CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Biological Evaluation Of Seeds Of *Trigonella Foenum* (Methi) Plant And Their Silver Nanoparticles

by

Nabgha Nosheen

A thesis submitted in partial fulfillment for the degree of MS BIOSCIENCES

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Dedicated to Allah Almighty, Hazrat Muhammad (SAW) and my father, my mother whom prayers always protect me, also dedicated to my father and mother in law. And Also to my husband and my beloved son Muhammad Zarrar.

CERTIFICATE OF APPROVAL

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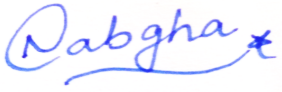

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Abstract

This study is about the synthesis of silver nanoparticles by using green chemistry from the seeds of *Trigonella foenum-graecum* (commonly known as methi plant) which is used as reducing and stabilizing agent. The nanoparticles were then characterized by UV–vis, SEM, EDX, XRD and FTIR analysis and evaluated for their anti-microbial, anti-oxidant and cytotoxic activities. Change in color indicated AgNP formation with absorption peak at maximum 400 nm. Average size of the synthesized silver nanoparticles was about 43 ± 4 nm according to Scanning Electron Microscopy analysis. Shape of AgNPs was found to be sphere. EDS analysis confirmed the presence of silver as a major constituent in the sample. Crystalline nature of these AgNPs was confirmed through X-ray Diffraction analysis. FT-IR analysis revealed the presence of functional group amine which were involved in reduction and stabilization of AgNPs. Significant antimicrobial activity was observed against various pathogenic bacteria (*Micrococcus luteus, Staphylococcus aureus, Bacillus subtilis* - gram positive and *Agrobacterium tumefaciens, Enterobacter aerogenes* - gram negative) and fungi (*Mucor species, Aspergillus flavis, Aspergillus fumigatus, Aspergillus niger, Fusarium solani*). Cytotoxic activity was determined by Brine shrimps lethality assay and antioxidant activity was assessed by DPPH method. These ecofriendly synthesized silver nanoparticles can be further used for various therapeutic needs such as anti-inflammatory, anti-diabetic, anti-tumor, anti-depressant and hormone therapy.

Keywords: *Trigonella foenum-graecum*, Silver nanoparticles, Antibacterial, Antifungal, Antioxidant, Cytotoxic.

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Abbreviations

A. flavis	*Aspergillus flavis*
A. fumigatus	A*spergillus fumigatus*
$AgNO_3$	Silver Nitrate
AgNPs	Silver Nanoparticles
A. niger	*Aspergillus niger*
B.subtilis	*Bacillus subtilis*
DPPH	(1,1-diphenyl-2-picrylhydrazyl)
FTIR	Fourier Transform Infra Electron Spectroscopy
KBr	Potassium Bromide
min	Minutes
mV	Mili Volt
M.luteus	*Micrococcus luteus*
nm	Nano-meter
rpm	Rotation Per Minute
SEM	Scanning Electron Spectroscopy
S.aureus	*Staphylococcus aureus*
UV-vis	Ultra Violet Visible Spectroscopy
XRD	X-ray Diffraction
μl	Micro Litter

Chapter 1

Introduction

One dimensional synthesis of nanoparticles ranging in size from 1-100 nm will results in high ratio of surface to volume. As ratio in surface area to volume goes higher the particle size decreases. Decrease in surface area to volume ratio helps to improve chemical, biological and physical properties as compared to their bulk counterparts [2, 3]. Noble-metal are also used in nanobiotechnology and the nanoparticles produced from these metals have great biochemical, optoelectronic and physicochemical characteristics. They are widely applied in pharmaceutical industries [4, 5]. In nanostructure form only few metals such as silver, gold, platinum and palladium are synthesized [6, 7]. Silver nanoparticles, among these metals have gained much attention because they have unique properties. They are being used in textile industries, water detoxification, agriculture, air filtration as a oxidization reaction's catalyst and also in pharmaceutics [8-11]. Their major property is that they without effecting animal cells can kill a number of bacteria [11-13]. Resistance in bacteria against antibiotics is been established when make them multi drug resistance and this phenomenon make scientist to work on developing and exploring new compound which can be used against multidrug-resistant microorganisms. Antibacterial property of Ag Nanoparticles can be used in place of antibiotics as they do not harm human body cells [14, 15]. Medical devices, hospital masks and implants are being made disinfectant by using AgNPs [16,

17]. When supplemented with an antibiotic they provide safety against various infections [17, 18].

Various physical and chemical methods have been used for the purpose of synthesis of silver nanoparticles. As compared to chemical and physical methods are less useful as they add toxic and hazardous chemicals into environment. A number of hazardous chemicals were segmented on the surface of these particles which makes them very harmful to the surrounding environment. Drawbacks of physical methods are their high cost and utilization of large amount of energy and space [19-21].

Another method named biological method uses various animal cells, plant and microorganisms to synthesize silver nanoparticles [22-24]. Various plants have been studied recently for biosynthesis of silver nanoparticles such as *Bacillus brevis* [25, 26], *Gelidium amansii* [26], *Phanerochaete chrysosporium* [27], *Enteromorpha compressa* [28] and *Daucus carota* [29]. The above mentioned plants are being studied to check their antibacterial and antifungal properties. This process of biosynthesis is very cost effective, time affordable and ecofriendly. Along with this they do not possess any kind of hazardous material on their surfaces. They also contain bioorganic compounds which are coated on their surfaces and make them fit for medical purposes. These above mentioned properties make them advantageous over physical and chemical methods [30, 31]. In cancer therapy they are used as targeting devise which are not toxic for healthy body cells. Biomolecules coated on their surfaces helps them in making biocompatible [32, 33]. Biomolecules present in the plant material act as reducing agent for reduction in silver ions. These biomolecules can be phenols, proteins or flavonoids [34] controlled environment helps plants in producing specified molecules which are bioactive [35]. Metabolic production of such plant is independent of environmental changes as they have no exposure to turbulence of environment [36].

There are various plants which contains active substances used in pharmacology, named medicinal plants. These plants have diverse uses like cosmetics, fragrances, dying, repellents, and industrial uses. Prescription of drugs contains about 40% of herbs [37]. A plant of family Lamiaceae and genus salvia contains about 900

species is been cultivated all arount the world give a product which can be used for pharmaceutical purpose and also in culinary as spice [38-40]. Its metabolite content has been studied by various scientists thoroughly. Its major components are carnosic acid (phenolic diterpene), rosmarinic acid (Caffeic acid ester), carnosol and flavenoids [35, 41]. Plants grown in in-vitro environment may does not have the ability to produce nanoparticles.

Fenugreek (*Trigonella foenum-graecumis*) is an herbaceous annual plant in the family Fabaceae. It is majorly used as a medicinal plant mostly in North Africa and Middle East. Wide use of fenugreek is due to its medicinal values. Its seeds are very nourishing and lead to weight gain, commonly in the case of anorexia nervosa. Seeds can cause uterine contraction in pregnant women so should be avoided during pregnancy [42]. Strong mucilage is being yielded by the seed which is very useful for the stomach problems and intestine ulcers. Ground seed decoction helps to drain off the sweet ducts when taken internally. According to research the seeds of fenugreek are very important for inhibition of cancer. The leaves and seeds are anti-inflammatory, emollient, galactogogue, laxative, hypoglycaemic, expectorant, antitumor, carminative, demulcent, deobstruent, febrifuge, anticholesterolemic, restorative, uterine tonic and parasiticide [42].

FTIR, SEM, EDX and XRD analysis are used for characterization of silver nanoparticles produces from *Trigonella foenum-graecum*. Antibacterial, antifungal, antioxidant and cytotoxic assays are performed to study on their ability to fight against the microorganisms.

1.1 Problem Statement Of Reserch Study

Drug efficacy is a major concern in present days. Biologically synthesised Nps have theraputic significance and also enhance the therapeutic value of available drug as well as reduces the side effects of the drug. In this study silver nanoparticles were be synthesized by using *Trigonella foenum-graecum* (Fenugreek) seed extract as reducing agent and were evaluated for their therapeutic significance

1.2 Aims And Objectives Of Research Study

This research was aimed to synthesize and evaluate medicinal properties of silver nanoparticles, their anti-bacterial, anti-fungal, cytotoxic and anti-oxidant activities. Furthermore, characterization of the synthesized nanoparticles by UV-vis, Scanning Electron Microscopy (SEM), X-ray Powder Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FT-IR) techniques was also done.

1.3 Objectives Of Research Study

The objectives of the research included:

- Utilization of eco-friendly plant extract of *T. foenum-graecum* L. to synthesize silver nanoparticles
- Evaluation of the antimicrobial properties of the synthesized nanoparticles against selected bacteria and fungi.
- Evaluation of anti-oxidant and cytotoxic properties of silver nanoparticles.
- Characterization of synthesised nanoparticles by using UV-spectrophotometer, SEM, XRD, EDX and FTIR.

1.4 Scope Of This Research Study

The field of green nanotechnology has been advanced by time to time. The silver nanoparticles synthesized by green nanotechnology are far better than those synthesized by conventional physical and chemical methods. Their unique antimicrobial and optical properties lead them to be widely used in the field of medicines for the purpose of food sanitation, wound healing and drug delivery etc. The technique provides stable nanoparticles dispersions that resist growth of various

pathogenic bacteria, and have high oxidation resistance and cytotoxicity that are of significant importance.

Chapter 2

Literature Review

2.1 Nanobiotechnology

In biological fields, nanobiotechnology is an application of biotechnology. A versatile field, nanotechnology provides approach, technology and facility in many chapters of engineering, physics, chemistry and biology along with the conventional avenue [43]. Being a new scientific approach it includes those materials and apparatus which are able to change apparent and chemical characters of a substance at lower levels. On other account biotechnology involves knowledge and techniques of biology used to develop products and services. These techniques of biology are used in altering molecular, genetics and cellular processes in variety of fields from medicine to agriculture. A distinct fusion of biotechnology and nanotechnology is considered to be nanobiotechnology due to reason that classical micro-technology is fused with molecular biological approach in a reel. Through the aid of this method making of atomic or molecular grade machines is done by impersonating biological systems. For studying or modulating various characteristics of a biological system on the base of size of molecules and other parameters this technique is very effective. Therefore easing many fields of life sciences nanobiotechnology integrates cutting edge uses of information technology

and nanotechnology to present day biological issues. This technology is able to remove the striking differences among biology, physics and chemistry to some extent and make new thoughts. Which is the cause of arrival of several novel challenges in many fields, these fields include education, research and diagnostics also this technology is being widely used as the time passes [43].

Both biotechnology and nanotechnology are the most favorable technologies of 21^{st} century. The pattern, progress and use of material and devices with the minimum functional making on nanometer oftenly referred to as nanotech is called as nanotechnology [44, 45]. On general account nanotechnology is applied on developing materials, devices and other structures ranging in dimensional size from 1 to 100 nanometers. In the same time, biotechnology is applicable on metabolic and other physiological procedure of living objects which involves minute living organisms (i.e. the unicellular bodies). Nanobiotechnology, i.e. combination of the two technologies has an important role in designing and applying devices and tools of worth usage in studying phenomenon of life. It's been a very diversified field that ranges from conventional physics's extensions to a entirely new self-assembly based approach of molecules, from the development of dimensional materials with dimensions on Nano scale to investigating whether the matters can directly be controlled on atomic scale or not. The idea involves the applications in the fields of science in large variety as surface science, organic chemistry, molecular biology, semi-conductor physics, micro fabrication, etc.

2.2 Benefits Of Nanobiotechnology

The pathophysiological circumstances and the internal bodily modifications of infected tissues can probably generate a long range for required nano technological product development. This development is likely to produce following advantages:

1. Use of different pathophysiological characters of defected tissues makes the act of drug targeting easy to achieve [46].
2. Accumulation of several nano products at high concentration as compared to normal drug is done with the aid of this technique [47].
3. The efficiency of nano systems in the tumors or diseased tissues is enhanced due to increased vascular permeability paired with impaired lymphatic in tumors through retention and better transmission [48, 49].
4. On diseased bunch of cells the nano systems have been very particular in their mode of action [50].
5. Nanoparticles act as effective transport material for the delivery of related drugs to brain to overcome the presence of blood-brain barrier [51, 52].
6. Modification of cell and tissue distribution is done by drug loaded nanoparticles and a more precise delivery of active biological compounds which increases drug efficiency and lessens the toxic effects of the drug [53, 54].

2.3 Nanobiotechnology Applications In Clinical And Medical Fields

Disease diagnosis, target-specific drug delivery, and molecular imaging which are being strenuously investigated at present and a number of others are the clinical

applications of nanobiotechnology. Clinical trials are also being carried on several new promising products [55, 56]. Such an approach of advanced applications of nanobiotechnology doubtlessly alters the foundations of diagnosis, treatment, and protection from diseases in coming time. Some of these applications are discussed below (Fig 2.1).

