GREEN SYNTHESIS OF SILVER NANOPARTICLES AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

In the research field the green synthesis of metallic nanoparticle has attributed tremendous attention in recent years due to availability of plants and cost effective synthesis. Pisum sativum and Sauropus androgynus are plants with high biological activity due to its high content of proteins, vitamins, carbohydrates, lipids and secondary metabolites. In the present work we synthesized stable silver nanoparticles by reduction of silver nitrate solution (Ag^+ to $Ag^{0)}$ using Pisum sativum peel and Sauropus androgynus leaf extract as the reducing agent. The synthesized silver nanoparticles were characterized by UV-Visible spectroscopy, FT-IR and X-ray diffraction (XRD). The nanoparticles were analysed with SEM for high resolution image of surface structure. Antimicrobial activity of synthesized silver nano particles against six bacterial strains such as *Vibrio Cholerae, Salmonellatypi, Escheriacoli, Micrococcusluteus, Shigellaflexneri, Pseudomonas flurescens* was screened.

Keywords: Silver nano particle, UV-Visible, FT-IR Spectroscopy, X-ray diffraction, SEM, Antimicrobial activity.

I .INTRODUCTION

In recent years, green synthesis of silver nanoparticles has gained much interest from chemists and researchers.Biosynthesis of inorganic materials, especially metal nanoparticle using micro-organisms and plants nano silver has many important applications. It is used an antimicrobial agent [1]. The need for environmental non-toxic synthetic protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals as byproducts. Thus, there is an increasing demand for “green nanotechnology” [2]. Green chemistry is the “utilization of a set of principle that reduces or eliminate the use or generation of hazardous substances in design manufacture and application of chemical products”[3]. Green synthesis of nanoparticles also provides advancement over other chemical and physical methods as they are simple, one step, and cost-effective, environment friendly and relatively reproducible and often results in more stable materials [4].Nano science is the study of phenomena and manipulation of materials at atomic, molecular and macromolecular where proscales properties differ significantly between these on and the scales and major scales. Conversely, nanotechnology refers to the design, characterization, productionand the application of structures, devices and systems for the Greek word vavoc and a nanometer to one (nm) is equal to one billionth of a meter, 10-9nm [5].

1.1 NANOSCIENCE

Nano science is defined as the study of physical and chemical characteristics displaced by particles in the 1 to 999 nanometer range. Nano science study and manipulation of at the Nano scale [6]. Nano science is the study of structure and material on the scale of nanometers. It refers to the particular technological goal of precisely manipulating atoms and molecules for fabrication of macro scale product. It is defined the study of phenomenon and manipulation of material is at atomic, molecular and macro molecular scales where properties differ significantly from those at a larger scale. Nano science of object typical size of 1-100 nm [7].

The branch of Nano science and technology is truly multi-disciplinary and is an emerging technology with full of promises to have an impact on virtually every spectrum of civilization including communication terms one of computing, textile cosmetics, sports, therapy, auto motives, purification, food and beverage industry etc [8]. Human hair 10-4 meter, blood cells are 10-5, bacteria is 10-6, viruses are 10-7, DNA is 10-8, molecular structure are 10-9 and soon.

Three key of Nanoterm

- ✓ Nano-Scale
- ✓ Nano Science.
- ✓ SNano-technology.

1.2 Nano Material

Nanomaterials can be constructed with top down or bottom up approaches.With top down very small structures are produced starting with a large part of materials through processes of mass removal. On the contrary, with bottom up technique nano scales materials are produced building up atom by atom, molecules by molecules. The evolution of this technique is self- assembling to their natural properties. Another aspect of the bottom up technique is the use of tools that can move individual atoms or molecules [9]. Nanomaterials are defined as those structures that do not even have all dimension in the Nano scale.For example, there are some materials, such as films or coating surfaces of computer chips, which are Nano scale only in one dimension and the other two are two macroscopic.Nanotubes or nanowires are examples of 2- dimendional nanostructures Nanometer scale. Compounds that have 3-dimentional Nanosized are colloids, precipitates and quantum dots. In this definition it is possible to find also materials with microscopic scalene are Nano crystalline materials made of nanomaterials-sized grains.

Material that have well defined physical properties, at the nanometer scale, can expressed quite different; and example is silver malleable and ductile, which, if produced spheres or size below 50nm, completely loses its malleability and ductility and become a very hard materials. As already maintained, the two main factors that give different properties of Nano scale materials are the increase of ratio between surface and volume and quantum effects. Fundamental properties for a materials, such as surface reactivity, resistance and electrical characteristics are size-depend and that depends of atoms on the surface respect total amount of atoms while for a micro structure this between surface atoms and total atoms tends to zero. For instance a particle with size of 30nm has 5% of atoms on its surface, whiles at 10nm are 20% and for nanoparticles with size of 3nm on the surface atoms is the half of the total. So a materials will be more reactive in its nanometric form compared to the coarse one. Quantum effects modify optical, electrical and magnetic properties.

1.2.1 One nanometric dimension

Materials with one Nanometric dimension, such as films and active and used for decades in electronic, chemistry and engineering; probably greatest development occurred in the industry of silicon integrated circuits. The monolayers thickness of an atom or molecule, are frequently used in chemistry.The formation and properties of these layers can reasonably be understood from an atomic point view, even in the case of complex layers, such as lubricants.Progress has been made in controlling the composition of the surface surfaces, such as the choice of the size or modulation of activity, finds many applications as cells with fuels of catalyst. The large surface area, full of nanoparticles, can be used in many application, especially in the chemical and energy sector where the great reactivity and selectivity is exploited to save money reducing the main resources for the production of energy [10].

1.2.2 Two nanometric dimension

Research and investigation of nanostructures with the Nano scale dimensions is quite recent, about the last 15 years.The interest increased when the first results emphasized the great and mechanical properties of these structures. Carbon nanotubes (CNTs) are grapheme sheets and are of 2 types: single wall, a single tube or multi-wall, a series of concentric tubes. In both cases, the diameter is a few nanometers and the length ranges from several micrometers to few centimeters.Nanotubes are mechanically very strong indeed, the value of the young's modulus is greater than 1 terapascal, very similar to diamond.In addition mechanical strength, they are very flexible and are good electrical conductors; actually they behave like semiconductors or metals.Applications of carbon nanotubes are several: the reinforcement of composite materials, Nano electronics, sensors and displays.In addition to the carbon nanotubes they are nanotubes of inorganic compounds of molybdenum disulfide; they have excellent properties as lubricant, high impact resistance and high reactivity in catalytic capability to store oxygen and lithium.Nanotubes based of oxides, such as titanium dioxide, may instead find good applications in the field of catalysis, photo catalysis and energy storage. Another example of materials with two nano metric dimensions are nanowires.These structure are composed of incredibly thin cables, or even by a linear series of point, self-assembled.Nanowires can be produced by a wide range of materials, such as silicon, gallium nitrate, and the indium phosphide.These structures have been shown to have magnetic, electronic data at high density for magnetic heads for writing on electronic media, for electronic devices and optoelectronic and metal interconnections between quantum and Nano devices [11].

1.2.3. Three nanometric dimension

All three dimensions of this kind of material are nano metric and as a consequence the volume of these structures is contained in a sphere-form structures with a size less than 100 nm. The main structures of this type are fullerenes, dendrimers, quantum dots and nanoparticles.

1.2.4 Classification of nano materials

Nano materials can be classified dimension wise into following categories:

Examples

- ✓ Nano rods, Nano wires have dimension less than 100 nm.
- ✓ Tubes, fiberes, planet lets have dimensions less than 100 nm.
- ✓ Particles, quantum dots, hollow spheres have zero (or) three dimensions<100 nm.

Nano materials in different phases can be classified as the nano material is called single phase solids. Crystalline, amorphous particles and layers are included in these class. Matrix composites; coated particles are included in multi- phase soilds. Multi-phase systems of nano material include colloids, aero gels, Ferro fluids etc [12].

1.2.5 Application of nanomaterial

Most application represent evolutionary of existing technology

1.2.5.1 Sunscreen

The traditional chemical UV protection approach from its poor long-term stability. A sunscreen based on mineral nanoparticles such a titanium dioxide offer several advantages. Titanium dioxide have a comparable UV protection property. Titanium dioxide and zinc dioxide are currently used in some sunscreens, as they absorb and reflect (UV) rays and are transparent to visible light. Nano sized iron oxide is present in some lipsticks as a pigments. The use of nano in cosmetics has raised a no of concerns about consumer safety.

1.2.5.2 Paint

Nanoparticles in paints improve their performance, for example by making lighter and giving them different properties. Thinner paint coatings (‘light weighting’) used for example on aircraft, reduce their weight, which could be good effect to the environment.

1.2.5.3Display

The huge market for large area, high brightness, flat-panel displays, as used in television screens and computer monitors, is driving the development of some nanomaterials. Nano crystalline zincselenite, zinc sulphite, cadmium sulphite and lead tellurian synthesized by sol gel technique are candidates for the next generation of light emitting phosphores [13].

1.3 Nano Particles

Nano means one billionth (or) 10-9. Thus, one nanometer is one billionth of a meter. The set of materials useful for probing the fundamental nature of matter. These material have unique structures and tunable properties. Particle is further classified according to diameter [14]. Coarse particles cover a range between 10,000 & 2,500 nanometers. Fine particles are sized between 2,500 & 100 nanometers. Ultrafine particles (or) no particles are sized between 1&100 nanometers. The term nanoparticles used to describes a wide variety of materials so submicron size. Scale, a human hair is the order of the 10,000 and 50,000 nm. A single red blood cell has a diameter of around 5000 nm, viruses typically have a medium dimension 10 to 100 nm and a DNA molecules has an order of 2-12 nanometers [15]. Nanoparticles have a large range of applications: In a short time in the cosmetics industry, textile and paint industries, in long times.Such as contrast agents for diagnostic image or carriers for drug delivery and hyperthermia currently, nanoparticles are not produced for direct use, but they are exploited as additives or ingredients in already existing products, in order to improve some properties. The diffusion of nanoparticles is very low compared to other Nano scale materials, because there is not yet in- depth information of their toxicity. Toxicity that can be decreased attaching them of the surface or binding with other composites. In fact, if nanoparticles are free or attached, you can have different impact on health and safety man or nature if the nanoparticles are free of bound to other molecules or structures [16].

