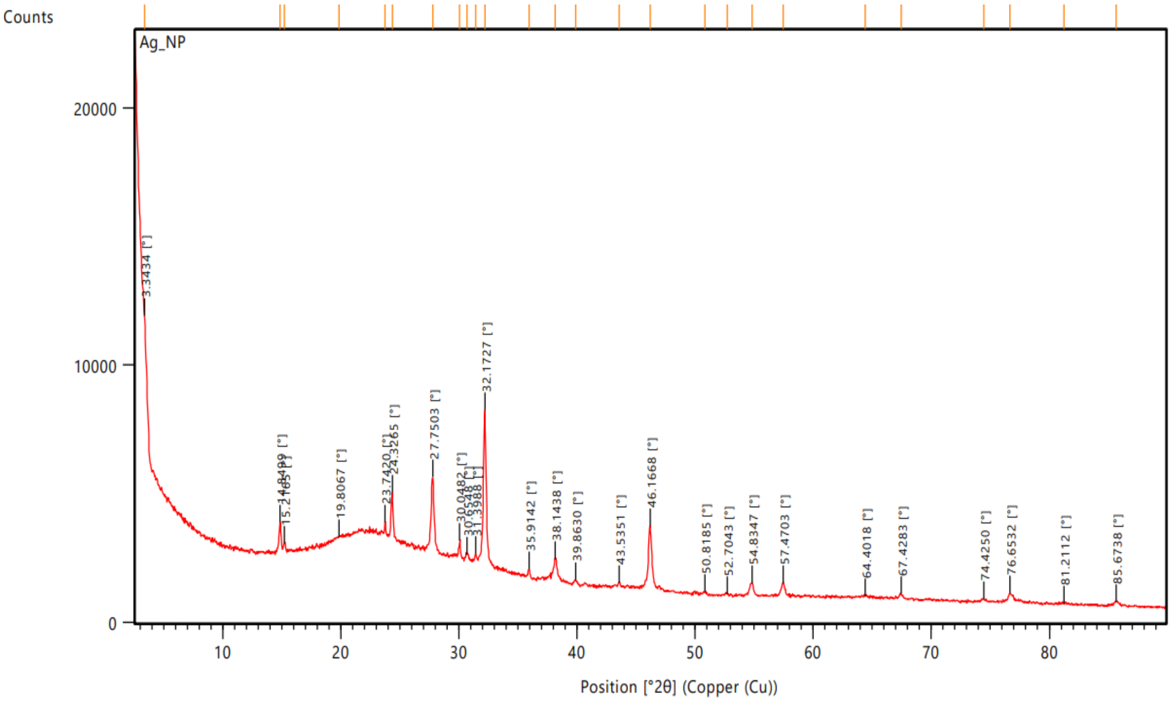


Figure no.35: Shows XRD of synthesized nanoparticles F4

In The XRD of the *Withania Somnifera* root powder and silver nanoparticles of *Withania Somnifera*, different formulations (F1, F2, F3 and F4). The F1, F2, F3 and F4 showed the biosynthesis of silver nanoparticles. But the characteristic peaks showed by the F4 formulation (27.7^0, 32.1^0,39.8^0,$43.5^{0)}$ indicates the best formulation for the biosynthesis of silver nanoparticles of *WithaniaSomnifera*root extract. Hence the F4 formulation is chosen for the further investigations.