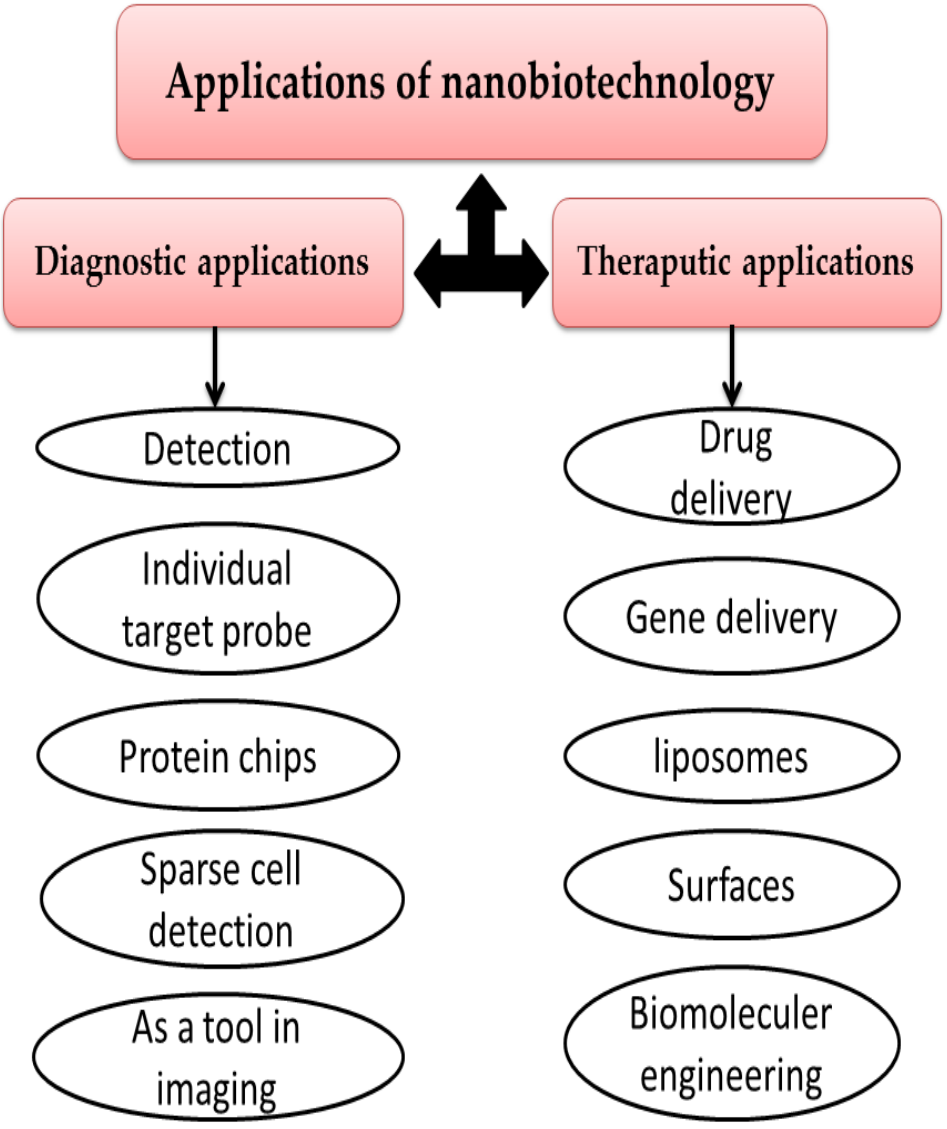

FIGURE 2.1: Overview of applications of nanobiotechnology.

2.3.1 Diagnostic Applications

Demonstration of visible symptoms is the basis of present day diagnostic methods for most of diseases, before the doctors could acknowledge that a man is suffering from a disease of specific type. With the passage of time the treatment provided to the appearing symptoms would have reduced efficiency. Therefore detection of a disease in early stages leads to a better cure for it. Ideally the diagnosis and cure of disease must be done before the appearance of symptoms in tissues or cells. An important role in this regard is played by nucleic acid diagnostics because this allows the detection of disease causing organisms and infection/infected tissues at an earlier symptomless stage of disease progression that makes treatment easy. Present day technology as polymerase chain reaction (PCR) progress toward such tests and devices, but nanotechnology is broadening the options presently available, which will result in greater sensitivity and much better efficiency and economy.

2.3.1.1 Detection

Revealing the presence of a molecule or a pathogen is done by currently used conventional clinical tests by uncovering the attachment of a particular antibody to infectious tissues i.e. target. Combining the antibodies with inorganic/ organic dyes and observing signs in samples by the use of fluorescence microscopy or electronic microscopy are a traditional way of performing these tests. Most often the practicality and specificity of the detection methods is limitized by dyes. A solution to this is offered by nanobiotechnology by the use of nanocrystals also called "quantum dots". The minute probes are able to withstand significantly agitations and emission of light as compared to distinctive organic molecules; these organic molecules degrade more readily as compared to those minute probes [57].

2.3.1.2 Individual Target Probes

Although there are other ways like magnetic detections, optical and calorimetric detection still medical community chose nanobiotechnology for identifying individual target probe. Doctors use methods developed by a well company to uncover genetic makeup of a living entity and the company is named nanosphere (Northbrook, Illinois). Nano gold particles along with small pieces of DNA are dispersed for the sake of easy to read genetic makeup of scattered with short segments of DNA form the basis of the easy-to-read test for the occurrence of any given genetic sequence. A heavy web of detectable gold balls is observed in case sequence of interest is about to occur in the sample. This thick web is formed when nanogold particles found sequence of interest in sample and binds with the arms of complementary DNA at several nanospheres. If sequence of interest are found in the samples, it binds to complementary DNA arms on multiple nanospheres and a dense web of visible gold balls is formed. Disease causing agents are being tracked down by this technique. It is most effective in tracking symptoms of anthrax and has given high sensitiveness than techniques presently used [43].

2.3.1.3 Protein Chips

Proteins play an important part in the establishment of physical characters of a living entity either in good physical state or infected state and are responsible for performance of several functions. Hence knowledge of proteins is essential for diagnosis and treatment of the diseases, for developing medications to alter the signal patterns. On treatment of chemical groups or acute modular protein components having the specificity of binding to the proteins having a certain structural or biochemical design [43, 58]. Both Agilent, Inc and NanoSInk, Inc are the two institutes working in this application of nanotechnology by the time now. Printing oligoes and complementary DNAs on slides made of glass at nanoscale with the aid of a non-contact ink-jet technique is being used by Agilent for the production

of microarrays. On contrary organizing shape on a nanoscale of measurement using dip-pen nanolithography (DPN) is done by NanoInk [43]. Both the institutes use two different techniques of nanotechnology for production of their respective products.

2.3.1.4 Sparse Cell Detection

The scattered cells e.g. cancer cells, lymphocytes, fetal cells and HIV-infected T-cells are most commonly rare under normal physiological conditions as well as physically different from the cells present nearby. Several genetic problems/disorders can easily be detected as the infected cells are different from the surrounding cells also they are sparsely located and aid in diagnosis of disorder. There are new chances for advancement in this regard provided by nanobiotechnology. There are efficient nanosystems been developed by scientist, which are able to sort out scattered cells from blood and other tissues. This technique has benefited with the ability of exploiting the distinct characteristics of the dispersed (infected) cells. Surface charges, affinity for special receptors and ligands along with apparent malformation/distortion are characters of these sparse cells for example cells can be differentiated on accordance with surface charges by the insertion of electrodes into micro channels. The process of sorting these cells can be done by the use of biocompatible surfaces with absolute nanopores. These techniques have been providing with the developed ways for the purpose of separating and detection of various infections by the Cornell University's center of nano-biotechnology [59].

2.3.1.5 Nanotechnology As A Tool In Imaging

Labeling of target group of cells with quantum dots (QDs) or artificially made chromophores is possibly done for intracellular imaging, like direct inspection of intracellular signaling complex by the aid of fluorescent proteins through optical techniques, i.e. confocal fluorescence microscopy or parallel imaging [60].

2.3.2 Therapeutic Applications

Nanotechnology has several therapeutic applications also and it is very beneficial in providing entirely new drugs with reduced side effects and pathway for delivery of that drug.

2.3.2.1 Drug Delivery

Ordinary drugs are not transported very easily to the site of action. But the activity of drug is enhanced to larger extent when binded with nano particles. These nano particles will manage the path of drug to the site of delivery. For example, a remedial chemically bound with nanoparticles finds its way to the avenue of infection with the aid of radio or magnetic signals. External triggers are those external factors, the presence of whome cause these nanoparticles to unload drug particles at the avenue of drug delivery, An example of external trigger is infrared heat, the presence of which causes release of drug. There are several harmful effects besides the treatment with the drugs. The severity of these side effects is being reduced by lessening the effective amount of drug taken along with nanoparticle. For more précised release of these drugs, encapsulation (such as organic dendrimers, hollow polymer capsules, and nanoshells) of drug particles in nanosized particles was done. This adds to the efficiency of drug release. These drugs are able to take medicinal charge along with imaging applications, and this all is the cause of their distinctive shaping/plotting [61]. There are several medicinal agents with poor bioavailability and cannot be administered orally. Administration of these medicinal agents is done by loading them along with the nanoparticles [62, 63]. Nano-formulations protect several agents which are highly vulnerable to degradation. These agents undergo denaturation under circumstances if exposed to high PH. Another application of nano-formulations is prolonging half-life of a drug by increasing bioadhesive properties of drug, so that they can long for more time [64, 65]. Transport of antigens for the purpose of vaccination is also an application of nano-formulations [66, 67]. Many research studies have been progressing

in this regard which leads to development of advanced encapsulation, also newly developed animal models depict that microparticles and nanoparticles are able to increase immunization [68].

2.3.2.2 Gene Delivery

Gene therapy at present is suffering from several problems. These include innate complexities of efficient medicinal processing and development, also the susceptibility of an engineered mutant to reverse back to the mutant type. The ability of viral vector to generate immunological response is also a problem [69, 70]. To overcome these hurdles, techniques of nanotechnology are being useful in two ways. One is the use of nanotechnological tools for human gene therapy and the second is the transfer of plasmid DNA with the aid of nanoparticle based nonviral vectors (usully 50-500 nm in size). Therefore substituting defected viral vectors with successful nanosized, immunogenic particles carrying gene of interest would be of worth importance for repairing and replacing defected genes in man [71].

2.3.2.3 Liposomes

Liposomes are the enzymes which could also be used in gene therapy. This is due to several abilities they possess. Among them one is that they could travel through lipid bilayer and cell membranes of required materials. This ability of liposomes is due to their structural composition i.e. lipid bilayer. Now-a-days many groups of liposomes are being used in gene therapy and make the localized delivery of genes [72, 73]. Use of liposomes would help us to attain required therapy. Linkage of monoclonal antibody (the antibody is for human insulin reporter) and nanoparticle (such as polythene glycol) loaded liposomes leads to reporter expression on broader scale in Rhesus monkeys brain. This all was described by Zhang et al [74]. These successful trials reflect the future of targeted therapy and the importance of nanometer-sized constructs for the advancement of molecular medicine.

2.3.2.4 Surfaces

Naturally molecules and surface used to show complex relationship among them. The relationship among blood cells and brain, fungal infectant and effected avenue are examples and depends on complicated interactions between cells and cell surface characters. Nanofabrication lessens the intricacy among these interactions. This can be done by modification of surface features with the aid of nanoscale resolutions leading to hybrid biological systems. The hybrid stuff can be used for various purposes like screening drugs, as sensors or as medical devices and implants. Nanosystems protected by an Irish drug company Elan, prepared polymer coat which could be able to alter the drugs surface with hydrophobic nature/ solubility of water.

2.3.2.5 Biomolecular Engineering

Traditional biomolecular designing may have limitized the presence of bioactive molecules with time and expenditure involved. Synthesizing methods and the nanoscale assembly had provided a substitute to conventional means. Biological and chemical reactions on solid substrate are improved as compared to traditional solution based processes. Ability to manipulate precisely and less waste is achieved by the use of solid substrate. Biomolecular engineering field is pioneered by Engene OS (Waltham, Massachusetts). Programmable bimolecular machines which are created by company developed engineered genomic operating system are used to employ natural and artificial building blocks. Commercial applications as biosensors, chemical synthesis and processing, as bioelectronics devices and materials, in nanotechnology, in functional genomics and in drug discovery biomolecular machines are used.

Along with the above mention there are various other applications of silver nanoparticles as shown in Figure 2.2.

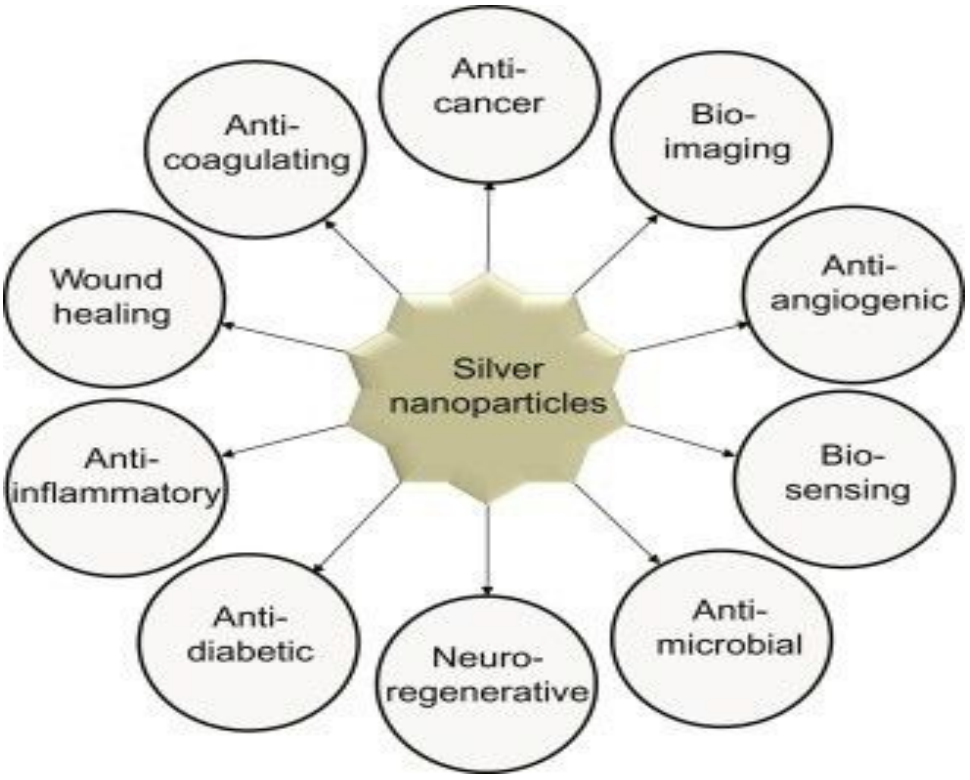

FIGURE 2.2: Applications of nanobiotechnology.