1.3.1 Silver nanoparticles

Silver nanoparticles have unique, optical, electrical, and thermal properties and are being incorporated into products that range from photovoltaic to biological and chemical sensors (antimicrobial application, bio sensor material, composites fibers, cryogenic superconducting, cosmetic products, and electronic components). Several physical and chemical methods have been used for synthesizing and stabilizing silver nanoparticles. Most popular chemical approaches, including chemical reduction using a variety of organic and inorganic reducing agents, electrochemical technique, physicochemical reduction, and radiolysis are widely used for the synthesis of silver nanoparticles. Recently, nanoparticle synthesis is among the most interesting scientific areas of inquiry, and there is growing attention to produce nanoparticles using environmentally friendly methods (green chemistry). Green synthesis approaches include mixed-valence polyoxometalates, polysaccharides, Tollens, biological, and irradiation method which have advantages over conventional methods involving chemical agents associated with environmental toxicity.

This chapter presents an overview of silver nanoparticle preparation by physical, chemical, and green synthesis approaches. Example includes conductive inks, pasts and fillers which utilize silver nanoparticles for their high electrical conductivity, stability, and low sintering temperatures. Additional applications include molecular diagnostics and photonic devices, which take advantage of novel optical properties of the nanometrials. An increasingly common application is the use of silver nanoparticles for antimicrobial coatings, and many textiles, key boards, wound dressings, and bio medical devices now contain silver particles that continuously release a low level of silver ions to provide protection against bacteria. Many technique of silver nanoparticles synthesis are extremely expensive and also involve the use of toxic, Hazardous chemicals which may possess potential environmental and biological risks. Bio inspired synthesis of nanoparticles provides advancement over chemical and physical methods as it is a cost effective and environment ; friendly and in this method there is no need to use high pressure, energy, temperature and toxic chemical.The plant contains a variety of photochemical compound such as phenol, amino acids, flavones and these molecules are expected to self-assemble and cap the metal nanoparticles formed in their presence and there by induce some shape control during metal ion reduction. Thus, it is of interest to study the use of plant extracts for synthesis of silver nanoparticles in order to search for the high efficiency preparation. In addition, antibacterial activity of the synthesized silver nanoparticles was also determines and reported [17].

1.3.1 Method for Nanoparticle Synthesis

1.3.1.1 Physical Approach

Most important physical approaches include evaporation-condensation and laser ablation. Various metal nanoparticles such as silver, gold, lead sulfide, cadmium sulfide, and fullerene have previously have previously been synthesized using the evaporation-condensation method. The absence of solvent contamination in the prepared thin films and the uniformity of nanoparticles distribution are the advantages of physical approach with in comparison with chemical processes [18,19]. It was demonstrated that silver nanoparticles could be synthesized via a small ceramic heater with a local heating source [20]. The evaporated vapor can cool at a suitable rapid rate, because the temperature gradient in the vicinity of the heater surface is very steep in comparison with that of a tube furnace. This physically method can be useful as a nanoparticle generator for long-term experiments for inhalation toxicity studies, and as a calibration device for nanoparticle measurement equipment. Silver nanoparticles could be synthesized by laser ablation of metallic bulk materials in solution [21,25].The ablation efficiency and the charact6eristics of produced Nano silver particles depend upon many factors such as the wavelength of the laser impinging the metallic target, the duration of the laser pulses (in the fmeta-, pico, and nanosecond regime), the laser fluence, the ablation time duration and effective liquid medium, with or without the presence of surfactant [26-29]. One important advantage of laser ablation technique compared to other methods for production of metal colloids is the absence of chemical reagents in solution. Therefore, pure and uncontaminated metal colloids for further applications can be prepared by this technique [30].

1.3.1.2 Chemical Approach

The most common approach for synthesis of silver nanoparticles is chemical reduction by organic and inorganic reducing agents. In General, different reducing reducing agents such as sodium citrate, ascorbate, sodium borohydride ($NaBH_4$), elemental hydrogen, polyol precess, Tollens reagent N,N-dimethylformamide (DMF), and poly (ethylene glycol)- block copolymers are used for reduction of silver ions (Ag^+) in aqueous or non-aqueous solutions. The aforementioned reducing agents reduce silver ions (Ag^+) and lead to the formation of metallic silver (Ag^0) which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to formation of metallic colloidal silver particles [31-33]. It is important to use protective agents to stabilize dispersive nanoparticles that can be absorbed on or bind on to nanoparticle surfaces, avoiding their agglomeration. The presence of surfactants comprising functionalities (e.g., , amines, acids, and alcohols) for interactions with particle surfaces can stabilize particle growth, and protect particles from sedimentation, agglomeration, or losing their surface properties. Recently, a simple one-step process, Tollens method, has been used for the synthesis of silver nanoparticles with a controlled size. In the modified tollens procedure, silver nanoparticle films (50-200 nm), silver hydrosols (20-50 nm) and silver nanoparticles of different shape

1.3.1.3 Biological Approach

In recent years, the development of efficient green chemistry methods employing natural reducing, capping, and stabilizing agents to prepare silver nanoparticles with desired morphology and size have become a major focus of researchers. Biological methods can be used to synthesize silver nanoparticles without the use of any harsh, toxic and expensive chemical substances [34,36]. Many studies have reported successful synthesis of silver nanoparticle using organisms (microorganism and biological systems [37,38].

1.3.1.4 Synthesis of Silver nanoparticles by Bacteria

The first evidence of bacteria synthesizing silver nanoparticles was established using the Pseudomonas stutzeri AG259 strain that was isolated from silver mine. There are some microorganisms that can survive metal iron concentrations and can also grow under those conditions, and this phenomenon is due to their resistance to that metal. The mechanisms involved in the resistance are efflux systems. Alteration of solubility and toxicity via reduction or oxidation, biosorption, bioaccumulation, extracellular complex formation or precipitation of metals, and laSck of specific metal transport systems [39] . The most widely accepted mechanism of silver biosynthesis is the presence of the nitrate reductase enzyme. The enzyme converts nitrate into nitrite. In in vitro synthesis of silver using bacteria, the presence of alpha-nicotinamide adenine dinucleotide phosphate reduced from (NADPH) dependent nitrate reductase would remove the downstream processing step that is required in other cases [40].

1.3.2 Nanosilver

- ✓ One of the substances used in nano formulation is silver (nanosilver). Due to its antimicrobial properties, silver has also been incorporated in filters to purify drinking water and clean swimming pool water.
- ✓ Nano-silver particles are mostly smaller than 100 nm and consist of about 20- 15,000 silver atom. In addition, nanostructures can be the silver particles exhibit deviating physical-chemical properties

(like PH depend partitioning to solid ad dissolver particulate matters) and biological activities compared with the regular metal [41].

- ✓ Due to the properties of silver at the nanoscale, nanosilver is nowadays used in an increasing number of consumer and medical products. Because, silver is a soft white lustrous element, an important use of silver nanoparticles is to give a products a silver finish.
- ✓ Still, the remarkably strong antimicrobial activity is the major direction for development of nano-silver products. Examples are food packaging materials and food supplement, odour-resistant textiles, electronics, household appliances, cosmetics and medical advices, water disinfectants and room sprays.

1.3.3 Importance of Silver

Silver is one of the basic element that makes up our planet. It is a rare, but naturally occurring element, slightly harder than gold and very ductile and malleable. Silver can be present in four different oxidation states: Ag0, Ag2+, Ag3+. Pure silver has the highest electrical and thermal conductivity of all metals and has the lowest contact resistance [42]. Metallic silver itself is insoluble in water, but metallic salts such as AgNO3 and silver chloride are soluble is water (WHO, 2002). Metallic silver is used for the surgical prosthesis and splints, fungicides and coinage. Soluble silver compounds such as silver slats, have been used in treating mental illness, epilepsy, nicotine addition, gastroenteritis and infectious diseases including syphilis and gonorrhea. Although acute toxicity of silver in the environment is dependent on the availability of free silver ions, investigation ns have shown that these concentrations of Ag+ ions are too low to lead toxicity (WHO,2002) [43]. The wide variety of uses of silver allows exposure through various routes of entry into the body. Ingestion is the primary route for entry for silver compounds and colloidal silver proteins. Since silver in any form is not thought to be toxic to the immune, cardiovascular, nervous or reproductive system an it is not considered to be carcinogenic. Dietary intake of silver is estimated at 70-90ug|day. Silver is not toxic for human being. It is not affect the immune system, especially cardiovascular, nervous and reproductive system. Therefore silver relatively non-toxic. Now a day silver is very demand. It is used in textiles, plastics and medical industries. Silver products diffuse through the global economy. A major drawback of using microbes to synthesize silver nanoparticles is that it is a very slow process when in comparison with plant extracts. Hence, the use of plant extracts to synthesize silver nanoparticles becomes an option that is feasible.

1.3.3.1 Synthesis of Silver nanoparticles by plant

The Major advantage of using plant extracts for silver nanoparticles is that they are easily available, safe, and nontoxic in most cases, have a broad variety of metabolites that can aid in the reduction of silver ions, and are quicker than microbes in the synthesis. The main phytochemicals involved are terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids. Flavones, organic acids, and quinines are water-soluble phytochemicals that are responsible for the immediate reduction of the ions. Studies have revealed that xerophytes contain emodin, an anthraquinone that undergoes tautomerizing, leading to the formation of the silver nanoparticles. In the case of mesophytes, it was found that they contain three types of benzoquinones: cyperoquinone, dietchequinone, and remirin. It was suggested that the phytochemicals are involved directly in the reduction of the ions and formation of silver nanoparticles [44].