9.8. ANTI-BACTERIAL ACTIVITY

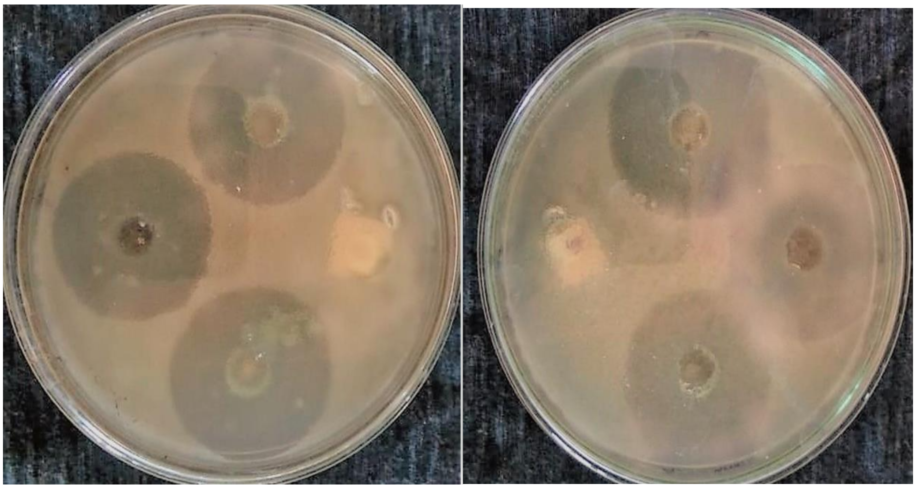
Antibacterial activities of the *Withania Somnifera*root extracted biosynthesized silver nanoparticles (F4) were carried out. The antibacterial activities of *Withania Somnifera* root extract and formulated silver nanoparticles of *Withania Somnifera* root extract against four bacterial strains *S.aureus, B.substilis, E.coli* and *K.pneumonia* were studied by measuring the diameter of the inhibition zone.

Figures no.36 & 37 shows the inhibition zone of the root extract of *Withania Somnifera* and formulated silver nanoparticles against the four bacterial strains. It is clear from the obtained results that as the concentration of the *Withania Somnifera* root extract and formulated silver nanoparticles were increased, the zone of inhibition was increased gradually.

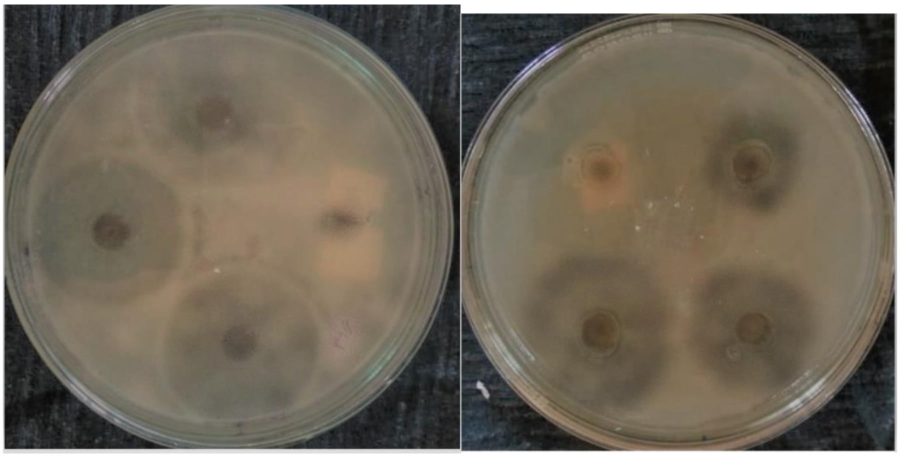
Table no.11: Diameter of the inhibitory zone of *Withania Somnifera* root extract against *S. aureus,B.substilis, E.coli,* and *K.pneumonia*

Sl.no	Concentration (µg/ml)	Diameter of inhibition zone (mm) - Gram Positive		Diameter of inhibition zone (mm) – Gram-Negative	
		S.aureus	*B.substilis*	*E.coli*	*K.pneumonia*
1	0	0	0	0	0
2	250	11	14	13	14
3	500	19	18	14	15
4	1000	22	20	18	17
5	Standard (Amoxyillin) 250	23	21	19	20