2.4 Silver Nanoparticles

Novel properties of silver nanoparticles depend on their shape, size and morphology which increase their interaction with animals, plants and microbes. Excellent bactericidal properties were observed against a number of pathogenic microbes. A number of perspectives are used to prepare nanoparticles of various physical characteristics or morphology [75]. Fungi, bacteria, actinomycetes, yeast and plant extract involves in the synthesis of AgNPs biogenically. Parts of plants like leaves, fruits and flowers are also used, along with a no of enzymes [76]. Methods of preparation, strength and concentration of reducing agent, temperature and nature of solvent depict the morphology, size and stability of synthesized nanoparticles [75-77]. Out of all the nanoparticles synthesized the AgNPs are assume a significant position owing to their inherent characteristic of acting as an antimicrobial agent even in solid state. Though their significance was recognized much earlier, it was not well exploited except for its use in oriental medicine and in coins. It is estimated that nearly 320 tons of AgNPs are manufactured every year and used in

nanomedical imaging, biosensing and food products [78, 79]. A continuous increase in in a number of multidrug resistant viral and bacterial strains due to pollution, mutation and changing environmental conditions. Scientists were trying to circumvent this predicament and developing drugs for microbial infection treatment. Many metal nanoparticles and metal salts are effective for inhibition of infectious microbial growth. Silver metals and their nanoparticles are in top of list due to their strong antimicrobial activities.

2.5 Therapeutic Uses Of AgNPs

Nanotechnology and nanoscience changed the way of diagnosis, treatment and helps in prevention of diseases in various fields of life. AgNPs are known to be most fascinating and vital among all the metallic nanoparticles. The most important role of silver nanoparticles is their importance in the field of medicines which is known as nanomedicines. There are various metals used for the purpose obtaining nanoparticles are in use in a number of fields, but the AgNPs are in top priority due to their potential applications in diagnosis of cancer.

2.6 Synthesis Of AgNPs

Different chemical, physical and biological methods (as shown in Fig. 2.3.) are used for the purpose of the nanoparticle synthesis and these nanoparticles are widely used for different purposes.

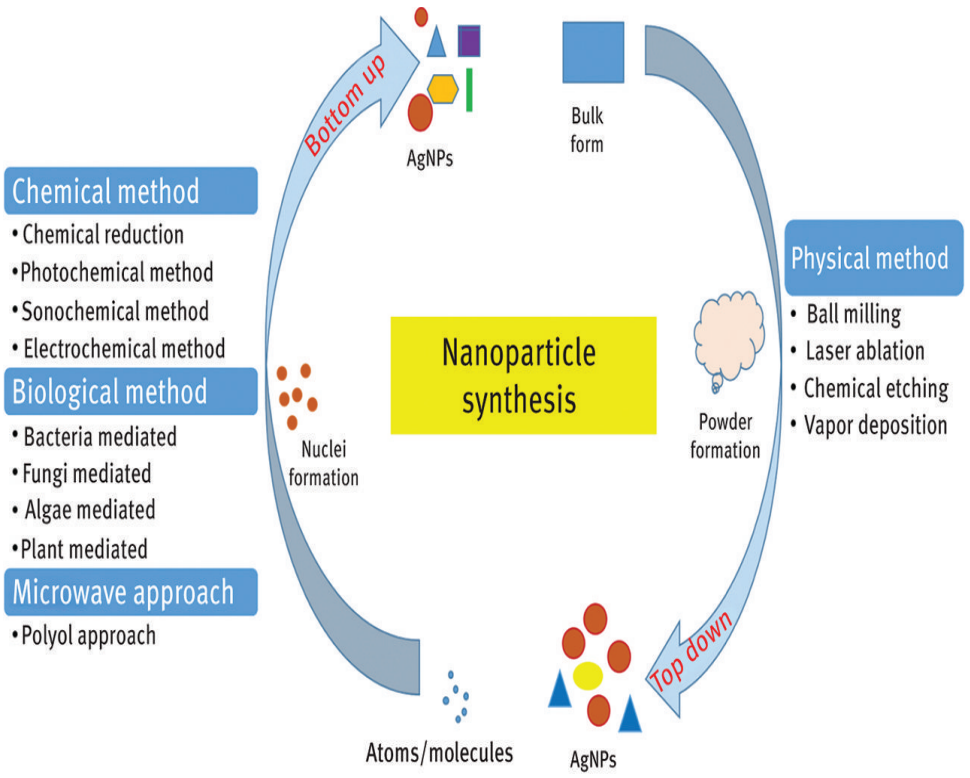

FIGURE 2.3: Green synthesis of silver nanoparticles from biological sources.

2.6.1 Physical Methods

Physical methods are that in which the tube furnace is used for evaporation-condensation at atmospheric pressure. Pyrolysis and spark discharging are physical methods also called as conventional methods and are very helpful in AgNP synthesis.

Advantages of physical methods are that these methods have no hazardous chemicals. They provide good speed and proper radiation for the reaction. They also provide strong reducing agents. But there are some disadvantages that are these

methods consume high amount of energy but have lower yield. These methods also have lack of uniform distribution and contamination of solvent is their major problem.

2.6.2 Chemical Methods

In chemical methods organic solvents and water is being used for silver nanoparticles formation. Reducing agent, silver metal precursor and stabilizing/capping agent are three major components in chemical methods for AgNPs synthesis. The following two stages are involved in reduction of silver salts which are:

- Nucleation
- Subsequent growth

Generally bottom up and top down methods are used to synthesize NPs

2.6.2.1 Top Down

This process involves in reduction in bulky materials. Processing of solid state materials is involved in top down routes. Bulky materials are crushed, milled or grind to make them smaller. Uniform shaped materials are difficult to prepared by using this route because small particles are difficult to realize even with consumption of high energy surface structure imperfection is major issue in this approach. Nanomaterials and nanostructure's surface and physical properties can be affected by this kind of imperfection. Major crystallographic damage can be caused by this conventional top down method.

2.6.2.2 Bottom Up

This process involves the synthesis of large materials from atomic level. From atom to molecule and cluster the materials are formed from bottom. Nanoscale

materials synthesized from this route have great characteristics of uniform size, distribution and shape. Chemical synthesis of nanoparticles is being effectively covered and inhibition of reaction for the purpose of controlling the particular growth is also precisely controlled by this route of nanoparticles synthesis. This approach is not a new area but it is quiet useful in nanoparticles and nanostructures processing and fabrication. It has always been a challenge for researchers to bitterly control over nanoparticles shape, distribution, quality, morphology, size and quality of synthesized nanoparticles. High yield of NP is the major advantage of the chemical methods contrary to the low yield of physical method. Chemical and physical methods of nanoparticles are quiet expensive and above that the chemicals used like borohydride, 2-mercaptoethanol, thio-glycerol and citrate are very hazardous and toxic, nanoparticles synthesized from these methods are not pure as there are chemical sediment on their surfaces [81]. Synthesis of well define sized nanoparticles is also difficult as it requires aggregation of particles, along this this various hazardous and toxic substances as byproducts excised out in environment [82]. Various techniques like electrochemical reduction, lithography, cryochemical synthesis, laser irradiation, chemical reduction, laser ablation, thermal decomposition and sono decomposition are used during the synthesis by chemical and physical methods [83]. These techniques give rise to harmful reducing agents. But the chemical method for nanoparticle synthesis is advantageous due to its high and easy yield [84].

2.6.3 Biological Methods

Biological methods help in overcoming the chemical methods shortcomings. Biological methods are emerging as viable options. These methods are simple, dependable, cost effective and environmental friendly. In biological methods various plants, bacteria, fungi and a number of small biomolecules such as amino acids and vitamins(as shown in Fig. 2.3) are been alternative to chemical methods which helps to produce high yield of AgNPs and gain a lot of attention.

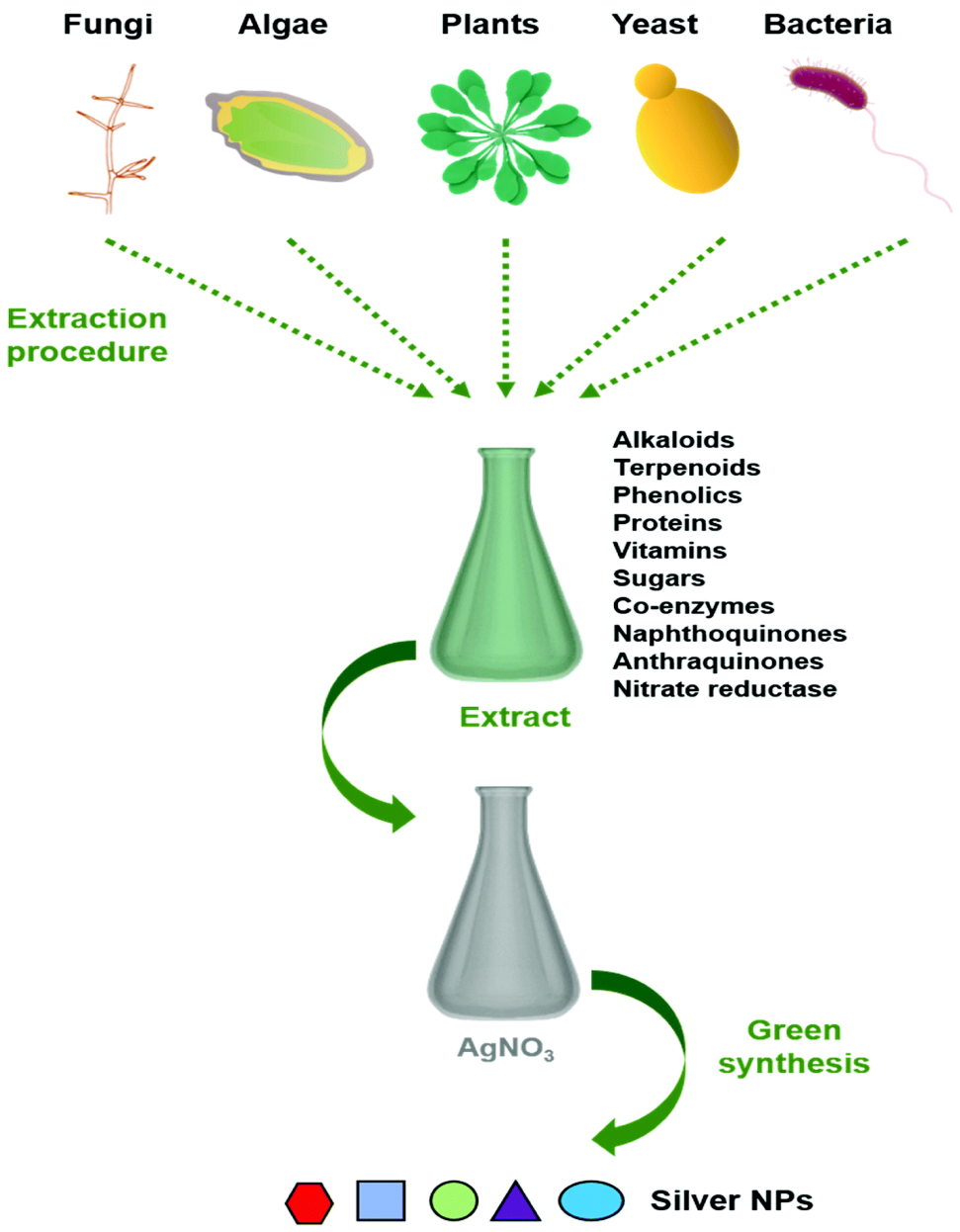

FIGURE 2.4: Biological synthesis of silver nanoparticles.

These methods not only helps in producing high amount of AgNPs but also important for many other nanoparticles like graphene and gold [85-87]. Gram-positive and Gram-negative bacteria help in metals bio-sorption. Bio-sorption of metals by provided an indication for the synthesis of nanoparticles before the flourishing of this biological method; however, the synthesized nanomaterials were as aggregates not nanoparticles [88]. Several studies reported the synthesis of AgNPs using green, cost effective, and biocompatible methods without the use of toxic chemicals in biological methods.

2.6.3.1 From Bacteria

Bacteria which is isolated from silver mines and named *Pseudomonas stutzeri*, AG259 helps in producing AgNP inside the cells [89-91]. Several other gram positive and negative strains like *B. amyloliquefaciens, A.calcoaceticus, B. fexus, S. aureus* and *B. megaterium* were been used for biosynthesis of AgNP both intracellular and extra cellular environment [92, 93]. The nanoparticles produced are of different shapes like triangular, hexagonal, disk, spherical and cuboidal. These nanoparticles were used in fabrication using cells, aqueous cell free-extract or culture supernatant [94]. Culture supernatant of *B. subtilis* and microwave irradiation are used in combination to produce silver nanoparticles in extracellular conditions [89]. Culture supernatants of *Enterobacter, E. coli* and *K. pneumonia* helps to synthesize AgNPs rapidly within 5 min of reaction time. Saravanan et al. studied on *B. megaterium* to produce extracellular AgNP from culture supernatant of *B. megaterium*. Reaction takes place in the presence of aqueous solution of Ag^{+} ions in 5 mins [95].

2.6.3.2 From Fungi

An extensive investigation is being made to study pathogenic and nonpathogenic fungi that can help in AgNPs Biosynthesis [75, 96-99]. Fungi help to reduce the silver ions extracellularly which results in the production of AgNPs in water which are strong and stable [97, 99]. Syed et al [100] reported about the thermophilic fungus helping in AgNPs synthesis extracellularly.Some of the bacteria, plants and fungi are enlisted in Table 2.1.

TABLE 2.1: Sources of biological synthesis of silver NPs.