1.4 Need for Green synthesis

The need for biosynthesis of nanoparticles rose as the physical and chemical processes were costly. Biosynthesis of nanoparticles is a kind of bottom up approach where the main reaction occurring is reduction oxidation. Often, chemical synthesis method leads to presence of some of the toxic chemical absorbed of the surface that may have adverse effect in the medical applications. Green synthesis provides advancement over chemical and physical method as it is cost environment, environment friendly. This method need not to use of high pressure, energy, temperature and toxic chemical.

1.4.1 Action of Silver nanoparticles on microbes

There are however various theories on the action of silver nanoparticles on microbes to cause the microbicidal effect. Silver nanoparticles have the ability to anchor to the bacterial cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell. The formation of free radicals by the silver nanoparticles may be considered to be another mechanism by which the cells die. There have been electron spin resonance spectroscopy studies that suggested that there is formation of free radicals by the silver nanoparticles when in contact with the bacteria, and these free radicals have the ability to damage the cell membrane and make it porous which can ultimately lead to cell death.

- ✓ The bacterial cell in contact with silver take in silver ions, which inhibit several functions in the cell and damage the cells. Silver is a soft acid, and there is a soft base. The cells majorly made up of sulfur and phosphorus which are soft bases.
- ✓ Another fact is that the DNA has sulfur and phosphorous as its major components; the nanoparticles can act of these soft bases and destroy the DNA which would definitely lead to cell death. The action of these nanoparticles on the cell can cause the reaction to take place and subsequently lead to cell death.
- ✓ The interaction of the silver nanoparticles with the sulfur and phosphorus of the DNA can lead to problems in the DNA replication of the bacteria and thus terminate the microbes. It has also been found that the nanoparticles can modulate the signal transduction in bacteria.

Dephosphorylation is noted only in the tyrosine residues of gram-negative bacteria. The phosphotyrosine profile of bacterial peptide is altered by the nanoparticles. It was found that the nanoparticles dephosphorylate the peptide substrates on tyrosine residues, which leads to signal transduSction inhibition and thus the stoppage of growth [45].

1.4 Application of Silver nanoparticle

- Silver nanoparticles are being used in numerous technologies and incorporated into a wide array of consumer products that take advantage of their desirable optical, conductive and antibacterial properties [46].
- Potential application of silver nanoparticle like diagnostic, biomedical, optical image biological implant (heart valves) and medical applications like wound dressing contraceptive devices, surgical instruments and bone prosthesis. Used as coating for solar energy absorption and inter calculation material for electrical batteries as optical receptor as catalyst, biolabilling and as antimicrobial activity.Nano silver lined refrigerators, air conditioners and washing machines.
- Silver nanoparticles are used as antibacterial agents in the health industry food storage and textile coating. Silver nanoparticles are used as water treatment [47]. Silver nanoparticles are used in biosensess and numerous assays where the silver nanoparticle materials can be used as biological tags for quantitative detection. Silver nanoparticles are incorporated in apparel, footwear, paints wound dressings, appliances cosmetics and plastics for their antibacterial properties.
- Silver nanoparticles are used in conductive inks and integrated into composites to enhance thermal and electrical conductivity.Silver nanoparticles are used to efficiently harvest light and for enhanced optical spectroscopies including Metal-Enhanced Fluorescence's(MEF) and surface-enhanced Raman Scattering [46].
- The size of silver nanoparticles 1 nm to 100 nm commonly used are spherical silver disease that occur on many commercially important plants like been, strawberry, peril and other crop plant. In vitro studies deal with the effect of silver nanoparticles on collie to trichomspecies under different concentrations and growth medium as well as the control mechanism of silver nanoparticles against colletotrichumin field trials [47]

1.6 Toxicity of Silver nanoparticles

Silver nanoparticles also proved to be toxic to in vitro mouse germ line stem cells as they impaired mitochondrial function and caused leakage through the cell membranes. It is estimated that tones of silver are released into the environment fro, industrial wastes, and it is believed that the toxicity of silver in the environment is majorly due to free silver ions in the aqueous phase. The adverse effects of these free silver ions on humans and all living beings include permanent bluish-grey discoloration of the skin (argyria) or the eyes (argyrosis), and exposure to soluble silver compounds may produce toxic effects like liver and kidney damage; eye, skin, respiratory, and intestinal tract irritations; and untoward changes in blood cells [48]. That silver ions cause changes in the permeability of the cell membrane to potassium and sodium ions at concentrations that do not even limit sodium, potassium, ATP, mitochondrial activity. The literature also proves that nanosilver can induce toxic effects on the proliferation and cytokine expression by peripheral blood mononuclear cells. Nanosilver is also known to show severe toxic effects on the male reproductive system. In vivo studies on the oral toxicity of nanosilver on rats have indicated that the target organ in mouse for the nanosilver was the liver [49]. Nanosilver also has toxic effects on aquatic animals because silver ions can interact with the gills of fish and inhibit basolateral Na+-K+-ATPase activity, which cab in turn inhibit osmoregulation in the fish. The nanosilver toxicity were done in vitro conditions which are drastically different from in vivo conditions and quite high concentrations of nanosilver particles.

1.7 Nanotechnology

"Nano-technology is the production technology to get the extra high accuracy and ultra- fine dimensions, i.e. the preciseness and fineness on the order of 1 nm (nanometer), 10-9 meter in length." [50]. Nanotechnology is an important field of modern research dealing with design, synthesis and manipulation of particles structure ranging from approximately 1-100 nm in one dimension [51]. Nanotechnology is the design characterization, production and applications of structures devices and systems by controlling shape and size at Nano Meter Scale. Nano means the word in Greek language. One nm is one billionth of meter Nanotechnology is referred to as the ability to engineer the material precisely at the nanometer level the field of nanotechnology is emerging as a driving force as technologies becomes smaller, faster and more sustainable Nanotechnology is an advanced technology which deals with the synthesis of nanoparticles and processing of the nanomaterial and their application [7]. Nanotechnology is rapidly gaining importance in a number of areas such as health care, cosmetics food and feed, environmental health, mechanics, optics biomedical sciences, chemical industries, electronics optoelectronics catalysis, reprography, single electron transistor, light emitters, nonlinear optical devices and photoelectron chemical application [52]. Nanotechnology has the capacity to improve our ability to prevent, detect and remove environmental contaminant in air, water and soil in a cost effective and environmentally friendly manner. Nano science and nano technologies are revolutionizing our understanding of matter and are likely to have profound implications for all sectors of the economy, including agriculture and food, energy production and efficiency the automotive industry, cosmetics, medical appliance and drugs, household appliances, computer and weapon [53]. Nanotechnology is fundamentally changing the way in which materials are synthesized and devices are fabricated. Incorporation of Nano scale building blocks into functional assemblies and further into multifunctional devices can be achieved through a "Bottom-up approach". Research of the synthesis of Nanosized material is of great interest because of their unique propertsies like optoelectronic, magnetic and mechanical, which differs from bulk [54].

1.8 Nanotechnology For Crop Biotechnology

Nano capsules can facilitate successful incursion of herbicides through cuticles and tissues, allowing slow and regular discharge of the active substances. This can be act as 'Magic Bullets', containing herbicides, chemicals (or) genes which target exacting plant parts to liberate their substance.

1.8.1 Application of Nanotechnology in Medical Field

1.8.1.1Cure of cancer

Chemotherapy used in cancer is a very blunt tool. It consists of small molecules, which art toxic enough to kill cells in body. Although they are meant to kill only the cancer cells, they kill hair cells too, and cause all sorts of other havoc. This tiny microchip just two (or) three cells in diameter and tenth of a millimeter in length is important in a humans tumors, where they would remain for days of weeks. A small Nano sized drug capsule designed to seek & destroy malignant cells.

1.8.1.2 Future Implication

The impact of nanotechnology of wealth health and lines of people will be at least the equivalent of the combined influences of microelectronics, medical imaging, and computer aided, engineering and manmade polymers developed in this century.

1.8.1.3 Nanotechnology in research space

NASA in planning to use nanotechnology in its space projects. One main goal is a significant increase space craft capability with simultaneous mass reduction and miniaturization. A new era robotic exploration of the solar system is to be proposed by application of nanotechnology, through the development of small economical space craft with high autonomy and improved capability.

1.8.1.4 Environmental Conservations

Nano machines can and protect environmental damage by putting soil in its most perfect state. Therefore eliminating starvation in lands that have intolerant soil for growing food and solve rainforests issues, revive extinct plants, animals and cleanup Pollution.

1.8.1.5 Nano Computer

It could be placed in our bodies to augment our own brains. The size of the Nano computers will be similar to the size of a human cell and will have same raw computing power as the whole human brain. In the beginning such computer screens that were part of the skin or by projecting image on their eyelids.

1.8.1.6Automating Cleanings

Retractable walls, doors, paper thin televisions, automatic cleaning floors, trash would also be automatically removed, assemblers could rearrange the atoms in garbage to recycle and produce clean water or food [55].

II. EXPERIMENTAL METHOD

2.1 Materials and Chemicals

- Pisum sativum Peel
- Sauropus androgynous Leaf
- Silver nitrate
- Distilled water

2.2 Preparation the plant Extract

Fresh leaves of Pisum sativum peel, Sauropus androgynus leaf were collected from periyakulam. 25 gms of collected green leaves and peel were thoroughly washed with tap water and then distilled water, cut into fine pieces, and boiled in 100 ml of distilled water, for half an hour. The aqueous extract thus obtained was filtered through whattman No.1 filter paper to obtain a clear extract. The extract was collected in clean and fried 100 ml beaker. Then the filtrates were collected and refrigerated for further experiments.

2.3 Synthesis of Silver nanoparticles using leaf Extract

Aqueous solution of silver nitrate (AgNO3) at concentration of 0.02 mmol/ml was prepared and used for the synthesis of silver nanoparticles. The 10 ml of the above prepared Sauropus androgynus leaf extract under normal concentration was taken in a test tube and 10 ml of the silver nitrate solution is added (10:10) and used for stirred method. The change of colour takes place within few minutes and the precipitate is formed. The precipitate is separated using whattman No.1 filter paper.