9.8.1. Inhibition zone of *Withania Somnifera* root extract against *S.aureus, B.substilis, E.coli* and *K.pneumonia*



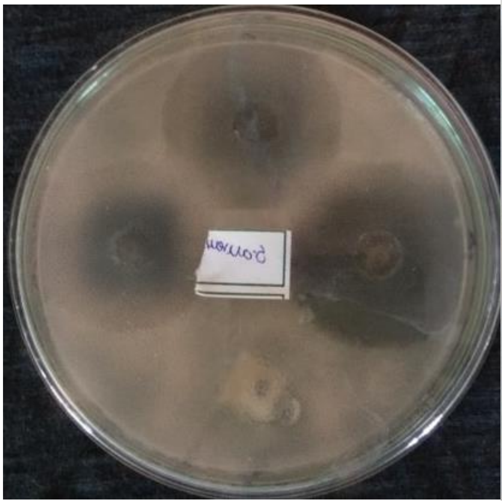
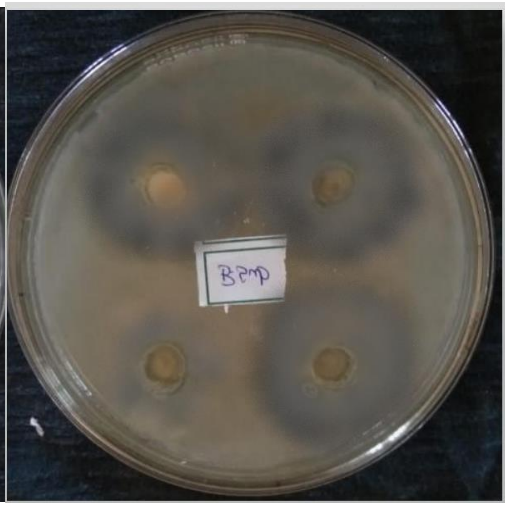
S.aureus ***B.substilis***



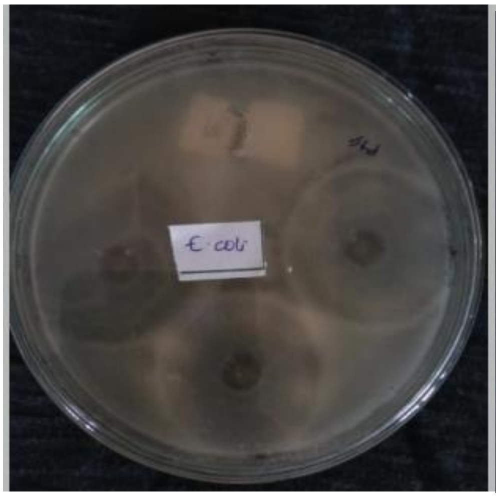
E.coli ***K.pneumonia***

Figure no.36: Inhibition zone of *Withania Somnifera* root extract against *S.aureus, B.substilis, E.coli* and *K.pneumonia*

9.8.2. Inhibition zone of silver nanoparticles of *Withania Somnifera* root extract against *S.aureus, B.substilis, E.coli,* and *K.pneumonia*

S.aureus *B.substilis*




E.coli *K.pneumonia*

Figure no.37: Inhibition zone of silver nanoparticles of *Withania Somnifera* root extract against *S.aureus, B.substilis, E.coli,* and *K.pneumonia*

Table no.12: Diameter of the inhibitory zone of silver nanoparticle of *Withania Somnifera* root extract against *S.aureus, B.substilis*, *E.coli,* and *K.pneumonia*

Sl.no	Concentration (µg/ml)	Diameter of inhibition zone (mm) - Gram - Positive		Diameter of inhibition zone (mm) – Gram-Negative	
		S.aureus	*B.substilis*	*E.coli*	*K.pneumonia*
1	0	0	0	0	0
2	250	18	16	15	14
3	500	21	20	17	18
4	1000	24	21	20	19
5	Standard (Amoxyillin) 250	25	23	21	20

From the study, we concluded that formulated silver nanoparticles show more anti-bacterial activity compared with that of the extract. The potent antibacterial properties of the silver nanoparticle may be attributed to the released silver ions, which could have an interaction with microorganisms using their attaching to the surface of the cell membranes of bacteria, penetrating the bacterial cells, and attaching the membrane permeability and respiration. In the bacterial cells, silver nanoparticles could even interact with sulfur- and phosphorus-containing compounds like DNA to give rise to the deadly impairment of microorganisms.

9.9. *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY

The in vitro anti- inflammatory activity was studied for *Withania Somnifera*root extract and formulated silver nanoparticles of *Withania Somnifera*root extract (F4). The in-vitro bioassay results of the anti-inflammatory effect of *Withania Somnifera*root extract and formulated silver nanoparticles assessed against denaturation of bovine serum albumin are summarized in **Table no.13**.