Bacteria	**Plants**	**Fungi**	**Algae**
Lactobacillus strains	*Cinnamomum camphora*	*Phaeneroechaete chrysosporium*	*Gelidiella acerosa*
Klebsiella pneumonia	*Azadiracta indica*	*Humicola sp*	*Botryococcus braunii*
Aeromonas sp. SH10	*Aloe vera* leaf extract	*Aspergillus fumigatus*	*Spirulina platensis*
Enterobacter colacae	*Pinus desiflora*	*Aspergillus flavus*	*Caulerpa racemosa*
Corynebacterium	*Pelargonium graveolens*	*Fusarium reductases*	*Sargassum spp*
Pseudomonas stutzeri	*Emblica officinalis*	*Verticilium sp.*	*Ascillatoria willei*

2.6.3.3 From Plants

It is very cost effective to synthesize nanoparticles from plants. For the purpose of production of nanoparticles at large scale, the source is very valuable and economic [101]. For the production of silver nanoparticles, reducing and stabilizing agents can be taken from the green tea plant extract. Its scientific name is *Camellia sinensis* [102]. It contains phenolic acid biomolecules example caffeine and theophylline, which are used for nanoparticles formation and stabilization. For NPs synthesis leaf extract of black tea is also used [103]. The NPs produced from plants are of various shapes and very stable. The shapes of Nps can be rod, prisms, trapezoids and spheres. The molecules which are known to be responsible for the biosynthesis of NP are polyphenols and flavonoids.

For the production of silver nanoparticles the plant extracts from *Medicago sativa* (alfalfa), *Cymbopogon flexuosus* (lemongrass), and *Pelargonium graveolens* (geranium) a source of green reactant leaf extract of *Datura metel* is a good and rapid source of stable AgNPs which are of size 16-40 nm [104]. Proteins, amino acids and alcoholic compounds and poly-saccharides of the leaf extracts of the *Datura metel* act as reactant and leaf react with silver ions to direct the silver nanoparticles formation. Nanoparticles of size 15-500 nm can be synthesized from broth of *Diospyros kaki, Pinus desiflora, Magnolia Kobus, Platanus orientalis* and *Ginko biloba*. Broth of these plans was studied by Song and colleagues, extracellularly and they found that the rate of synthesis of NP and conversion of silver ions into nanoparticles is much faster in the case of the leaf broth of *M . Kobus* and *D. Kaki* were used as the source of reducing and stabilizing agent . But their size decreased from 50 nm to 16 nm and increase in temperature is noticed from 25^{o}C to 90^{o}C [105]. They studied that the 90% convention of NP at 25^{o}C requires only 11 minutes [105]. *Nelumbo nucifera* (an aquatic medical plant) leaf extract also produces silver NPs of about 45 nm size and in various shapes [106].

Other than these plants *Capsicum annuum* is also a good source for green synthesis of silver nanoparticles [107]. The amine group present in the Proteins can act as reducing agent for silver ions and during synthesis of Nps play a role of controlling agent. The secondary structure of the proteins will be altered after their interaction with the silver ions. The polycrystalline phase of nanoparticles will be changed into crystalline phase and as long as the reaction time increases the size of nanoparticles will also be increased. To demonstrate the mechanism of formation of AgNP from *Capsicum annuum* L. plant extract, a recognition reduction limited nucleation and growth model is suggested [107].

Euphorbia hirta leaf extracts synthesized nanoparticles of spherical shape which are 40-50 nm in size. Antibacterial properties of spherical silver nanoparticles was studied and results shows that strong antibacterial activity against *Staphylococcus aureus* and *Bacillus cereus* [108].

Silver nanoparticles of 20-30 nm were produced from the leaf extracts of a Euphorbiaceae plant *Acalypha indicain* the reaction time of 40 min [34]. Strong

antibacterial activity of these AgNP was observed against *Vibrio cholerae* and *Escherichia coli* These NPs had excellent antimicrobial activity against water borne pathogens, (MIC (minimum inhibitory concentration) of them is about 10 μg/ml). Silver NPs were synthesized from leaf extract of *Moringa oleifera* when 10 ml of plant extract and 90 ml of aqueous salt of $AgNO_3$ were mixed and heated for about 20 min at 60-80^{o}C. strong antibacterial activity was observed against the pathogenic microorganism like *Candida tropicalis*,*Staphylococcus aureus*, *Candida krusei* and *Klebsiella pneumoniae* [109].

Silver NPs produced from *Ficus bengalensis* and *Eucalyptus citriodora* and they were been loaded on cotton fibers, excellent antibacterial activity was observed for *Escherichia coli* . Burns/wounds, dressings, antibacterial textiles fabrication and finishings are the areas of utilizations for the fibers coated with [110]. Leaf extract of *Garcinia mangostana* also play a vital role of reducing agent to produce 35 nm sized NPs.

These highly effective Nps with their strong antimicrobial activity were used to fight against the pathogenic microorganism [111]. Leaf extract of *Osmium sanctum* was reported for producing 4-30 nm NP in almost 8 min of reaction time. Proteins present in plant extract helps in synthesizing strong and stable Np as these proteins act as capping agents. Ascorbic acid present in leaf extract of *Ocimum sanctum* act as reducing agent for silver ions. These Nps also shows strong antibacterial activity against pathogenic microorganism like *Staphylococcus aureus* and *Escherichia coli* [24].

Cacumen platycladi was also studied for silver NPs synthesis; components of plants that act as reducing agent are flavonoids and reducing sugars. The nanoparticles synthesized are quiet effective against positive and also negative pathogenic bacteria (*Escherichia coli* and *Staphylococcus aureus*) [112]. The bark extract of *Cinnamon zeylanicum* was used to produce hexagonal and cubic nanocrystals of silver which are of 31-40 nm in size [8]. With the varied amount of bark extract of *Cinnamon zeylanicum* the size of particles also varies. With increased number of particles the dosage also increases as the reductive biomolecules amount varies. High PH helps to produce Np of smaller size. More spherical sized NPs

are produced at high PH.

2.7 *Trigonella Foenum-Graecum*

2.7.1 Scientific Classification Of Fenugreek

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyte
Division	Magnoloophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Fabales
Family	Fabaceae
Genus	*Trigonella* L.
species	*T. foenum-graecum* L.

2.7.2 Description Of Plant

Common name of *Trigonella foenum-graecum* is Fenugreek. Fenugreek is an herbaceous annual plant in the family Fabaceae. Its leaves and seeds are being used as a spice. It may be a single stem or a branched stem (at the base of main stem) plant. Erect growth habit has been shown by plant. It has sweet and strong aroma and habit of even growth. It has small leaves with leaflets which are trifoliate and oval in shape. Color of leaves is purple. The flower color is purple and pale white. Flower is soliditary which has a staight or sometimes curved pod. One pod usually contains 10-20 seeds which are bilobed. Seeds are small smooth and brown in appearance. Normal height of fenugreek is 60 cm (23.6 inch). Because it is an annual herb, it can only survive for one growing season.

2.7.3 Seed Of *Trigonella foenum-graecum*

Habitats of *Trigonella foenum-graecum* are Field verges, uncultivated ground, dry grasslands and hillsides [113]. Leaves, Seed and Seedpod are used as Coffee; Condiment; Tea. Seeds can be eaten raw or cooked and sprouted [114, 115]. Flavor of seed is spicy which is very strong and different from celery [116]. Ground seed of fenugreek in the form of powder is basic component of curries and mango chutney Spices and pickles also uses fenugreek seed as their basic component [42, 116]. For the purpose of reducing the bitterness the seeds are usually roasted slightly [42]. The size of the seed is almost 3 mm and one pod contains about 10-20 seeds (as shown in Fig 2.5). Various essential elements like iron, phosphorus and Sulphur are

FIGURE 2.5: Seeds, seed pod and leaves of Fenugreek.

present in seed and there are about 6% moisture, 23% protein, 10% carbohydrate, 8% fat, 10% fiber and 4.3% ash [117]. Flavor of maple syrup is also obtained from seed [116]. after sprouting the seed can be used in salads and also be eaten after cooked [42]. A food flavor named maple syrup is obtained from essential oils of

fenugreek seed [116]. It also has medicinal importance [118]. The seed which are grounded up are alternative of maple syrup [115, 119].

2.7.4 Leaves Of *Trigonella foenum-graecum*

Leaves of this plant can be used when cooked but sometimes raw leaves. Due to the aroma in seeds they are being used in salads but in very small quantities. Due to its fragrance of leaves it is used in pot herbs. Its leaves are also an important ingredient of curries [42, 116].

2.7.5 Seedpod Of *Trigonella foenum-graecum*

Seedpods are used after being cooked [120]. When seeds are roasted they can substitute coffee [116, 121]. Leaves and seeds can give a very soothing effects [116].

Nutrient Composition: Nutrient can be figured in milligrams (mg) or grams (g) per 100 g of food.
Components of Seed: Fresh weight of seed contains about 0% Calories per 100 g.
Other components: like water is 6.2%, Proteins are 23.2 g, Fat is 8 g, Carbohydrate 10 g, Fiber: 9.8 g; Ash: 4.3 g.
Vitamins and minerals are not present in fenugreek seed [95].

2.7.6 Medicinal Uses

There are various uses of fenugreek plant but most importantly it is used as an Antiinflammatory. It is also used as carminative, diuretic, Galactogogue, Hypoglycaemic, Anticholesterolemic, Antitumor, Appetizer, Cardiotonic, Carminative,

Demulcent, Deobstruent, Diuretic, Emollient, Febrifuge, Hypotensive, Laxative, Parasiticide, Restorative and Expactorant.

It is majorly used as a medicinal plant mostly in North Africa and Middle East. Wide use of fenugreek is due to its medicinal values. Its seeds are very nourishing and lead to weight gain, commonly in the case of anorexia nervosa. Seeds can cause uterine contraction in pregnant women so should be avoided during pregnancy [42]. According to research the seeds of fenugreek are very important for inhibition of cancer. The leaves and seeds are anti-inflammatory, emollient, galactogogue, laxative, hypoglycaemic, expectorant, antitumor, carminative, demulcent, deobstruent, febrifuge, anticholesterolemic, restorative, uterine tonic and parasiticide [42]. A strong mucilage is being yielded by the seed which is very useful for the stomach problems and intestine ulcers. Ground seed decoction helps to drain off the sweet ducts when taken internally. For body building seeds are very nourishing and are very effective for physical ability. In the case where anemia cause physical debility and nervous factors are going to be involved the seeds of fenugreek are quite helpful. The seeds are very effective in the case of diabetes, insufficient lactation, poor digestion, labour pain [42] and in painful menstruation treatments, seeds of fenugreek are used. Bad breath or dulled sense of taste can also be cured with fenugreek seeds. Powder of seed can be used to cure burns, boils, ulcers and poultices of abscesses .They are also helpful for the excessive vaginal discharge douche. During the harvesting season the seeds can be used freshly or can be dries and preserved for later use, only fully ripen leaves are kept and dried [42, 122]. Plant provide compounds which possess various kinds of activities such as hypoglycaemic, antiphlogistic cardiotonic, diuretic, and hypotensive [122]. Trigonlline is one of the alkaloids of the seeds and has special potential in cancer therapies. A saponin diosgenin present in the seed has quite potenials for oral contraceptives and sex hormones synthesis. There are a number of pharmaceutical products that uses saponins which are derived from a plant source [42]. *Trigonella foenum-graecum* is being approved for the treatment of loss of appetite, inflammation of the skin by a German Commission E. Monographs (an herbal medicine therapist). Some of the benefits of fenugreek are shown in Fig. 2.6.

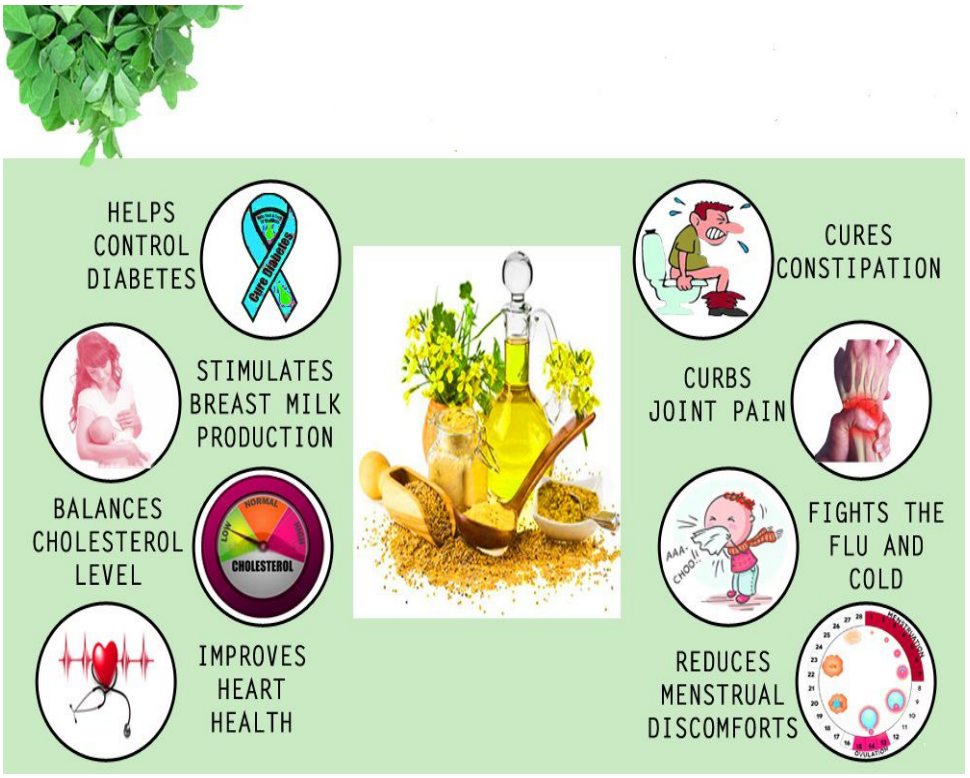

FIGURE 2.6: Health benefits of fenugreek.

2.7.7 Other Uses

In cosmetics and hair dyes *Trigonella foenum-greacum* is oftenly used as a source of dyes. Other than medicinal, pharmaceutical and edible uses of fenugreek it plays very important role in the green manure and parasiticide. It also helps in food flavoring. Seeds in crushed form and their oil are good help to obtain glossy and shiny hairs. Due to its hay aroma it is being very famous in cooking. Skin lotion made from the seed infusion is good for skin complexion. It helps in environmental nitrogen fixing as it is a fast growing and vigorous green manure crop that can produce a large quantity of bulk. Seeds can produce a yellow color dye [117].