2.4 Synthesis of silver nanoparticles peel extract

Aqueous solution of silver nitrate (AgNo3) at concentration of 0.02 mmol/ml was prepare and used for the synthesis of silver nanoparticles. The 10 ml of the above prepared Pisum sativum peel extract under normal concentration was taken in a test tube and 2 ml of the silver nitrate solution is added and used for stirred method. The change of colour takes place within few minutes and the precipitated is formed. The change is separated using whattman No.1 filter paper.

2.5 Characterization

A variety of characterization techniques are currently available some which precede the advent of nanoscience and technology and mostly drawn from material science.The development of new and integrated methods suited to probe nanomaterials is, however, a continuous process.The common techniques used in the characterization of nanoparticles are ultraviolet-visible (UV) spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-Ray diffraction studies (XRD), Inductively coupled atomic optical emission spectroscopy (ICPAES/OES), fluorescence spectroscopy (FS), X-ray photoelectron spectroscopy (XPS), scanning/transmission electron microscopy (SEM/TEM), dynamic light scattering (DLS), atomic force microscopy (AFM) and energy dispersion and analysis of x-rays (EDAX) [56].

2.5.1 UV-Visible Spectroscopy

UV-Vis spectroscopy is based on the absorbance of photons in the visible, near-UV and near-infrared regions of the electromagnetic spectrum. UV-Vis spectroscopy, as a technique of characterization, also involves the transition of electrons, and it complements fluorescence spectroscopy which deals with transitions of electrons from excited state to ground state [57]. Generally, spectroscopy is used to identify elements and compound for structural elucidation of matter at the atomic and molecular levels, the most common form being ultraviolet-visible (UV/Vis) spectroscopy [58]. Nanoparticles of silver have been extensively characterized by this technique due to their plasmonic nature and optical properties which are sensitive to size, shape, concentration and aggregation state. Although the wavelength for UV/Vis spectroscopy is within the (i.e. <1 um), some nanomaterials have much smaller dimensions and may require other nanoscale electroscopic technique for characterization [59].

2.5.2. FT-IR Spectroscopy

The Infrared region of the electromagnetic spectrum extends from the red end of the visible spectrum to the micro wave region include at the wavelength between 0.7 and 500 micrometer(or) wave number between 14,000 and 200 cm-1. Infrared spectroscopy (IR spectroscopy) is the spectroscopy that deals with the infrared region of the electromagnetic spectrum that is light with a longer wavelength and lower frequency than visible light. It covers a range techniques, mostly based on absorption spectroscopy. It can be used to identify and steady chemicals. The infrared portion of the electromagnetic spectrum is usually divided into three regions: the near, mid and far-infrared. The higher energy near IR, approximately 1400-4000cm-1 (0.8-2.5 µm wavelength). The mid-infrared, approximately 400-4000cm-1 (2.5- 25µm) may be used to study the fundamental vibrations and associated rotational-vibrations structure [60].

2.5.3 X-Ray Diffraction studies

The X-ray diffraction analysis investigates structure through the use of diffraction. The method of X-ray diffraction analysis are used to study, for example, metals, alloys, minerals, inorganic and organic compounds. Polymers, liquids, gases and the molecule of proteins and nuclei acids. The X-ray diffraction analysis has been used to establish the atomic structure is crystalline substance. The X-ray Diffraction patterns of silver nanoparticle were recorded according to the description of Wang. Samples were air dried, powdered and used for XRD analysis. X-ray Diffraction patterns were recorded in the scanning mode on Miniflex 600 Desktop (Fist support) analytical instrument operated at 40 KV current of 30 mA [61].

The average size of the nano particles can be estimated using the Debye-Scherrer equation

$$D=K\lambda/\beta\cos\theta$$

$$D\ (nm)= 0.9\lambda/\beta\cos\theta$$

2.5.5 Scanning electron microscopy

SEM is recorded by JEOL model 6390 computer- controlled microscope. The image obtained by SEM of the sample for Ag show quasi spherical like nanoparticles. The Ag nanoparticles have been distributed well within the range of ~100nm. The most important in scanning electron microscope is the use of electrons. Much logically flows from this, such as that a vacuum is needs to generate an electron beam, that electrons are used for imaging, and that we need to understand the interactions of electron beam with the sample in order to interpret the images. The use of electrons also impacts on image resolution and image color, and also explains the 3D nature of the micrographs (photos).The second most important concept is that we are looking only at the surface of the sample, and penetrating only on a small way into the sample with the electron beam shows that the SEM instrument images are displayed as monochromatic gray scale digital images in which each pixel carries only intensity information in shade of gray varying form be black at weakest intensity to white at the stronger. Sometimes these gray scale images be post-produced to display false color i.e. colorized gray scale [62].

2.5.6 Antimicrobial activity

Silver is powerful and natural antibiotic and antibacterial agent. Silver nitrate has long been considered as powerful and natural antibacterial agents. Synthesized silver nano particle were tested for antimicrobial activity against pathogenic bacteria organisms like Escherichia coli, Pseudomonas aureus and staphylococcus by standard disc diffusion methods. Luria Bertani (LB) broth/agar medium was used to cultivate bacteria. Fresh over night culture of inoculums (100µl) of each culture spread on to Muller Histon Agar (MHA) plates. Sterile paper disc of 5mm diameters containing 10mg/liters silver nanoparticles standard amikacin (100µg/ml) containing disc were placed in each plate as control. The plates were incubated at 37oc for overnight. Next day the inhibition zones around the discs were measured. 10 mg/litres silver nanoparticles served as standard and Amikacin (100mg/ml) containing disc were placed in each plate as control [62].

III. RESULTS AND DISCUSSION

3.1Synthesis of Silver Nanoparticles

Two different plant extracts were used to produce silver nanoparticles, the reduction of silver ions silver nanoparticles occurred after mixing silver nitrate with different plant and species extract, followed color change of solutions due to reduction of silver ion, which may be indicating of formation silver nanoparticles were characterized by UV-Visible spectroscopy, Scanning Electron Microscopy (SEM), X-Ray diffraction (XRD), Infrared (IR) was used to characterize the crystal structure, morphologies, impurities and optical properties of silver nano structure.

3.2 UV-Visible Spectroscopy

The bioreduction of silver in aqueous solution was monitored by periodic sampling of aliquots of the mixture and subsequently measuring UV-Visible spectra. UV-Visible spectral analysis was done by using shiatsu UV-1800 double beam spectrophotometer. The absorption peaks are measured in the rang 200-800nm [63]. The UV-Visible spectrum is recorded in acetone solvent by shimadzu 1800 UV Double beam spectrophotometer. UV-Visible spectrum has been widely used to characterize the semiconducter nanoparticles. As the particles size decreases absorption wave length will be shifted to shorter wave length and the band gap increases for the nano sized particles. This is the quantum confinement effect of semiconducter nanoparticles. **UV**-Visible spectroscopy analysis showed that the wave length of silver nanoparticle synthesized using pisum sativum, Sauropus androgynus extract centered at 400-500nm due to the excitation of surface plasmon vibrations in the silver nanoparticles. The figures 3.2 (a) and 3.2 (b) represent the UV-Visible spectrum of silver nanoparticles.