The table significantly shows, the *Withania Somnifera*root extract also showed the anti -inflammatory activity of about (25% ,37% ,50% ,59% ,68%) respectively. It also indicates that by increasing the concentration of *Withania Somnifera*root extract the anti-inflammatory activity was also increased, so we came to know the *Withania Somnifera*root extract shows anti-inflammatory activity.

The formulated silver nanoparticles of *Withania Somnifera*root extract (F4) formulation showed the percentage protein denaturation as (30% ,44% ,53% ,63% ,71%). it indicated by increasing the concentration of silver nanoparticles the anti-inflammatory activity also increases.

This activities of *Withania Somnifera*root extract and formulated silver nanoparticles of *Withania Somnifera*root extract (F4) was compared with the Aceclofenac, antibiotic (standard). The **Table no: 13** shows the *in vitro* anti-inflammatory activity of silver nanoparticles of *Withania Somnifera* root extract,*Withania Somnifera* root extract and the standard. The statistical analysis of the anti-inflammatory activity of *Withania Somnifera* synthesised silvernanoparticles are shown in **Figure no:38.**

Table no.13: Shows *In-vitro* anti-inflammatory activity of silver nanoparticles of *Withania Somnifera* root extract, *Withania Somnifera* root extract and the standard.

Sample	Concentration µg/ml	Absorbance	% Protein denaturation
Control	-	0.098	-
Test (Formulated Silver Nanoparticles)	20	0.14	30
	40	0.173	44
	60	0.21	53
	80	0.263	63
	100	0.334	71
Withania Somnifera root extract	20	0.131	25
	40	0.156	37
	60	0.195	50
	80	0.239	59
	100	0.314	68
Standard (Aceclofenac)	20	0.181	45
	40	0.218	55
	60	0.253	61
	80	0.322	70
	100	0.381	75