Chapter 3

Methods And Materials

The research was carried out in wet lab of department of Bioinformatics and Biosciences, Faculty of Health and Life Sciences, Capital University of Science and Technology, Islamabad.
Following materials were utilized for research work:

3.1 Materials

3.1.1 Microogranisms Used

3.1.1.1 Fungal Strains

1. *Mucor species*,
2. *A.flavis*,
3. *A.fumigatus*,
4. *A.niger*,
5. *Fusarium solani.*

3.1.1.2 Bacterial strains

- **Gram positive**

1. *M.luteus*,
2. *S.aureus*,
3. *B.subtilis*

- **Gram negative**

1. *A.tumefaciens*,
2. *E. aerogenes*

3.1.2 Chemicals Used

Chemical name:	**Company name**
Silver nitrate	Sigma aldrich
Nutrient agar	//
Luria broth	//
Sabourad dextrose agar	//
Brine shrimps eggs	//
Sea salt	//
DPPH reagent (2,2-diphenyl-1-picrylhydrazyl)	//
Ascorbic acid	//
Streptomycin	//
Terbinafine	//
Ethanol	//
Petri plates	//
Test tubes	//
Glass vials	//
Micropipette	//
Micropipette tips	//
Cotton plugs	//
Cotton swabs	//
Aluminium foil	//
Falcon tubes 50ml	//
Eppendorf tubes	//
Beakers 100ml, 500ml	//
Test tube racks	//
Para film or masking tape	//
Forceps	//
Discs	//

3.2 Methods

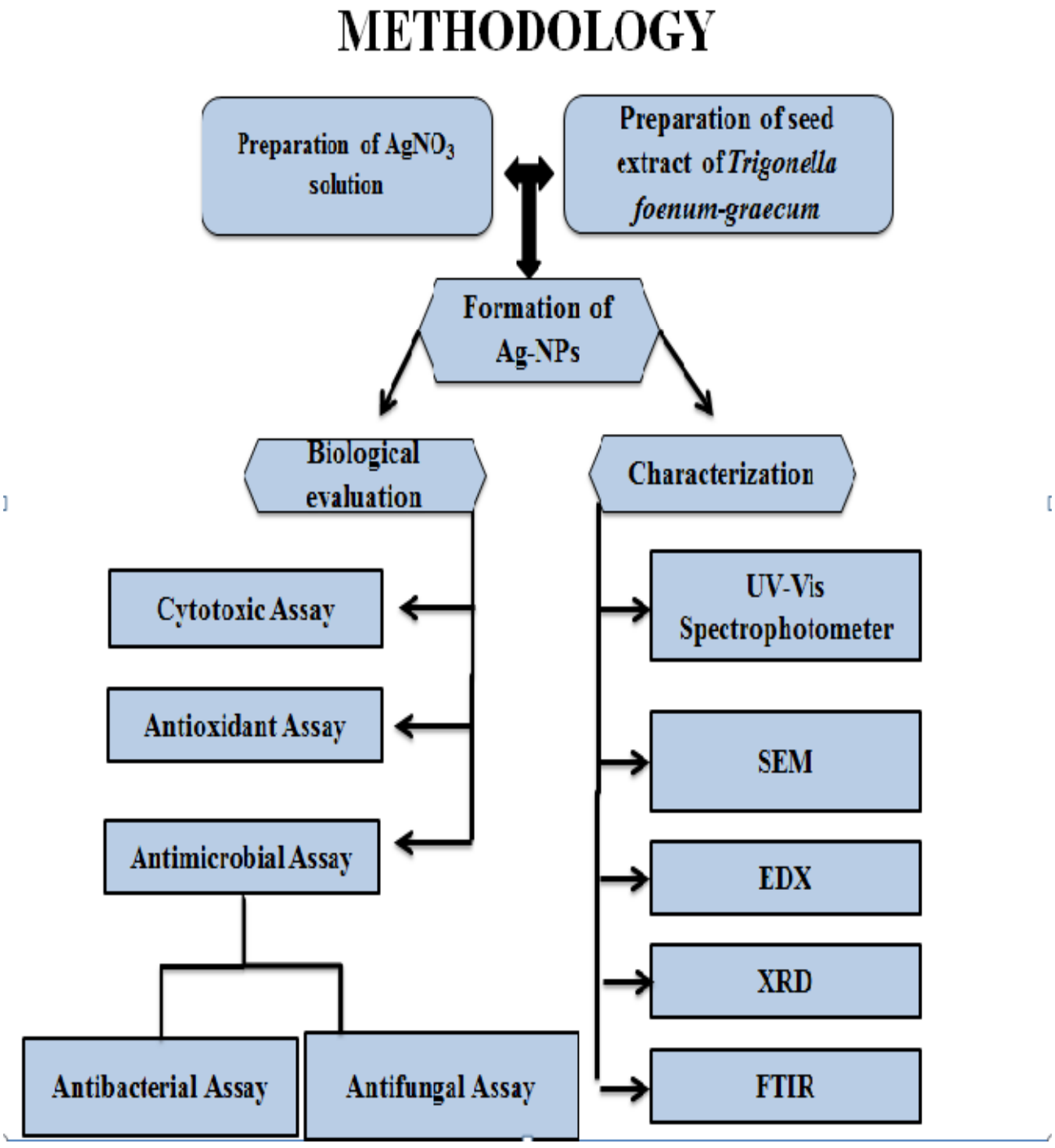

FIGURE 3.1: Overview of methodology.

3.3 Silver Nanoparticles Synthesis

3.3.1 Preparation Of Plant Extract

About 20 g of seeds were taken and washed with distilled water to remove dust particles. Then boiled in 100 ml distilled water for about 15 minutes. After boiling kept for some time to cool it down then filtered with Whatmann No. 1 filter paper. The seed extract with final concentration 200 mg/ml was stored at 40^oC for further use. This extract act as reducing and stabilizing agent [34, 123].

3.3.2 Synthesis of Silver Nitrate solution

Silver nitrate (0.1 M) was prepared in laboratory by dissolving 8.49 g of $AgNO_3$ in 500 ml distilled water under magnetization at room temperature for about 30 minutes [124, 125].

3.3.3 Reaction for the Synthesis of Silver Nanoparticles

45 ml solution of silver nitrate solution was taken and 5 ml of *Trigonella foenum* seed extract was added in the solution to make final volume 50 ml. The process was repeated in triplicate. The reaction tubes were kept in incubator for about 1 hour at 30^oC. After 1 hour the suspension was centrifuged at 6000 rpm for 40 minutes.

The pellets obtained were added in distilled water and supernatant was discarded. Due to by products and various toxic chemicals were being adsorbed on the surface of silver nanoparticles so this process was repeated several times about 3-4 times to remove them [124, 125]. The pellets of nanoparticles obtained were thus dried in oven for 48 hours and the powdered silver nanoparticles were obtained which were collected and preserved in eppendrof tubes.

3.4 Characterization Of Silver Nanoparticles

3.4.1 UV-Vis Analysis

3.4.1.1 Sample Preparation

After centrifugation, the supernatant was discarded and pellets were collected for sample preparation. The pellet (20 mg) was dissolved in 20 ml of distilled water and stocked in refrigerator at about 40^oC. The concentration of stock was 1000 ppm. This stock was later used for UV-Vis analysis [126].

3.4.1.2 Experimental Procedure

UV-Vis spectrophotometer (UV-1602) was used to determine optical properties of silver nanoparticles. About 4 ml of prepared sample was taken and water was set as a blank reference then AgNPs were subjected to spectrophotometer to confirm the synthesis of silver nanoparticles. The spectras were observed at 300-500 nm [127].

3.4.2 Scanning Electron Microscope

The morphology of prepared silver nanoparticles was determined using Scanning Electron Microscope (SEM). The samples were monitored at voltage of 20KV with the frequency of 2838 cps (max). Smear of solution was made on the slides and then they were arranged. SEM slides were prepared by coating gold on the smears to make them conductive. The nanoparticles samples were analyzed at different resolutions for determining size and shape [128].

3.4.3 X-ray Diffraction Spectroscopy

X-ray diffraction technique is commonly used for studying structure of crystals. First of all X-rays were generated using cathode ray tube and then these x-rays were subjected on the targeted sample and x-ray spectrum was obtained using X-ray diffractometer [128].

3.4.4 FTIR Analysis

FTIR spectrometer was used to study chemical composition of synthesized nanoparticles. The solution was dried at 75^{o}C and characterization was done in the 4000-400 cm^{-1} range using KBr pellet method [128, 129].

3.4.5 Energy-dispersive X-ray Spectroscopy

Elemental composition and their proportion in sample in addition to this chemical characterization of synthesized silver nanoparticles was executed by using energy-dispersive x-ray spectroscopy. Semiconductor sensors are used in EDX which measures energy of photons. Liquid nitrogen is used in EDX in order to keep it chilled and to maintain detector resolution and integrity. At energy level of 3 KeV signal for silver nanoparticles was detected which marks that silver nanoparticles are correctly recognized [105].

3.5 Biological Evaluation Of Synthesized Nanoparticles

Antimicrobial Assays:

For evaluating biological activity of prepared silver nanoparticles, two types of

antimicrobials assays were performed which are as under:

- Antibacterial assay
- Antifungal assay

3.5.1 Antibacterial Assay

Five bacterial strains were used in this assay. Disc diffusion method was used to determine bactericidal activity as described by Ruparelia et al [130].

3.5.1.1 Bacterial Strains Used

Following bacterial strains were used to determine antibacterial activity of silver nanoparticles:

Gram Positive Strains

- *Bacillus subtilus*
- *Staphylococcus aureus*
- *Micrococcus luteus*

Gram Negative Strains

- AT-10
- *Enterobacter aerogenes*

3.5.1.2 Sample Preparation

After centrifugation, the pellet (20 mg) obtained was dissolved in distilled water (20 ml) and final concentration of 10,000 ppm was made. Stock was diluted and different concentrations i.e 10, 20, 30, 40, 50, 60 and 100 ppm were used.

3.5.1.3 Media For Bacterial Growth

For bacterial growth in petri plates Luria broth agar was used. Its composition is as following:

a)	NaCl	5 g/ 500 ml
b)	Yeast	2.5 g / 500 ml
c)	Agar	7.5 g / 500 ml
d)	Bacto-tryptone	5.5 g / 500 ml

3.5.1.4 Experimental Procedure

Petri plates were autoclaved at 121^{o}C for 20 minutes. After this luria broth agar was poured in petri plates in equal quantity and allowed it to solidify. After solidification, bacterial strains were streaked by using cotton swabs and discs were put on the media. Then samples of AgNPs were poured on the discs at different concentrations in triplicates. Petri dishes were sealed and incubated for 24 hours at 37^{o}C. Streptomycin (100 ppm) was used as positive control and distilled water was used as negative control. After 24 hours, zones of inhibition were identified around each disc and these zones were measured. Experimental procedure was done in triplicate.

3.5.2 Antifungal Assay

Antifungal activity of silver nanoparticles was determined by following tube dilution method [131].

3.5.2.1 Fungal Strains

The five fungal strains were used which are as follows:

- *Aspergillus flavis*
- *Aspergillus fumigatus*
- *Aspergillus niger*
- Mucor species
- *Fusarium solani*

3.5.2.2 Preparation Of Sample

After centrifugation, the pellet (20 mg) obtained was dissolved in distilled water (20 ml) and final concentration of 10,000 ppm was made.

3.5.2.3 Media Preparation

Sabouraud dextrose agar was prepared for growth of fungal strains. Its composition is given below:

Sabouraud dextrose agar	6.50 g/100 ml distilled water

3.5.2.4 Procedure

SDA (Sabouraud dextrose agar) was prepared and autoclaved. Test tubes were taken and autoclaved at 121^{o}C for 20 minutes. After this test tubes were marked to 10 cm. 5ml of sabouraud dextrose agar was added in the test tubes and cotton plugs were used to cover these test tubes. After that 100 μl of sample was added

and slant was made to the 10 cm mark at room temperature(final working concentration was 20 ppm). The media was allowed to solidify. After solidification, test tubes were inoculated with fungal strains and covered with cotton plugs. Experiment was done in triplicates. Then incubation of test tubes was done at 37^oC for 4 days. Terbinafine was taken as positive control and distilled water was used as negative control. Fungal growth in slanting position was determined by calculating percentage growth inhibition with reference to the negative control (distilled water) and readings were calculated. The following formula was used to calculate the percentage inhibition:

$$\% \text{ inhibition} = \left[\frac{\text{Linear growth in negative control} - \text{Linear growth in samples}}{\text{Linear growth in negative control}}\right] \times 100$$

3.5.3 Antioxidant Assay

Antioxidant activity of nanoparticles and plant extract was assessed by following DPPH method as described by Gyamfi et al, [132].

3.5.3.1 Sample Preparation

Stock was prepared by dilution of nanoparticle pellet with water and further dilutions were used for cytotoxic assay (100, 50, 25 ppm).

3.5.3.2 Preparation Of DPPH Solution

12mg of DPPH was added in 100ml of ethanol to prepare standard DPPH solution

3.5.3.3 Procedure

Glass vials were taken and about 200 ul for each concentration of nanoparticles was added along with 2.8 ml of DPPH solution. Vials were placed in dark for

45 minutes. Ascorbic acid as positive and distilled water was used as a negative control. Readings were taken at 517 nm. Following formula was used to calculate the free radical percentage scavenging:

$$\% \text{ Scavenging} = \left[\frac{\text{Control absorbance–Nanoparticle sample absorbance}}{\text{Control absorbance}}\right] \times 100$$

3.5.4 Cytotoxic Assay

Cytotoxic potential of the prepared silver nanoparticles was determined by using Brine shrimps lethality assay following the method of Bibi et al, [132].

3.5.4.1 Sample Preparation

Stock was prepared by diluting nanoparticle pellet with water and further dilutions were used for cytotoxic assay (100, 50, 25 ppm).