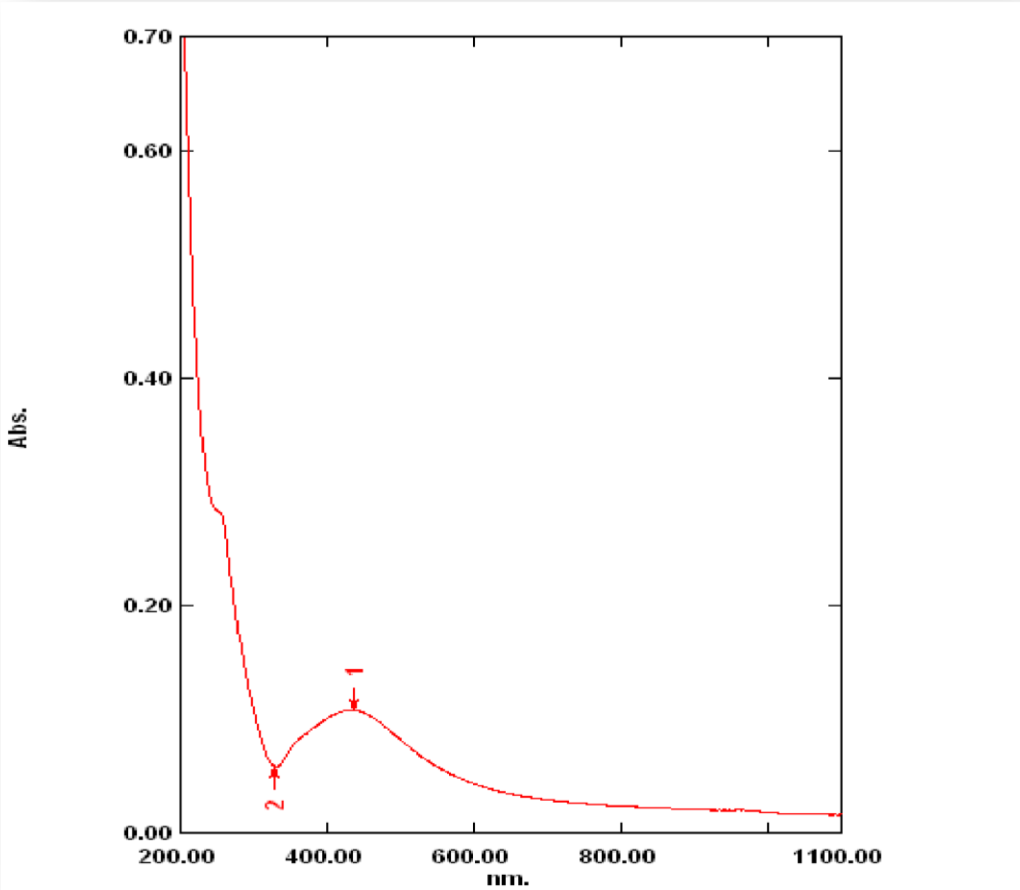

Fig.3.2 (a) UV-Visible spectrum of silver nanoparticles from pisum sativum

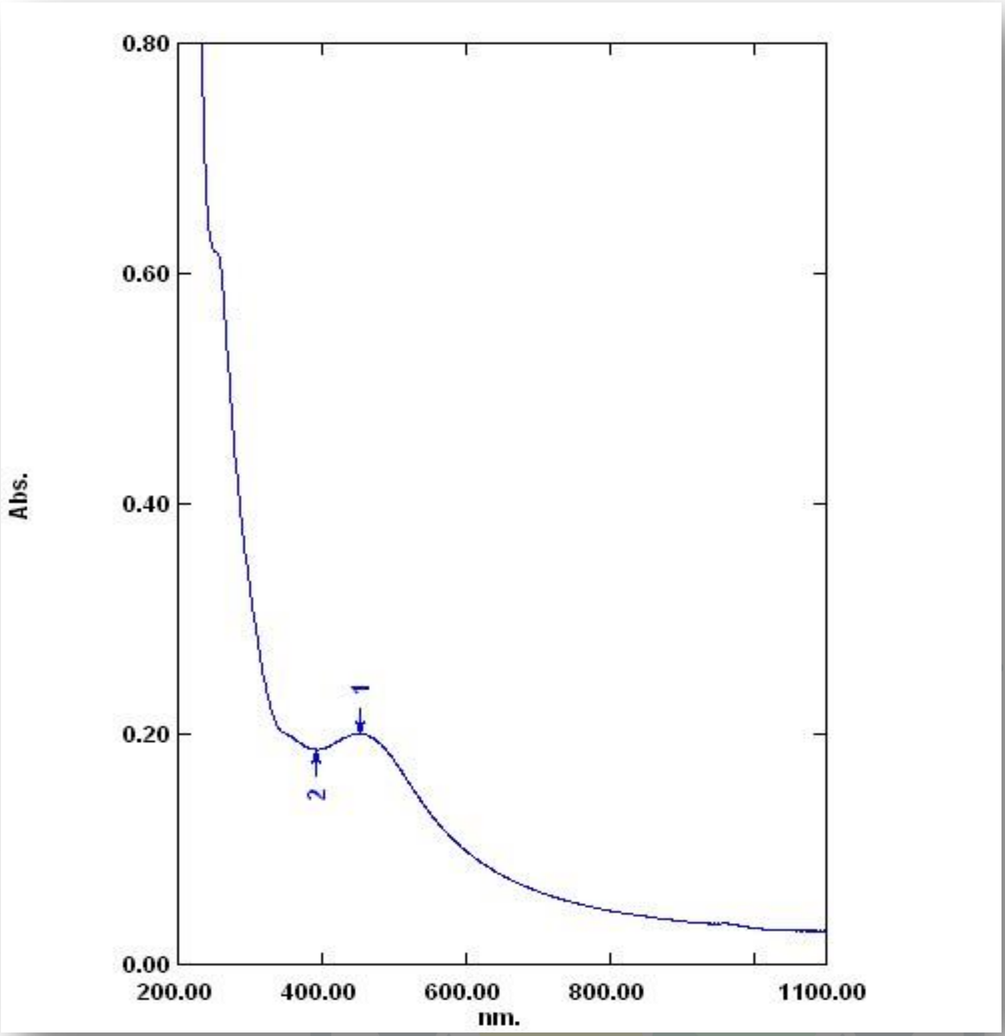

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Fig 3.2 b Uv-Visible spectrum of Silver nanoparticle from Sauropus androgynous

WAVELENGTH (nm)	ABSORPTION
451.00	0.20
391.0	0.19

In this spectra for silver nanoparticle is observed at 435 nm. This indicates the absorption shift towards the shorter wavelength, because of the particle size reduction. From these spectra, it is evident that resultant nanoparticles were embedded in silica matrix and exhibited the significant blue shift. This is an indicating of strong quantum confinement. The bulk value for Ag is at 400-500nm.

3.3 FT-IR Analysis

The FT-IR spectrum is recorded in acetone solvent by shimadzu 1800 UV double beam spectrophotometer. For FTIR measurements, Silver nanoparticle solution was centrifuged at 20,000 rpm for 20 minutes the pellet was washed three times with 20 ml of de-ionized water. The samples were dried and analyzed on IRPrestige-21 [SHIMADZU] operating at a resolution of 2cm -1. Further the FTIR analysis of the peel extract mediated silver nanoparticles was performed. The spectra gave maximum peaks at 3415cm-1, 1645cm-1, ,1388cm-1, 1024cm-1, which indicate the presence of alcohols/phenols, aldehydes, carbonyls, alkanes and aliphatic amines.

Infrared spectroscopy (IR) is the spectroscopy that deals with the Infrared region of the electromagnetic spectrum that is light with a longer wave length and lower frequency than visible light. It covers a range of techniques, Mostly based on the absorption spectroscopy. As with all spectroscopic techniques, it can be used to identify and study chemicals [64] . A common laboratory instrument that uses this technique is a Fourier Transform Infrared *(FT-IR) spectrophotometer. FT-IR spectra for silver shown in figure 3.3(A) and 3.3(B).

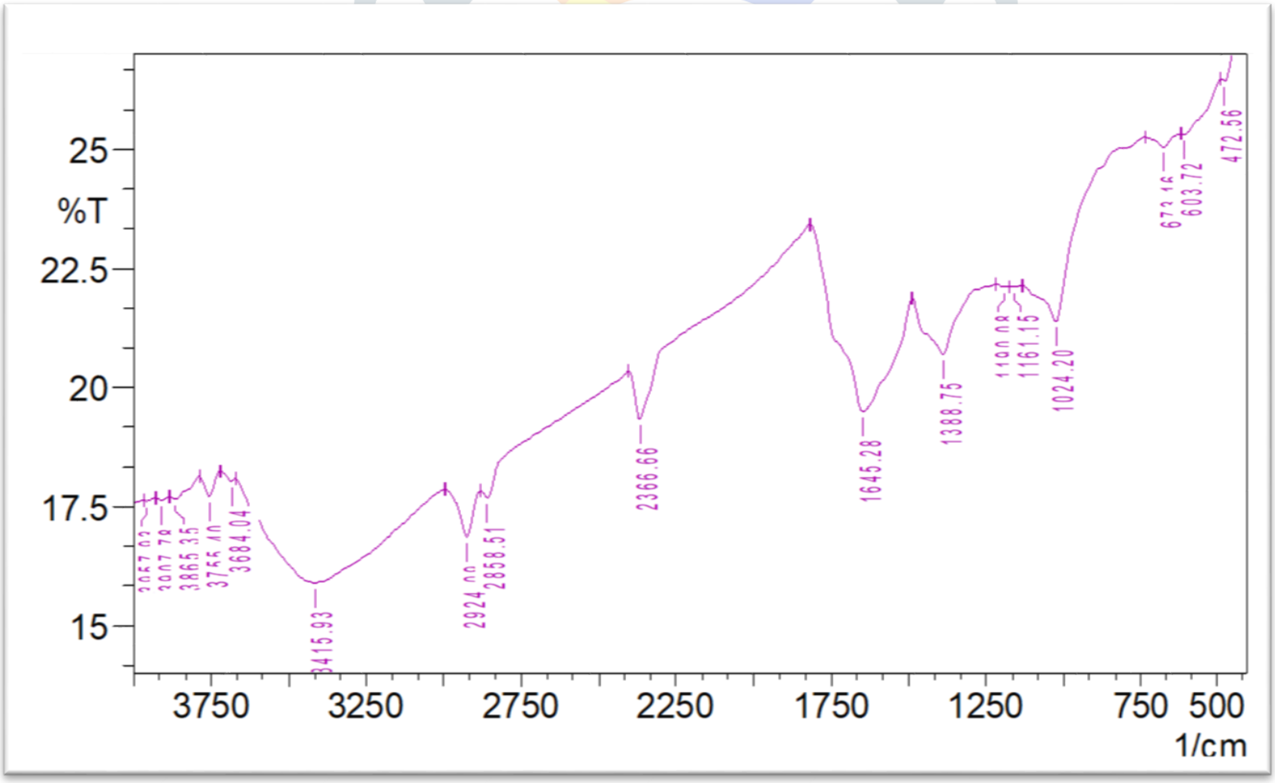


Figure 3.3(A) FT-IR spectrum for silver nanoparticles from pisum sativum

Frequency(cm^{1})	ν (C=C) stretch	ν (O-H) stretch	ν(C-O) stretch	ν (N-H) stretch	ν($N-O_2$) stretch	ν(C-O-C) stretch	ν($R_2C=NR_2$) stretch
PISUM SATIVUM	1645 Cm^{-1}	3415Cm^{-1}	1190Cm^{-1}	1024Cm^{-1} \	1388Cm^{-1}	1161 Cm^{-1}	1645 Cm^{-1}