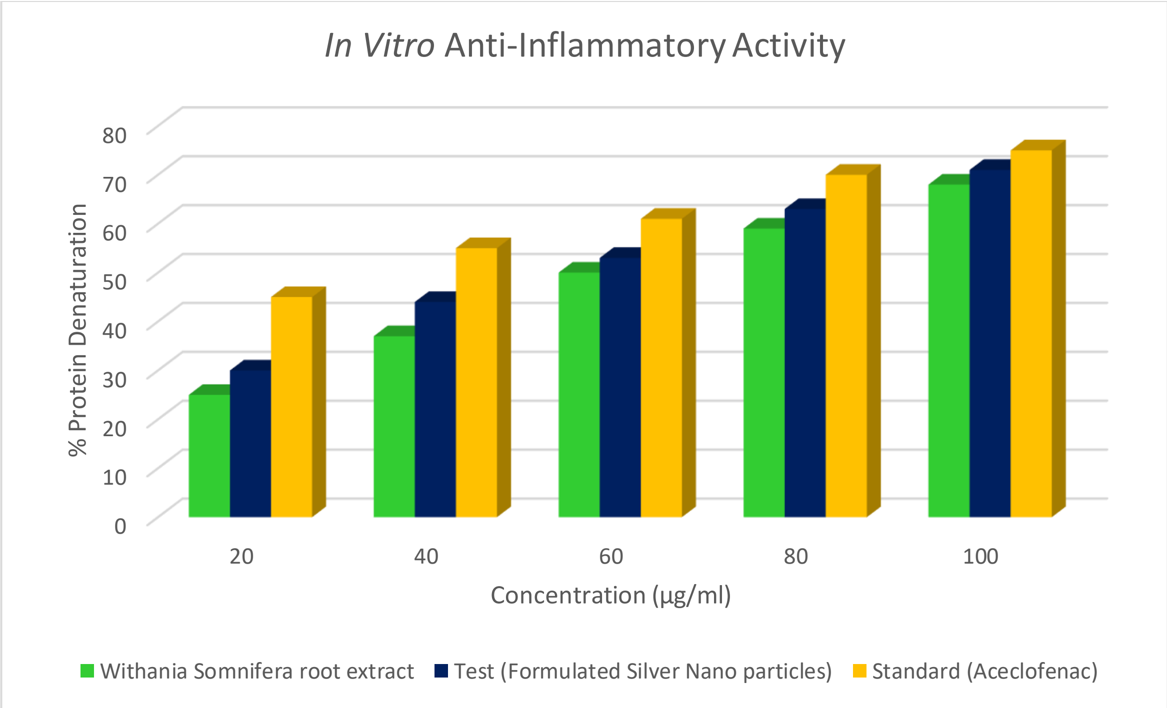


Figure no.38: *In-vitro* Anti-inflammatory activity of silver nanoparticles of *Withania Somnifera* root extract.

In the study, by comparing the*in vitro* anti-inflammatory activity of *WithaniaSomnifera* root extract and the synthesized silver nanoparticles of *Withania Somnifera* root extract(F4), the synthesized silver nanoparticles of *Withania Somnifera* root extract(F4)showed more percentage protein denaturation. Thus, the F4 formulation of the synthesized silver nanoparticles of *Withania Somnifera* root extract shows best anti-inflammatory activity.

SUMMARY AND CONCLUSION

10. SUMMARY AND CONCLUSION

This study represents, green synthesis is a safe, inexpensive, and sustainable method for the synthesis of nanoparticles. The green synthesis of metal and metal oxide nanoparticles is extremely appealing in the present and future eras. Various kinds of natural extracts like bacteria, fungi, yeast, and plant extracts have been used as effective supplies for the fabrication of materials. Among them, the plant extracts made by biological methods, have proven to possess chiefly stable products that do not cause any adverse reactions, and the waste disposal is toxic-free and effortless.

The synthesized silver nanoparticles by green synthesis are having a wide range of applications that are applied extensively both in the field of environmental remediation and in other important areas like pharmaceutical, food, cosmetic industries, and many more.

In the study, the biological synthesis of *Withania Somnifera* silver nanoparticles was successfully prepared. The method was found to be simple and does not need any specialized equipment or isolation techniques. Different characterization methods prove the formation of silver nanoparticles. The synthesized silver nanoparticles were of an estimated size, the first indication was visual observation and further characterization using the UV-Visible spectroscopy showed a peak at **431nm** for F4 formulation, which confirmed the synthesis of silver nanoparticles. The FTIR result of formulated silver nanoparticles showed no interaction between silver nitrate and the extract. The particle size of the nanoparticles is homogeneous in size and the size distribution is compared with that of the extract. All the formulations showed lower particle sizes. Among these, the F4 formulation showed the lowest particle size range of **(64.14 d.nm).** Zeta potential is an indication of the stability of the formulations which should be around ±20 mV. The Zeta potential of F4 **(-14.9 mV)**indicated good quality and showed the best formulation. The image of the SEM result showed agood surface morphologyin small rectangular needle-shaped structure for F4 formulation when compared with that of *Withania Somnifera* root extract. The XRD technique for F4 formulation showed reduced diffraction peaks when compared with that of *Withania Somnifera*root extract and the XRD technique showed the faced centered cubic in nature of the nanoparticles.

The formulated F4silver nanoparticle was investigated for its Anti-Bacterial and Anti-inflammatory activity. The Anti-Bacterial activity against Gram-positive and Gram-negative bacteria was carried out by the agar diffusion method. The biosynthesized silver nanoparticles exhibited more excellent antibacterial activity against S. *aureus* and B. *substilis* than E. *coli* and K. *Pneumonia*. The formulated silver nanoparticle, F4 formulation also showed Anti-inflammatory activity, which was investigated by using in -vitro method of denaturation of protein albumin. Through this study, we summarized that the formulated silver nanoparticles showed good anti-inflammatory activity when compared to the extract.

The present study showed the potential of *Withania Somnifera* root extract to reduce the silver ions to synthesize silver nanoparticles that can be used for different applications like Anti-bacterial and Anti-inflammatory activity. In the future, it can be further formulated for various clinical applications as well. From the Scientific view, there is more scope for the synthesis of silver nanoparticles because of their simple method for manufacture and various applications, like eco-friendly, low cost, and used for large scale synthesis.

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