3.5.4.2 Sea Salt Preparation

Sea salt was prepared using following concentration;

Sea salt	34 g/L

3.5.4.3 Hatching Of Eggs

Sea salt water was used for hatching of brine shrimp eggs (34 g/L)

3.5.4.4 Experimental Procedure

Eggs were added in sea salt for hatching. Nanoparticle and plant extract samples were added in the vials at different concentrations 100, 50 and 25 ppm. Sea water

was added in the vials to make the final volume 5 ml. The distilled water was used as negative control. After 24 hours shrimp brine eggs were hatched and seen floating in the sea salt water. These shrimps were transferred to each vial (about 15 shrimps were added in each vial). Experiment was done in triplicate. Vials were kept in light at 25^{o}C for 24 hours. After 24 hours alive shrimps were counted with the help of pasture pipette. Following formula was used to calculate percentage viability.

$$\% \text{ Viability} = \left[\frac{\text{No. of alive shrimps in -tive control} - \text{No. of alive shrimps in test}}{\text{No. of alive shrimps in -tive control}}\right] \times 100$$

3.6 Statistical Analysis

All the experiments were performed in triplicate and S.D was calculated. Data for antioxidant and cytotoxic assay was also analyzed by two way ANOVA by prism graphpad.

Chapter 4

Results And Discussions

In this chapter the whole process of synthesis of silver nanoparticles and their characterization and biological evaluation is discussed. The characterization was performed by UV-Vis spectrophotometer analysis, SEM (Scanning electron microscope, EDX (Energy-dispersive X-ray Spectroscopy) XRD (X-ray Diffraction Spectroscopy) FT-IR(Fourier Transform Infrared Spectroscopy). And biological evaluation was performed for their antibacterial, antifungal, cytotoxic and antioxidant activities. Results are discussed in this chapter.

4.1 Synthesis Of Silver Nanoparticles

Silver nanoparticles were synthesized by using $AgNO_3$ as source of silver ions and seed extract of *Trigonella foenum-graecum* as a reducing agent for silver ions. When $AgNO_3$ solution and seed extract were mixed the reaction proceeds and the color of the solution become dark brown after about 20 mins as shown in Figure 4.1. The color change indicated the reduction of silver ions and synthesis of AgNPs. This color change is due to surface plasmon resonance (SPR) phenomenon [133]. Another study stated the formation of brownish red color indicated the formation

AgNPs [125]. Another group of researcher indicates the periodic color change of the *Trigonella foenum-graecum* seed extract when treated with 1 mM silver nitrate solution from pale yellow to dark brown within 72 hours of incubation which is an indication of the formation of silver nanoparticles [134]. In another similar study the seed extracts of the *T. foenum-graecum* were mixed with 1 mM $AgNO_3$ which resulted in the formation of brownish red color revealed the synthesis of the AgNPs [125].

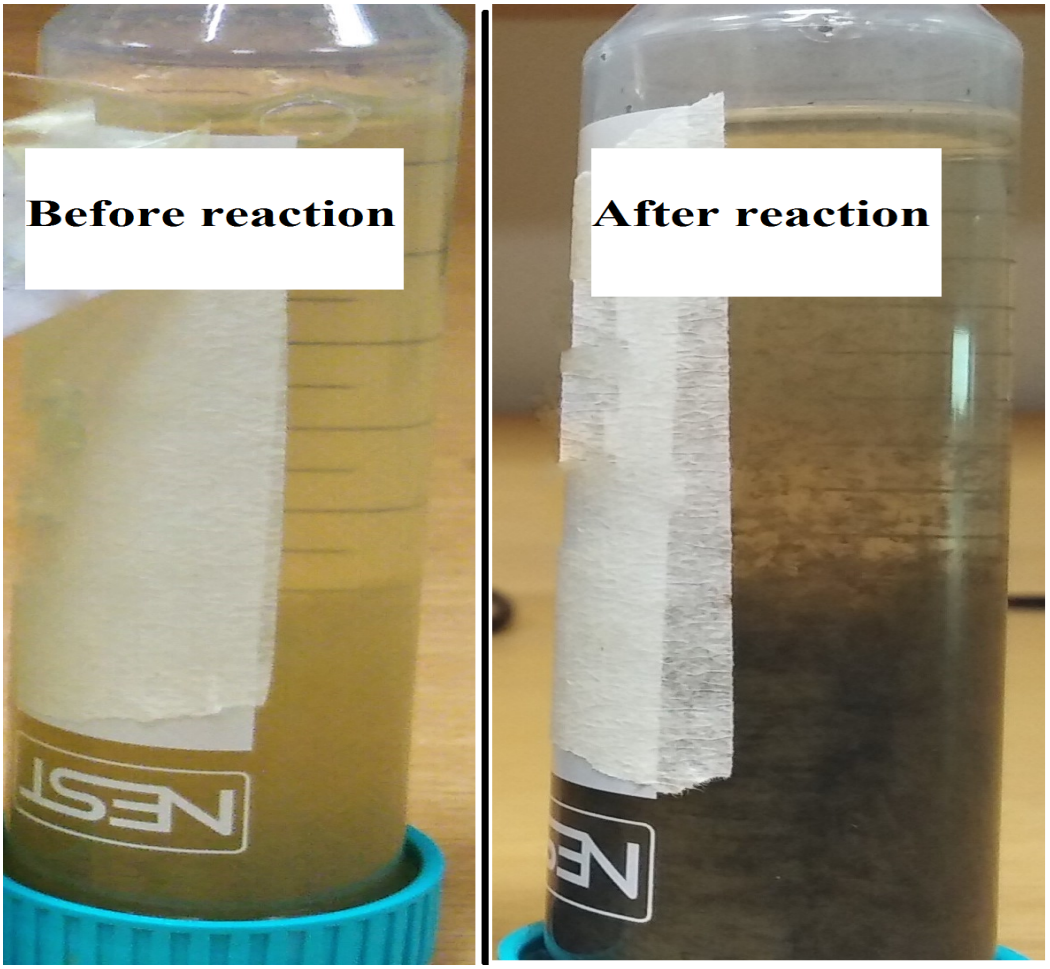

FIGURE 4.1: Change of colour shows synthesis of silver nanoparticles.

4.2 Characterization Of Silver Nanoparticles

4.2.1 Silver Nanoparticles Analysis through UV-Vis Spectrophotometer

Synthesis of silver nanoparticles and their optical properties were confirmed by UV-Vis spectrophotometer. Peak in the range 390-410 was observed which indicated the presence of silver nanoparticles as shown in Figure 4.2. For determination of formation of the silver nanoparticles, their stability and optical properties, UV-Vis absorption spectroscopy is commonly used. This analysis is highly significant. Metallic nanoparticles exhibit surface plasmon resonance (SPR) by exhibiting spectra of electromagnetic waves due to strong absorption in the visible range. When nanoparticles are exposed to visible light conductive electrons starts oscillating collectively and they exhibit the SPR phenomenon. Depending upon the particle size it is well established fact that AgNPs exhibit a characteristic yellowish-brown color in aqueous solution when viewed through UV-Vis spectrophotometer. This can be attributed due to excitation of free electrons [117]. In a study, synthesized silver nanoparticles from *Trigonella foenum graecum* showed the appearance of brownish red color indicating the formation NPs with the absorption maximum at 420 nm [125]. In a similar study, UV–visible spectrum purified from AgNPs showed an absorption peak at 420 nm [135]. Another group of researchers reported the peaks at 450 nm and 460 nm of silver nanoparticles synthesized from seed extract of *Trigonella foenum-graecum* [136]. In another study, AgNPs obtained from seeds of S*ilybum Marianum* observed that the maximum absorbance of Ag nanoparticles occurs at 425 nm, indicating that AgNPs were produced [137]. In another similar study of Ag nanoparticles a solid broad peak was perceived at 450 nm [138].

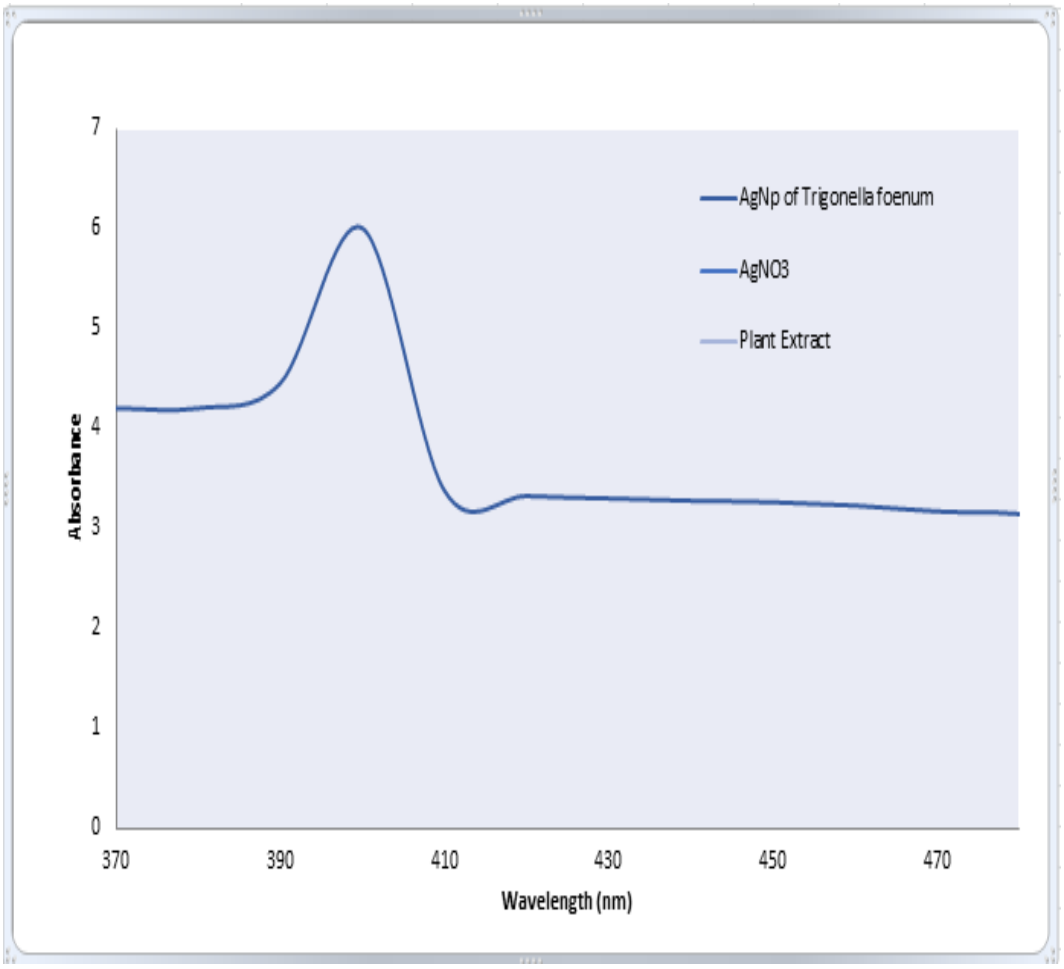

FIGURE 4.2: UV-Vis spectra of plant extract and silver nanoparticles.

4.2.2 Silver Nanoparticles Analysis Through SEM

SEM analysis is used to determine the morphological features and size of synthesized nanoparticles. SEM analysis shows the size of nanoparticle between 43 ± 4 nm (Figure 4.3). SEM works by bombardment of electrons (which are highly activated) on the sample which results in signals production. This electronic interaction with the sample provides information regarding external morphology (texture), chemical nature, crystal structure and orientation of materials which are building blocks of the sample.

The magnification range of SEM is 4.03 kx and spatial resolution of 50 to 500 nm. The usage of SEM in most applications confers an advantage due to its ability to collect data specifically from one sample surface and then spatial differences among a sample can be studied by generating 2-dimensional image. This approach

is very important especially in various applications such as in forensics, identification of novel species, rock sampling, identification of new drug targets, vaccines testing and in assemblage of electronic components [139]. At the nano and micro scales the surface morphology, shape and size of a nanomaterial can be imaged by SEM technique [140]. The SEM along with energy-dispersive X-ray spectroscopy (EDX) is used to examine morphology of silver nanoparticles and also chemical composition analysis is performed. The SEM cannot give information on internal structure, but it can provide valuable information regarding the purity and the degree of particle aggregation The modern high-resolution SEM is able to identify the morphology of nanoparticles below the level of 10 nm.

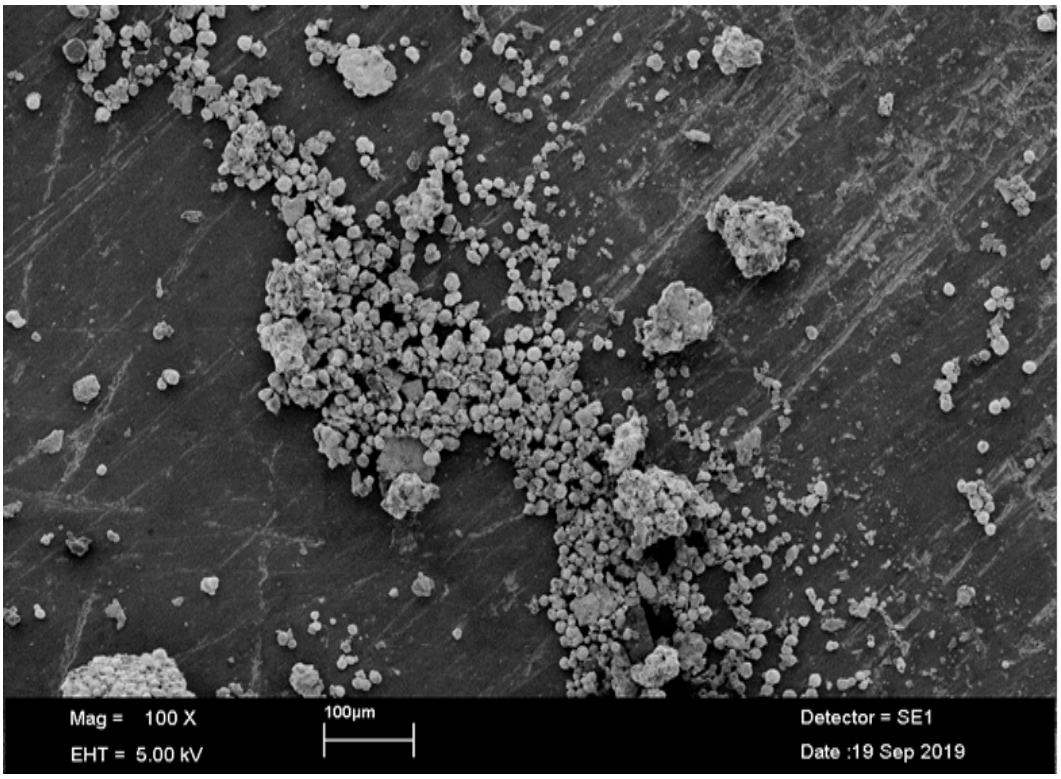

FIGURE 4.3: SEM analysis of synthesized nanoparticles.