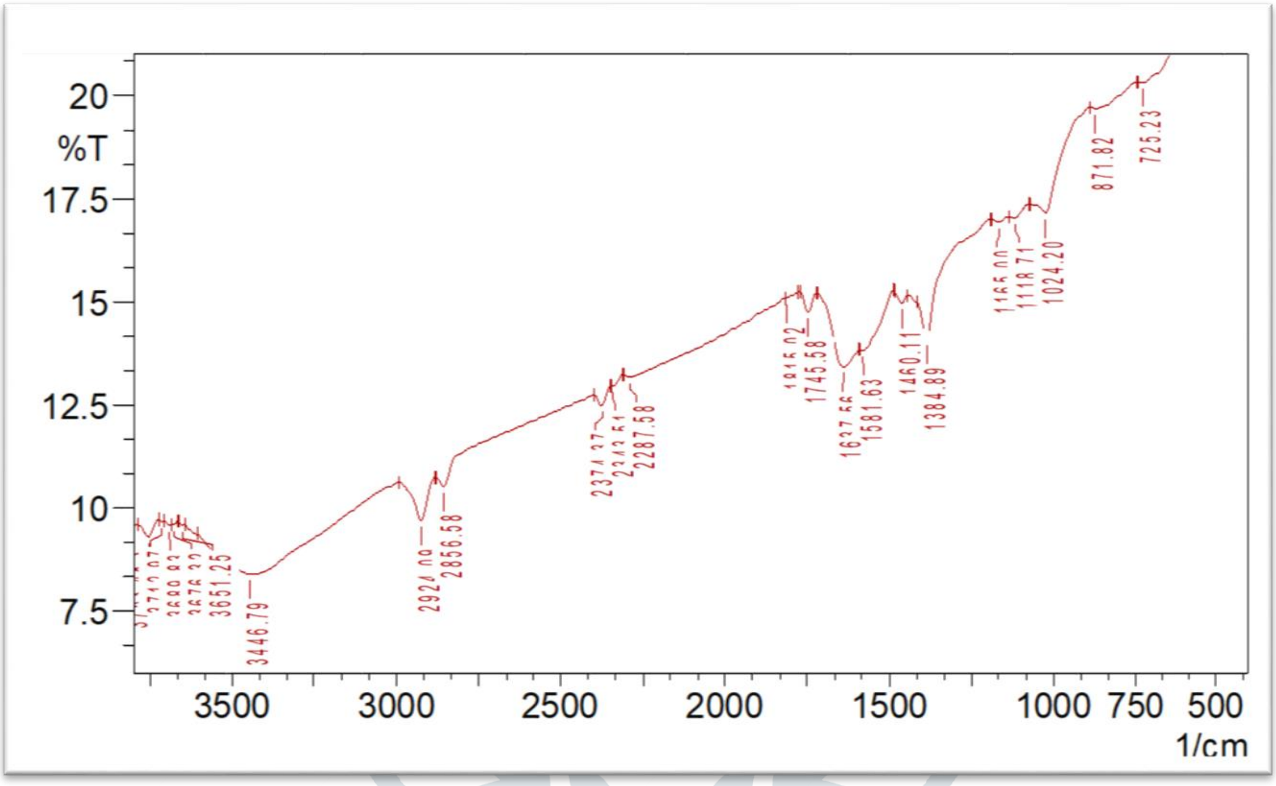


Figure 3.3(B) FT-IR spectrum of silver nanoparticles from Sauropus androgynus

Frequency ν	ν(C=C) Stretch	ν (O-H) Stretch	ν(C-O) Stretch	ν (N-H) Stretch	ν (C-H) Stretch	ν (C-O-C) Stretch	ν ($N-O_2$) Stretch
PATTANI RIVER	1637Cm^{-1}	3446Cm^{-1}	1165Cm^{-1}	1024Cm^{-1}	2856 Cm^{-1}	1118 Cm^{-1}	1384 Cm^{-1}

The FT-IR spectrum of the stretching frequencies are observed at 400-4000 cm^{-1}.

3.4 X-Ray Diffraction Studies

The silver nanoparticle solution obtained was purified by repeated centrifugation at 5000 rpm for 20 minutes followed by redispersion of the pellet of silver nanoparticles into 1ml of deionized water. After freeze drying of the purified silver nanoparticles, the structure and composition were analyzed by XRD. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the scherer formula. Figures 5.4 (A) and 5.4(B) show the XRD spectrum for silver nanoparticles [65].

The XRD spectrum is recorded by X-ray diffract meter with Cu kα radiation at 25°C. The average particle size is determined using **Debye-scherrer's equation** applied to major, peaks corresponding to maximum intensity in the XRD patterns of the sample.

The size of the synthesized silver nanoparticle were calculated from powder XRD pattern using Schetter"s formula.

D = k λ /βcos θ

D=0.94 λ/ β cos θ

Where

- D is the average crystallite domain size perpendicular to the reflecting planes,
- λ Is the X-ray wavelength,
- β Is the full width a half maximum (FWHM), and □ θ is the diffraction angle.
- To eliminate the addition instrumental broadening the FWHM was corrected, using the FWHM from a large grained SI sample.
- B corrected = (FWHM 2 sample-FWMH2si)

Determination of Structural Parameter

From the XRD profiles, the inter planar spacing dlnk was calculated using Bragg"s relation, **dlnk = nλ/2 sinθ**

The crystalline size (D) was calculated using the formula from the full width at half maximum (FWHM).

$$D = k\lambda/\beta \cos\theta$$

Where,

- The constant K is the shape factor 0.94,
- "λ"is the wavelength of the X-ray (1.5406 A^0 for Cu kα), □ θ Is the the Bragg"s angle and ' β ' ' is the **FWHM**.

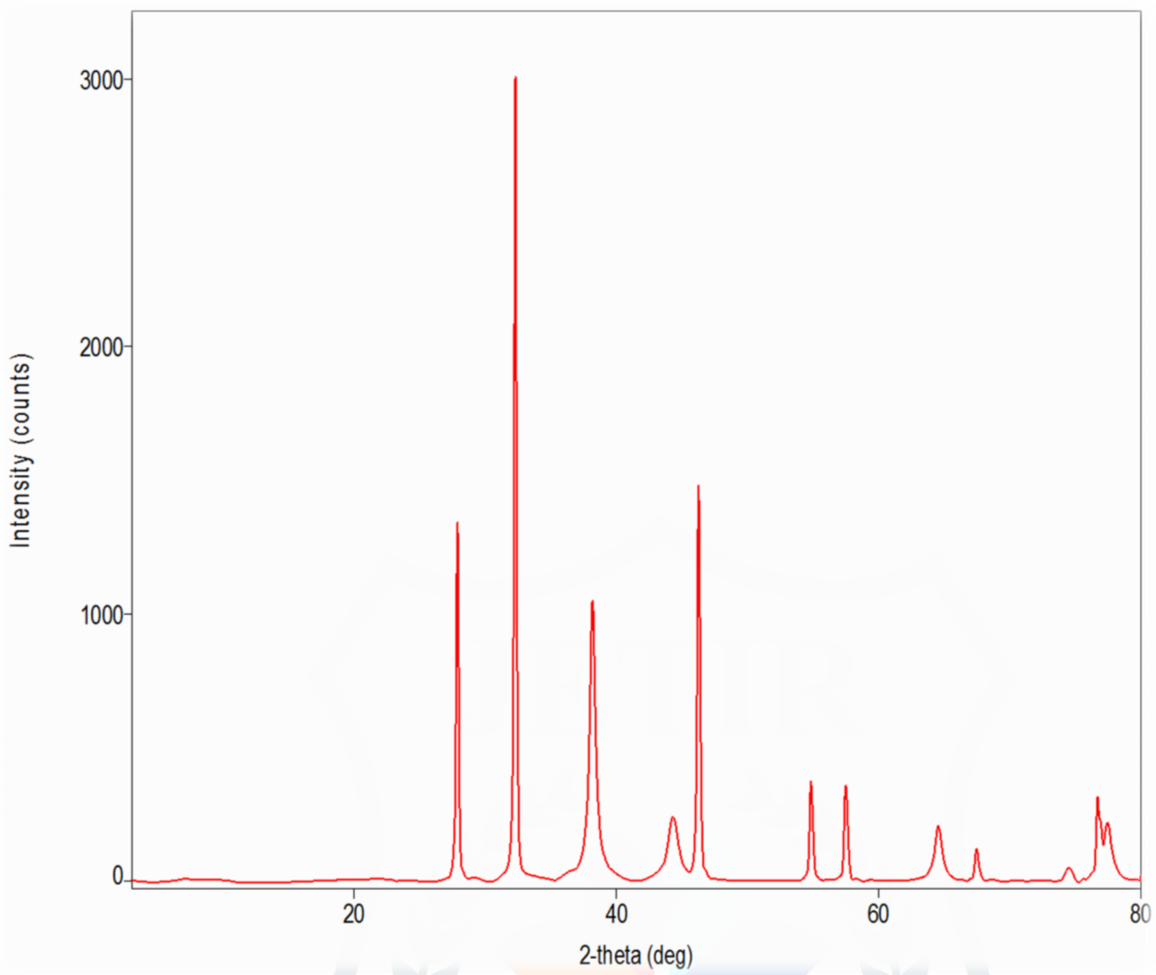


Figure 3.4 (A) XRD pattern of silver nanoparticles form pisum sativum

$2\ \theta = 44.3636$

$\theta = 44.3636/2$

$\theta = 22.1818$

$\cos\theta=0.9259$

D=0.94× 1.5406\0.9658×0.9259

D=1.448164/0.1648102 = 8.7868 nm

This XRD value confirms that the synthesized particles were nanomeric in the size. The size of the silver nanoparticles thus estimated was found to be 8.7868 nm.

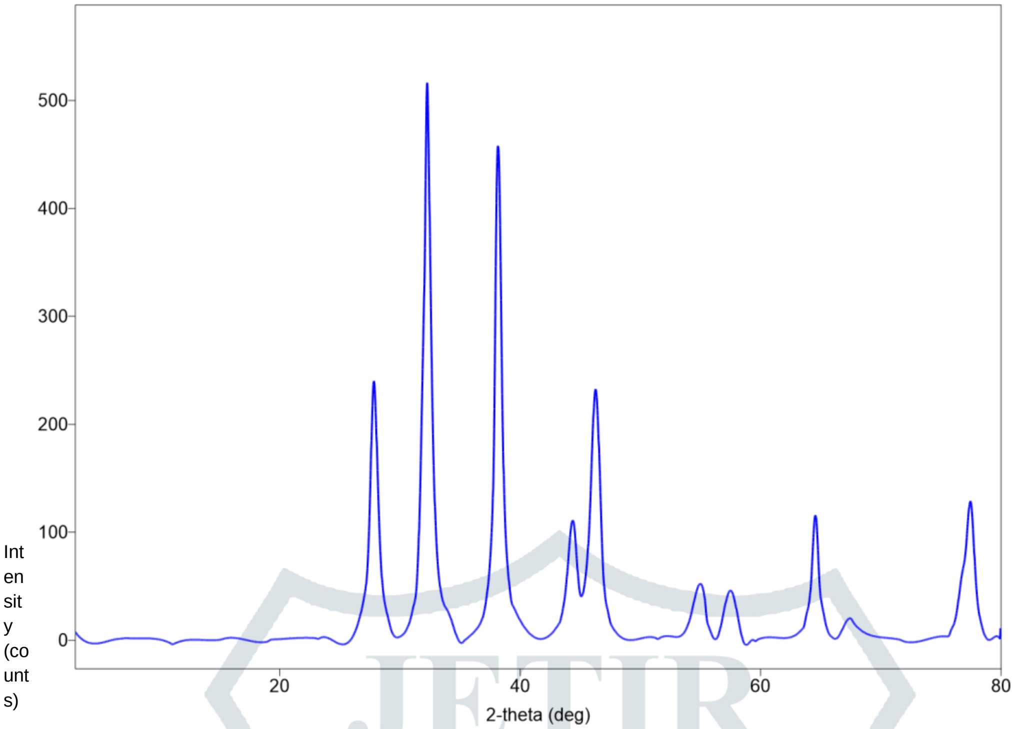
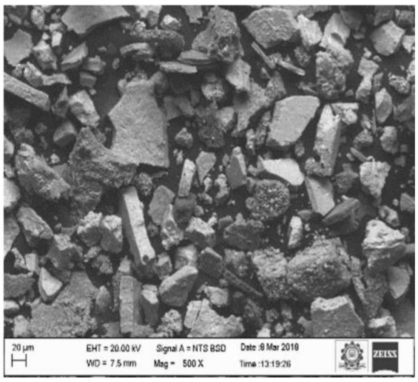


Figure 3.4(B) XRD pattern of Silver Nanoparticle form Sauropus androgynus .

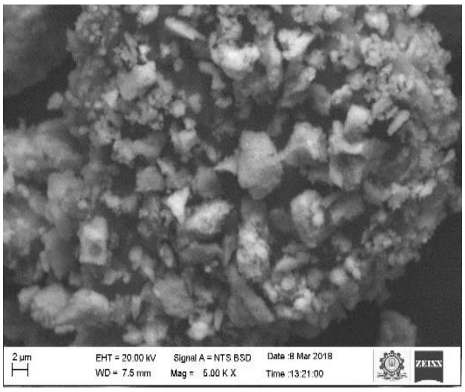
This XRD value confirms that the synthesized particles were nanometric in the size. The size of the silver nanoparticles thus estimated was found to be 2.6342 nm.

3.5 Scanning Electron Microscopy

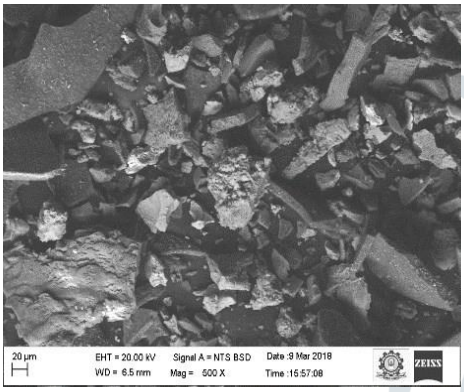
A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons. The electron interact with electrons in the sample, producing various signals that can be detected and that contain information about the sample‟s surface topography and composition. The electron beam‟s position is combined with the detected signal to produce an image. SEM can achieve resolution better than 1 nanometer. Specimens can be observed in high vacuum, low vacuum and environmental SEM specimens can be observed in wet condition. Size, shape and distribution of green synthesized silver nanoparticles were characterized by scanning electron microscope. The particle morphology of the silver nanoparticles fabricated using pisum sativum peel extract and sauropus androgynous leaf extract 3.5(a) and 3.5(b),3.5(c)and3.5(d)[65].



3.5 (a) Sauropus androgynus



3.5 (b) Sauropus androgynus

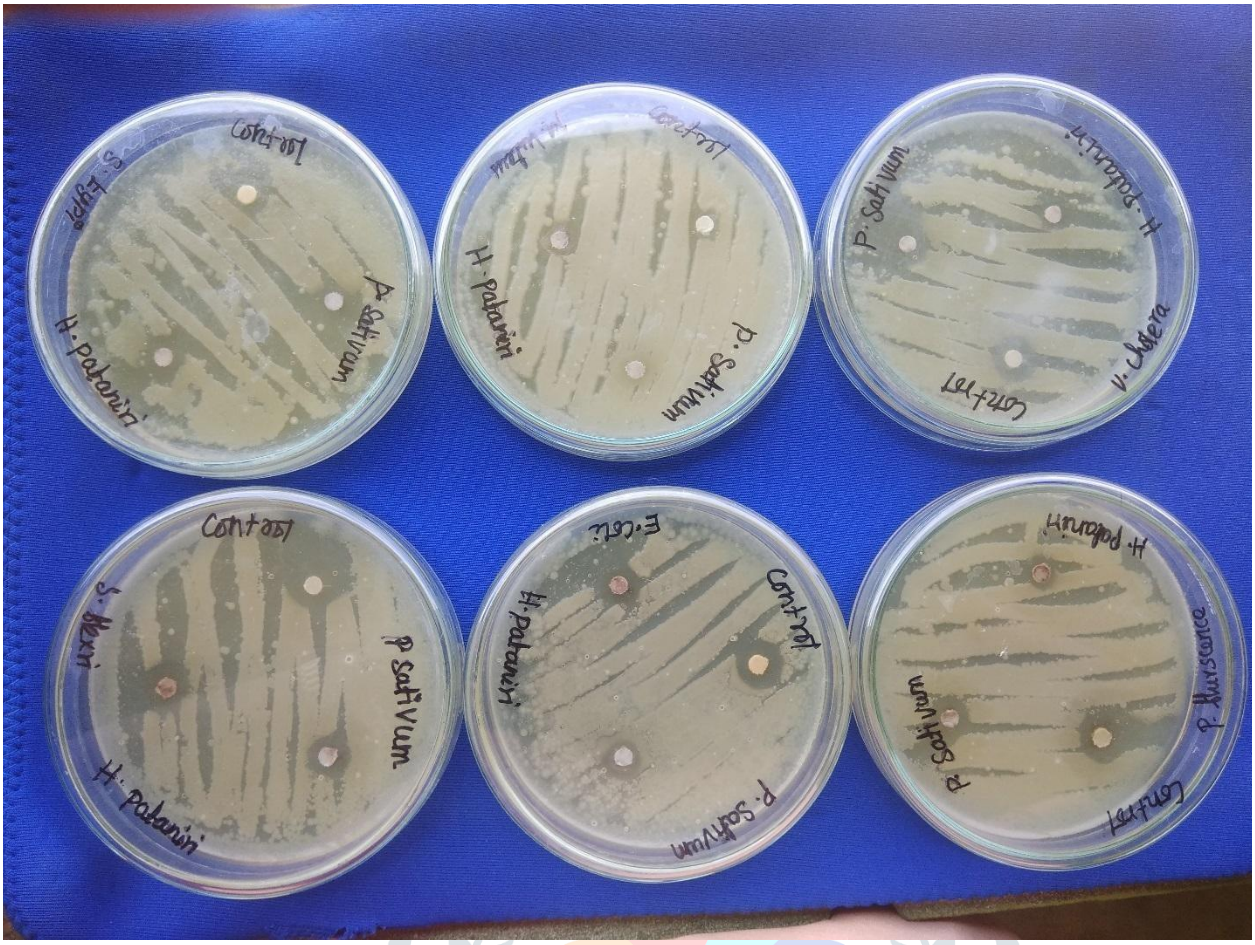


3.5 (c) pisum sativum

3.5 (d) sauropus androgynus

3.6 Antimicrobial Activity

The antimicrobial assay was done on human pathogenic *Vibrio Cholerae (mm), Salmonella typi (mm), Escheria coli (mm), Micrococcus luteus (mm) , Shigella flexneri (mm), Pseudomonas flurescens (mm)* by standard agar disc diffusion method. The pure cultures of bacteria were sub-cultured on nutrient agar medium. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Wells of 10 mm diameter was made on nutrient agar plates using gel puncture. Using a micropipette, 50μl of nanoparticles solution was poured onto each well an all plates. After incubation at 37^{0}C for 24 hours, the different levels of zone of inhibition were measured and it was tabulated 3.6.1.and the image showed 3.6.2.



3.6.2 Antimicrobial Activity of Bacteria.

TABLE 5.6.1: ANTIMICTOBIAL ACTIVITY FOR BIOSYNTHESIS OF SILVER NANOPARTICLES

BACTERIA	Silver Nanoparticles from Pisum Sativum	Silver Nanoparticles from Sauropus androgynus	Control

	(peel)	(leaf)	
Vibrio Cholerae (mm)	*8*	*9*	*10*
Salmonella typi(mm)	*13*	*12*	*10*
Escheria coli (mm)	*10*	*7*	*12*
Micrococcus luteus (mm)	*8*	*6*	*7*
Shigella flexneri (mm)	*10*	*12*	*15*
Pseudomonas flurescens (mm)	*12*	*5*	*10*

IV.CONCLUSION

A Critical need in the field of nanotechnology is the development of reliable and eco-friendly processes for the synthesis of metallic nanoparticles. Here, we synthesized a simple biological and low-cost approach for preparation of stable silver nanoparticles by reduction of silver nitrate solution with a bio reduction method using pisum sativum peel and Sauropus androgynus leaf aqueous extract as the reducing agent. Biologically synthesized silver nanoparticles could be of immense use in pharmaceutical field for their efficient antibacterial and antimicrobial properties. The synthesized silver nanoparticles were characterized by UV-Visible spectroscopy ,FT-IR spectroscopy ,XRD, SEM and Antimicrobial activities of synthesized nanoparticle were evaluated.The result confirmed the reduction of silver nitrate to silver nanoparticle with high stability and without any impurity. The formation of silver nanoparticles was confirmed by color changes from yellow to black (pisum sativum peel) and light green to brown color (Sauropus androgynus leaf) and it was confirmed by UV-Visible spectra showed a broad peak located at 400-500 nm for silver nanoparticle. FT-IR revealed that the resultant confirmed by alkynes , alkanes , alkenes, alkylhalides , dialkyl ethers, aliphatic Nitro groups, amine groups, phenol groups of pisum sativum peel extract and pattaniriver leaf extract are mainly involved in the reduction of silver ion to silver nanoparticles.X-ray diffraction pattern (XRD) revealed that the resultant nanoparticles were nanometric in size and the particle size was found to less than 20 nm. The SEM image revealed the morphology of the particles. This new method is rapid time scales for biosynthesis of metallic nanoparticles using environmentally benign natural resources as an alternative to chemical synthesis protocols as reductant for synthesizing silver nanoparticles. The synthesized silver nanoparticles using aqueous pisum sativum peel and Sauropus androgynus leaf extract shows good antibacterial efficacy against pathogens. Thus the antimicrobial activities of silver nanoparticles were established against *vibro cholera, salmonella typi, Escheria coli, micrococcus luteus , shigella flexneri and pseudomonas flurescens*. In the future, it would be significant to know the precise mechanism of biosynthesis and to technologically engineer the nanoparticles with the aim of attaining better control shape, over size and whole monodispersivity.