In a study, synthesis of silver nanoparticles from *Padina tetrastromatica* nanoparticles produced had average size of about 20 nm and they are spherical in shape [120]. In another study silver nanoparticles ranged in size from 25-50 nm and cubic structure nanoparticles were synthesized from *Argemone Mexicana* leaf extract [141]. Another group of researchers revealed the synthesized silver nanoparticles using *Trigonella foenum-graecum* seed extract sized between 95 and 110 nm [142].

4.2.3 Silver Nanoparticles Analysis Through EDX

The chemical and elemental composition of synthesized AgNPs was determined using Energy dispersive X-ray spectroscopy equipped with SEM (Scanning electron microscope). It was inferred from the EDX spectrum that silver is major constituent in the samples without presence of any contaminants (Figure 4.4). Corresponding peaks except silver are may be because it remains in the pallet and gold make the particles more conductive for SEM analysis. In a previous study of synthesis of silver nanoparticles from *Mentha piperita*, EDX spectra recorded for all treatments showed a strong Ag signal at 3 keV.

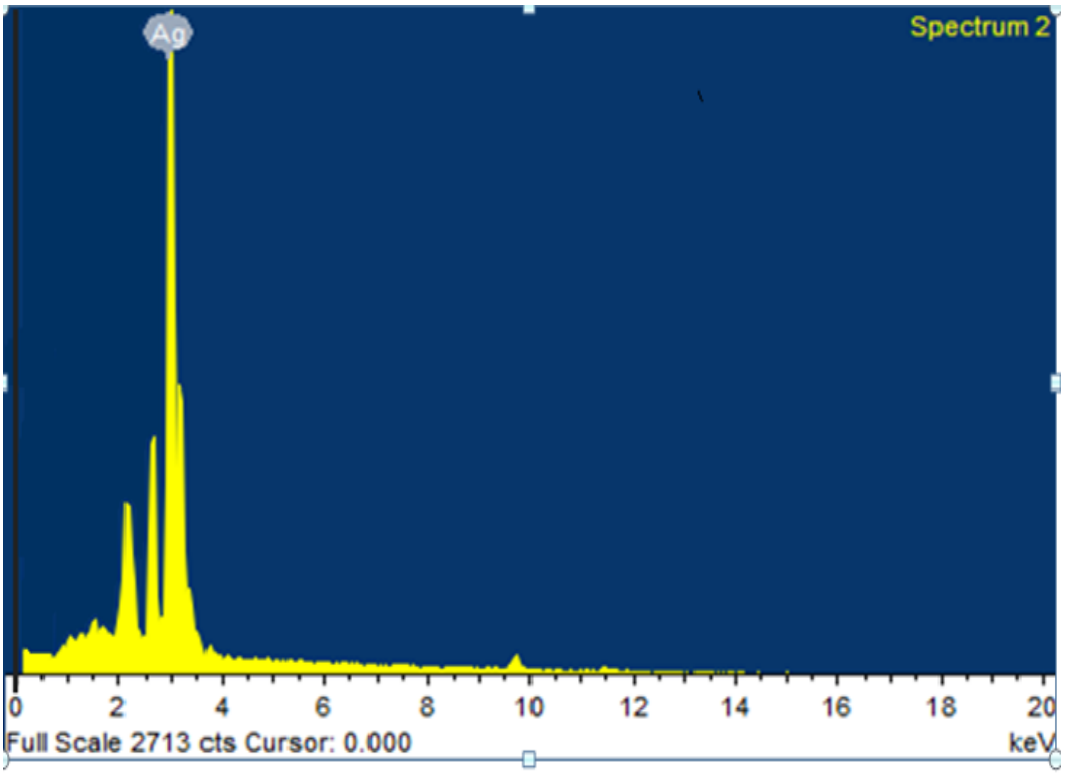

FIGURE 4.4: EDX spectrum of synthesized silver nanoparticles.

4.2.4 Silver Nanoparticles Analysis Through XRD

X-ray diffraction technique is commonly used for studying structure of crystals. First of all x-rays were generated using cathode ray tube and then these x-rays were subjected on the targeted sample and x-ray spectrum was obtained using x-ray diffractometer [143]. X-ray diffraction spectra expressed the crystalline nature of nanoparticles and the presence of strong peak values of 2θ with identified peaks [38.01 (1 1 1), 44.5 (2 0 0), 64.6 (2 2 0), 77.5 (3 1 1)] (Figure 4.5).

A study on *Silybum marianum* seed extract Intense peaks were observed at 2θ values of 38.098^o, 44.154^o, 64.674^o, and 77.544^o, corresponding to (111), (200), (220) and (311) Bragg's reaction based on the face-centered-cubic (fcc) crystal structure of AgNPs [137]. Another study on silver nanoparticles synthesized from seed extract of *Trigonella foenum-graecum* L., the X-ray diffraction spectra expressed the presence of strong peak values of 2 within the angle of 37.1^o, 44.7^o, 65.66^o, 78.6^o and 80.2^o [125]. The results were documented that the AgNPs have face centered cubic structure and crystalline in nature [125]. In another similar

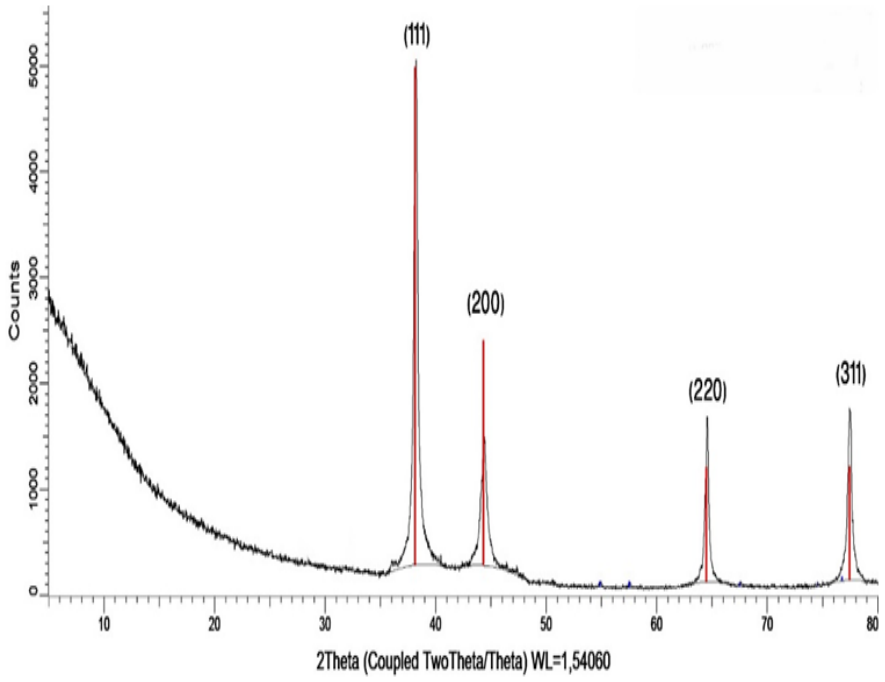

FIGURE 4.5: XRD peak diffractogram of silver nanoparticles.

study the crystalline nature of Ag nanoparticles was confirmed from X-ray diffraction (XRD) analysis. Four peaks were observed at 38.21, 44.31, 64.71 and 77.81 in the 2θ range 30–801 which can be indexed to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) [144].

4.2.5 Silver Nanoparticles Analysis Through FTIR

For determination of various biomolecules present in *Trigonella foenum-graecum* seeds extract which were involved in nanoparticles synthesis FTIR analysis was carried out by using FTIR spectrophotometer. Difference of peaks between frequencies 3200 - 2300 cm^{-1}(Figure 4.6) shows O-H stretching and 1600 - 1300 cm^{-1}(Figure 4.6) shows C-N stretching of N-H groups. Similar results were observed in a similar study of silver nanoparticles of seed extract of *Trigonella foenum-graecum* [145]. O-H and amide group stretching was also reported in another study of AgNPs [125]. These results represented the involvement of the primary amines in the formation of AgNPs.

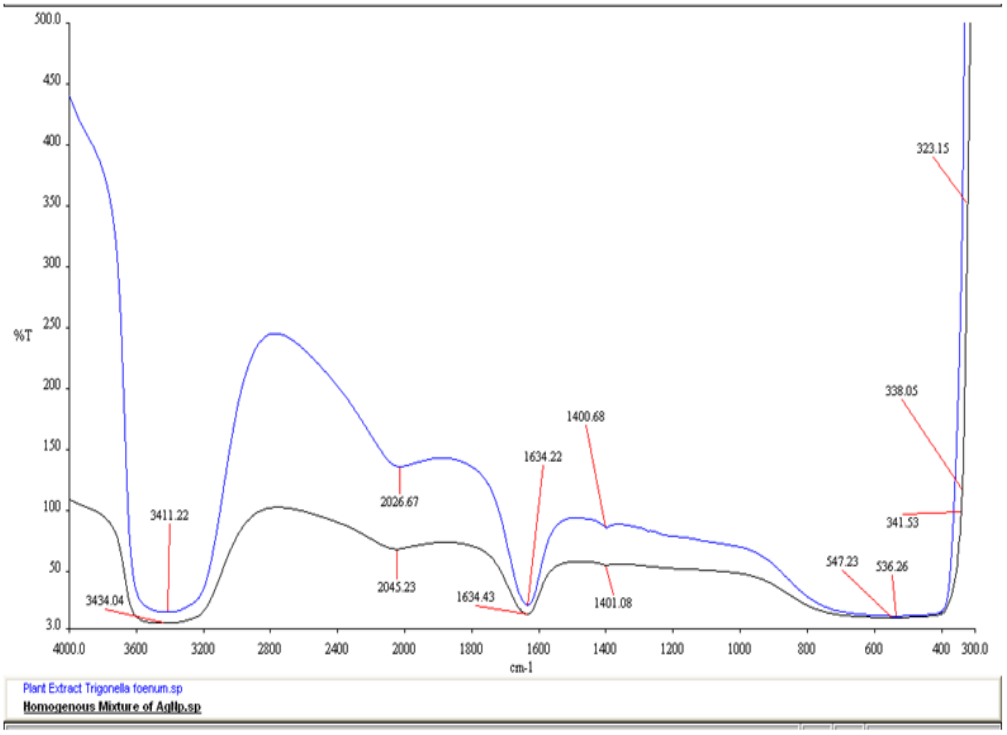

FIGURE 4.6: FTIR spectra of plant extract and silver nanoparticles.

4.3 Biological Evaluation Of Silver Nanoparticles

4.3.1 Antibacterial Assay

Antibacterial activity of synthesized nanoparticles was determined by following disc diffusion method. Six bacterial strains were used in this assay i.e three gram positive (*M. Luteus, S. aureus, B. subtilis*) and two gram negative (*A. tumefaciens, E. aerogenes*). Results are shown in the Table 4.1. The antimicrobial effects of AgNPs were determined by comparison with control treatments. Minimum inhibitory concentration (MIC) was determined against different concentrations of AgNPs 10, 20, 30, 40, 50, 60 and 100 ppm. Minimum inhibitory concentration is the lowest concentration of any chemical usually a drug that retards bacterial growth. After an incubation period of about 24 hours, clear zone of inhibition was observed.

Growth of all bacterial strains both gram positive (*M. Luteus, S. aureus, B. subtilis*) and gram negative (*A. tumefaciens, ,E. aerogenes*) was inhibited at 100 ppm. It was also found that there is rapid decline in bactericidal activity when concentration was decreased. Findings revealed that AgNPs are proved to be more effective against gram negative bacteria (MIC as low as 20 ppm) in comparison to gram positive bacteria (MIC as low as 30 ppm).

The antimicrobial properties of silver nanoparticles are highly dependent on superficial contact because silver has the potential to inhibit enzymatic systems of the respiratory chain and also cause disruption in DNA synthesis which leads to DNA modification. Owing to their small size and larger surface area as compared to other salts and even the silver particulate can interact with micororganisms effectively and can bind to the cell membrane and also penetrate inside [146]. AgNPs act primarily in three ways against gram-negative bacteria [146].

1. Nanoparticles have the ability to disrupt normal functioning of a bacterium by adhering to its cell wall such as gaseous exchange and transport of materials [147].

2. Nanoparticles have the potential to penetrate through the cell membrane of bacteria and cause damage inside because of its ability to interact with sulphur and phosphorous compounds thereby leading to DNA damage [147].

3. Release of ionic silver also show bactericidal activity [147].

It has also been reported that silver nanoparticles can rupture the outer membrane in case of gram negative bacteria hence affecting the permeability and these disruptions are termed as pits [148]. In another study by Lara et al, it was reported that antimicrobial activity of AgNPs was almost same on gram positive and gram negative bacteria which shows that AgNPs have a broad-spectrum bactericidal effect [149]. AgNPs can bind to the cell membrane of gram negative bacteria and

increasing permeability of membrane which leads to structural changes and ultimately cell lysis [150]. The silver which permeated through cell membrane can cause denaturation of proteins and other biomolecules because cationic silver are positively charged as compared to neutral silver ions [151].