V. Acknowledgment

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CHAPTER –II

REVIEW OF LITERATURE

An extensive review has been carried out on the synthesis of silver nanoparticles by chemical and green synthesis methods.

- **Eman H.Ismailet.al** synthesized silver nanoparticle using olive leaf extract and its antimicrobial activity by green method. The silver nanoparticles was synthesized analyzed by UV-Visible spectroscopy ,X –Ray diffraction ,scanning electron microscopy and thermal gravimetric analysis(TGA).The silver nanoparticles were with an average size of 20-25nm and mostly spherical [1].s
- **Balaprasad et.al., (** 2005)reported biosynthesis of gold and silver nanoparticle using emblicaoffianalis fruit extract ,their phase transfer and transmetallation in an organic solution on treating aqueous silver sulfate and chlorocuric acid solution with emblicaoffcinalis fruit extract rapid reduction of silver and chloronic ions are observed leading to the formation of highly stable Ag and Au nanoparticle in solution [2].
- **Nair et.al.,** synthesis and therapeutic application (2007)reported silver is nontoxic .safe inorganic antibacterial agent used for centuries and it has the capability of killing different types of dieases causing microorganisms [3].
- **Manisha D.Ret.al.,**reported biosynthesis of silver nanoparticles using aqueous flower extracts of the plant Catharanthus roseus .The reduction of Ag+ to Ag0 resulted in the formation of silver nanoparticles with spherical shape and size within the range 6nm-25nm. The spectroscopic analysis using UV-visible spectroscopy which gave a peak at 460nm proved the formation silver nanoparticles, FTIR analysis supported that the formation of silver nanoparticles due to molecular interactions. The antibacterial studies it has been evident that the synthesized flower mediated silver nanoparticles were toxic to different human pathogens [4].
- **Naheed ahmad** et.al (2012) reported biosynthesis of silver nanoparticles using extracts of Ananas comosus reducing aqueous silver nitrate. The silver nanoparticles were characterized by Ultraviolet-Visible (UV-vis) Spectrometer, Energy Dispersive X-ray Analysis (EDAX), Selected Area Diffraction Pattern (SAED) and High Resolution Transmission Electron Microscopy (HRTEM). TEM micro- graphs showed spherical particles with an average size of 12 nm [5].

- **Akinsiku, A.A, Ajanaku, K.O., Adekoya, J.A** reported in this work, a facile one-pot green synthesis of silver nanoparticles had been successfully carried out with the leaf extract of C. indica and S. occidentalis synthesis is cheaper and faster than the conventional methods. Energy dispersed analysis by X- ray (EDAX) indicated silver oxide peaks, and scanning electron microscopy (SEM) showed an amorphous phase of the nanoparticles as further proofs of nanosilver formation from both extracts [6].
- **Eman Mohamed Halawani** reported biosynthesized silver nanoparticles using Zizyphus spinachristi aqueous leaves extract (ZSE), and their antibacterial properties. The green synthesized silver nanoparticles were characterized using UV-visible spectrum a peak at 414 nm ,The transmission electron microscopy (TEM) ,Fourier Transform Infrared Spectroscopy analysis (FTIR) and X-Ray Diffraction patterns (XRD) showed that they could be indexed as face centered cubic structure of silver. Antibacterial activity of silver nanoparticles was against Staphylococcus aureus , Acinetobactersp , Pseudomonas aeruginosa and Escherichia coli respectively [7].
- **Divya P and Nithya** reported Green synthesis of silver nanoparticle using the aqueous solution of Azadirachta indica leaf extract and silver nitrate. silver nanoparticles were characterized by UV-vis Spectrophotometer, FTIR, DLS, Zeta Analysis, XRD, and SEM [8].
- **M. Prathap • A. Alagesan • B. D. Ranjitha Kumari** reported Green synthesis of silver nanoparticle using Abutilon indicum sweet leaf extract and their antibacterial agent. FTIR and XRD result confirmed crystal structure of nanoparticles,anti-bacterial agent to control pathogenic microorganisms of Klebsiella pneumonia, Sal- monella typhi, Bacillus subtilis, and Proteus vulgaris [9].
- **Kuldeep Sharma,et.al.,** reported Green synthesis of silver nanoparticles using five different Vegetable peel wastes of (Lagenaria siceraria, Luffa cylindrica, Solanum lycopersicum, Solanum melongena and Cucumis sativus) and their antibacterial activites. Silver nanoparticles were synthesized UV-Vis spectra showed maximum absorbance at 430 nm, TEM analysis revealed spherical shape silver nanoparticle having size up to 20 nm, FTIR spectra and antimicrobial activity of silver nanoparticle against Escherichia coli and Klebsiella pneumonia [10].
- **Justyna et.al., 2**013 synthesized gold and silver nanoparticle that fulfilled the objectives of green chemistry. The methodology of this process is simple, inexpensive and environmentally friendly. The result showed that the contain the extracts may act as both reducing agent and stabilizers resulting nanoparticle [11].

- **Geethalakshmi et.al**., reported green synthesis of silver nanoparticle from 1Mn agno3 solution through the extract of trianthema decandra as reducing as well as capping agent. Nanoparticles were characterized using UV-Visible absorption spectroscopy ,FTIR,XRD and SEM ,X-ray diffraction and SEM analysis showed the average particle size of 15nm as well as rebelled their cubic structure .further these biologically synthesized nanoparticle were found to be highly toxic against different multi drug resistant human pathogens [12].
- **A.P. Kulkarani et.al., (**2012) was reported bio synthesis of silver nanoparticles using Bryophytes as plant source. In this study, the plant extract was prepared in ethanol and treated with silver nitrate to obtain nanoparticles. The synthesis of nanoparticles was confirmed by change in color from pale green to reddish brown ,a peak between 400 to 440nm was obtain on UV-Vis spectrophotometer which conformed the biosynthesis of silver nanoparticle [13].
- **MD.Arshad farooqui et.al.,** was reported green synthesis of Silver nanoparticles using leaf extract of clerodendrum inerme.Nanoprticles weresynthesized from three different leaf conditions –fresh leaves, sun-dried leaves, and hot –air ovendried leaves, (AFM) analysisof the nanoparticle revealed difference in size for the nanoparticles synthesized from different leaf condition. [14]
- **Ibironke A.et.al.,** was reported to synthesize silver nanoparticles using plant seed extracts of Cyperus esculentus and Butyrospermum paradoxum and their Antimicrobial activities Synthesis of silver nanoparticles characterized by change in pH, FTIR spectrum and UV-Vis spectrum study of the colloidal solutions and further subjected to antimicrobial against other organism are in decreasing order starting from P. aeruginosa, K. pneumonia, A. niger, P. notatum and R. stolonifer. T. [15].
- **Sabinal Abidin Ali**[1] wasreported Green synthesis of silver nanoparticles using apple extract and their antimicrobial activites. Silver nanoparticles were characterized using X-ray diffraction pattern confirmed the presence of only Ag crystallites, and the dynamic light scattering estimates the average sizes of the silver nanoparticles to be 30.25 ± 5.26 nm. Furthermore, Fourier Transform Infrared as well as UV-vis spectroscopy identifies ethylene groups as the reducing agent and capping agent for the formation of the silver nanoparticles. Antimicrobial activity against Gram-negative and Grampositive bacteria with minimum bactericidal concentrations (MBCs) to be in the range from 125 μg/mL to 1000 μg/mL [16].
- **Annamalai et.al.,** (2011) was reported Green synthesis of Silver and gold nanoparticles were prepared using aqueous leave extraction of phyllanthus amarus

schum . The resulting silver and gold nanoparticles were characterized using UV-Visible spectroscopy Scanning Electronic Microscopy (SEM),XRay diffraction (XRD),energy dispersive X–RAY (EDX),atomic microscopy (AFM),particle size analyses and Fourier transform Infrared spectroscopy (FTIR) Methods [17] .

- **Banerjee et al.,** was reported Green synthesis of silver nanoparticle using three plant leaf extract musabalbisiana(banana), azardirachta indica (neem), ocimum tenuifloru (blocktulsi)and their antimicrobial activity. sThe silver nanoparticles were characterized by UV-visible spectrophotometer, particle size analyzer (DLS), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and energy-dispersive spectroscopy (EDS).

  Fourier transform infrared spectrometer (FTIR) analysis and antimicrobial activities against Escherichia coli (E. coli) and Bacillus [18].
- **Padmanaban sivakumar et.al,** was prepared silver nanoparticle through the extract of lantana camara fruit as reducing and capping agent from 1mM silver nitrate solution. The silver nanoparticles were characterized using UVVisible absorption spectroscopy, fourier transfer infrared spectroscopy (FTIR),Transmission electron microscopic (TEM) and synthesized nanoparticles are found to be highly effective against different human pathogenic bacterial species [19].

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