It has also been reported that bactericidal activity of AgNPs is due to the formation of free radicals such as ROS and it has been supported through the fact that the electron spin resonance (ESR) spectrum of AgNPs shows a peak of 336.33 [152]. Some researchers have attributed growth inhibition of microbes by interaction of positive charge and negative charges between AgNPs and cell membrane. It have also been reported in literature that AgNPs showed remarkable antibacterial activity on gram negative bacteria and this activity is concentration dependent [150].

TABLE 4.1: Results of antibacterial assay against different bacterial strains..

AgNPs Conc (ppm)	**Zone of inhibition (cm)±S.D**									
	Gram Positive Strains						**Gram Negative Strains**			
	M. luteus		***S. aureus***		***B. subtiles***		***AT-10***		***E. aerogenes***	
	AgNP	**Plant Extract**	**AgNP**	**Plant Extract**	**AgNP**	**Plant Extract**	**AgNP**	**Plant Extract**	**AgNP**	**Plant Extract**
100	1.3±0.9	1.2±0.7	2.0±0.05	1.3±1.8	1.7±0.1	1.0±0.1	1.8±1.0	1.0±1.0	4.0±0.12	1.9±0.1
60	1.2±0.5	1.0±0.15	1.6±0.1	1.2±1.7	1.5±0.1	0.8±0.1	1.82.0	8.0±1.0	3.0±0.31	1.2±1.0
50	1.0±0.03	-	1.7±0.05	0.7±0.05	1.2±0.1	-	1.7±1.6	-	2.5±0.21	0.8±0.1
40	0.9±1.2	-	1.5±0.4	-	1.0±0.1	-	1.5±1.7	-	2.0±0.1	-
30	-	-	1.0±0.1	-	1.0±0.1	-	1.2±0.15	-	1.5±0.05	-
20	-	-	-	-	-	-	0.8±0.1	-	0.8±0.1	-
10	-	-	-	-	-	-	-	-	-	-
Distilled H2O (-tv Control)	-	-	-	-	-	-	-	-	-	-
Streptomycin (+tv Control)	2.5		2		2		2		3.5	

4.3.2 Antifungal Assay

Fungal species are causative agents of variety of diseases and confer serious resistance against various fungal drugs. It is needed to design such alternatives which can combat this problem. In this study synthesized silver nanoparticles were tested against various fungal strains which are responsible for various diseases. The highest activity was observed in the case of *Aspergillus flavis* 62.5% (growth inhibition). In contrast to this least activity which was observed in the case of Mucor species 13%. *Solani, Niger* and *fumigatus* also showed considerable activity against silver nanoparticles which are 23%, 24% and 43% respectively (Table 4.2). The effect of silver nanoparticles was studied in comparison with plant extract and positive and negative control. Plant extracts did not showed significant activity except *Aspergillus fumigatus* and *Aspergillus flavis*. Highest activity was observed in the case of *Aspergillus flavis* 27% and least activity was observed in case of *Aspergillus fumigatu*s 15% (Table 4.2). Enhanced antifungal activity was observed with silver nanoparticles as compared to plant extract. Treating any fungal infection with conventional antibiotics such as amphotericin B causes a serious problem because of side effects of these drugs like renal and liver dysfunctions. With the passage of time, microorganisms become resistant to conventional antimicrobial drug. To cope with this problem AgNPs prove to be a best alternative because they show high antimicrobial activity [153].

From ancient times, it has been reported that silver has been used as an antimicrobial agent. It is also reported that silver-based compounds are much cheaper than gold based. Antimicrobial activity of silver nanoparticles have been reported through several studies against virulent fungal strains and most of the studies are focused on the Candida species [154]. Marcato et al. (2012) have also reported that silver nanoparticles produced by green method (disc diffusion test) show considerable antifungal activity against *Tricophyton rubrum* [155]. Moreover, conventional antifungal drugs have also various drawbacks which limits their usage and efficacy such as toxicity, resistance and high treatment costs [155]. Furthermore, it has been reported that effective antimicrobial activity of AgNPs depend on size and shape [156]. Another study reports that silver nanoparticles show higher degree

of antimicrobial activity as compared to silver ions. The antimicrobial action is basically due to is interaction of silver ions with thiol (sulfhydryl) groups as well as various other sites in the membrane proteins of microbes [157].

TABLE 4.2: Percentage inhibition of silver nanoparticles and plant extract against different fungal species

S.No	Samples	Percentage inhibition against Fungal Species (%)				
		Mucor.sp	***F.Solani***	**A.*fumigatus***	***A.Flavis***	***A.Niger***
1	AgNPs	13	23	43	62.5	24
2	Plant ectract	-	-	15	27	-
3	Distilled water (-ve Cont)	-	-	-	-	-
4	Terbinafine (+ve Cont)	100	100	100	100	100

4.3.3 Antioxidant Assay (DPPH)

The antioxidant ability of AgNPs was evaluated by DPPH assay. The free radical scavenging activity was exhibited by both AgNPs and plant extract. In this study, silver nanoparticles exhibited high free radical scavenging activity as compared to plant extract which means that nanoparticles proof to be more effective as compared to plant extract. At highest concentration (100ppm) silver nanoparticles showed 61 % free radical scavenging activity whereas plant extract showed 51%. At 50 ppm silver nanoparticles showed 51% whereas plant extract showed 46% free

radical scavenging activity. At lowest concentration of 25ppm silver nanoparticles exhibited 47% whereas plant extract showed 37% free radical scavenging activity (Figure 4.7). The results are also significant statistically as P<0.0001 (Table 4.4) and IC_{50} 17ppm for silver nanoparticles and 22 ppm for plant extract (Table 4.3). The less IC_{50} value of silver nanoparticles shows that they showed significant free radical scavenging activity as compared to plant extract. It was also found that the free radical scavenging activity of silver nanoparticles increases in dose dependent manner and by decreasing the concentration there is a gradual decline in the scavenging activity.

The DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical and has the ability to donate hydrogen atoms by reacting with various compounds. This assay is used to measure the reducing ability of various antioxidants by DPPH free radical. Moreover, this method is also feasible due to its short reaction time. The DPPH assay can also be used in combination with other techniques such as HPLC for screening a large number of antioxidants [158]. It can also be inferred that the antioxidant ability of silver nanoparticle is mainly due to the adherence of functional groups to the surface of nanoparticles from plant extract. Methi seeds are chief source of various antioxidant compounds such as phenolic acids, flavones and flavanones [159].

It is also reported that silver nanoparticles synthesized from spice blends showed remarkable scavenging activity as compared to a well-known antioxidant rutin [160]. Kanipandian et al., have also reported that color change was observed after adding silver nanoparticles in DPPH solution which also confirmed that silver nanoparticles exhibit strong antioxidant potential [161]. In another study silver nanoparticles synthesized from apple showed strong antioxidant activity [162]. On addition of silver nanoparticles formulation in the solution having DPPH, purple color of the solution changes to yellow which shows scavenging of free radicals [162].

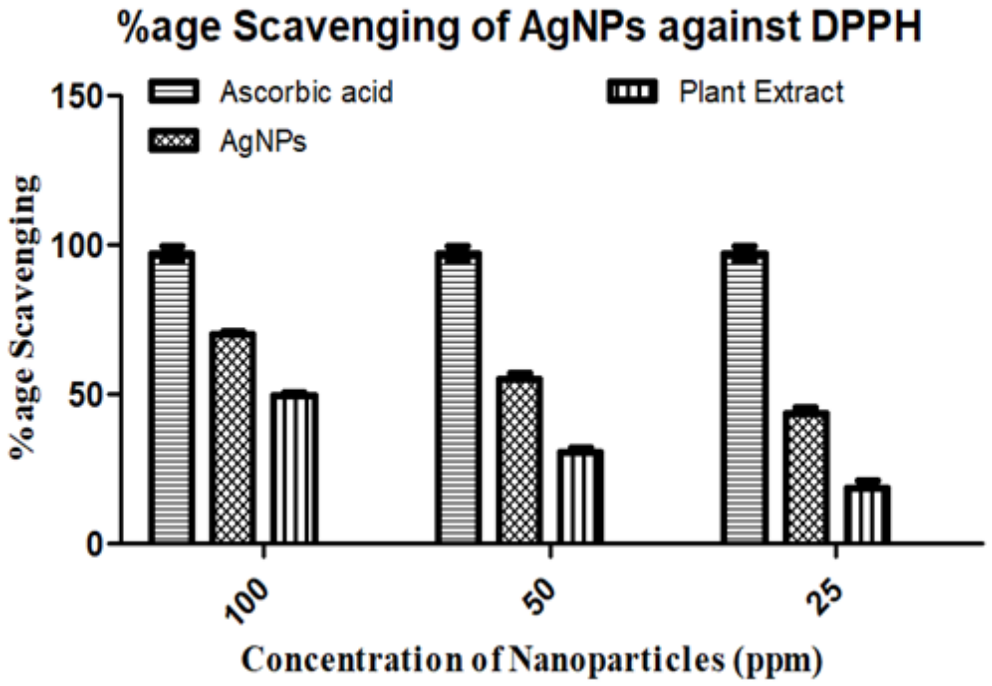

FIGURE 4.7: % age scavenging of silver nanoparticles and plant extract against DPPH

TABLE 4.3: % age scavenging and IC_{50} of silver nanoparticles and plant extracts against DPPH

Samples	**Percentage scavenging**			
	100 ppm	**50 ppm**	**25 ppm**	**IC_{50} (ppm)**
AgNPs	61	51	47	17
Plant extract	51	46	37	22

TABLE 4.4: Analysis of variance for factors affecting the free radical scavenging activity of Silver nanoparticles

Source of variation	**Df**	**Sum of squares**	**Mean square**	**F-Value**	**P-Value**	**Significant**
Interaction	4.0	846.5	211.6	27.25	<0.0001	Yes
Sample Type (AgNPs and Seed Extract)	2.0	19110	9553	1230	<0.0001	Yes
Concentration	2.0	1653	826.4	106.4	<0.0001	Yes
Residual	18	139.8	7.767			

4.3.4 Cytotoxic assay

Toxicity of AgNPs was evaluated using brine shrimp cytotoxic assay. Different concentrations of silver nanoparticles and plant extract i.e. 100 ppm, 50 ppm and 25 ppm were used and showed significant toxic effect. At 100 ppm silver nanoparticles showed 93% mortality whereas plant extract showed 78% mortality. At 50ppm silver nanoparticles showed 86% mortality whereas plant extract showed 61% mortality. At 25 ppm silver nanoparticles showed 73% mortality whereas plant extract showed 58% mortality. It was observed that mortality was increased by increasing the concentration and highest mortality 93% was achieved as compared to plant extract 78% at highest concentration i.e. 100 ppm (Table 4.5). In figure 4.7 %age mortality of silver nanoparticles against brine shrimps at different concentrations

is shown. It was also found that higher concentration corresponds to higher mortality rate (Figure 4.7). The results were also found quiet significant statistically (P<0.0001) (Table 4.6) and IC_{50} 701 ppm for silver nanoparticles. However, plant extracts had greater IC_{50} 956 ppm which proved that AgNPs showed more promising results as compared to plant extract and hence can be used on cancer cell lines (Table 4.6).

In an earlier study by Jacob et al (2012) it was found that cytotoxicity of AgNPs is governed by several factors which include dose, time and size of silver nanoparticles. Moreover, AgNPs shows dose dependent cytotoxicity against MCF-7 cells. AgNPs can cause death of cancerous cells by initiating different reactions. These include prevention of cancerous cells to proliferate in healthy cells, initiation of self-destruction (apoptosis) and causing damage to DNA [163]. Another report by Warheit et al (2004) stating the investigation of lungs toxicity in rats was found that carbon nanotubes which are inserted in intra-tracheal region instilled single-wall carbon nanotubes produced granulomas at very high doses [164].

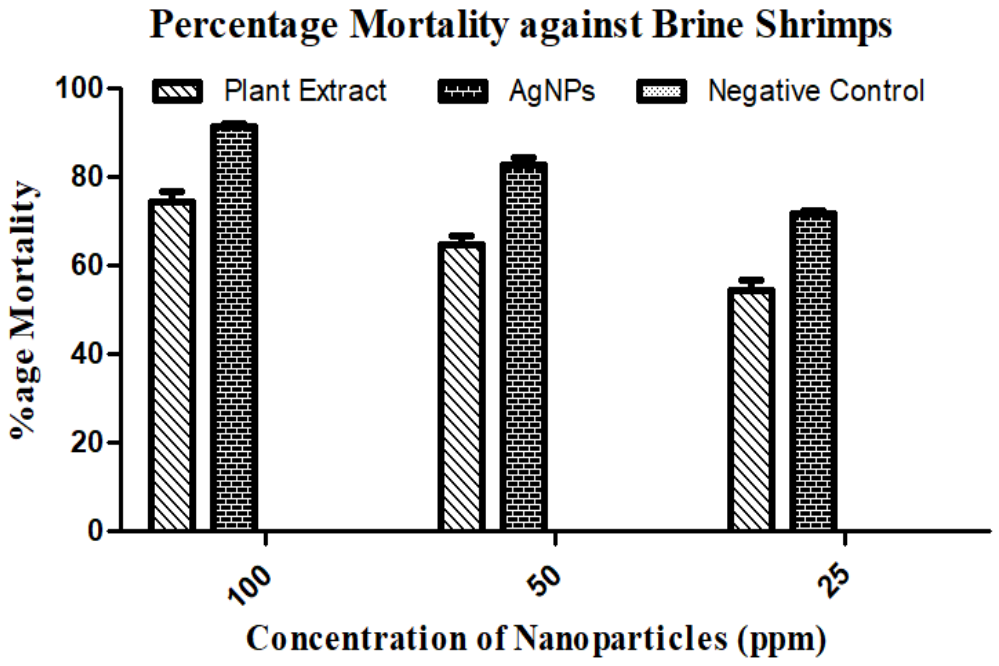

FIGURE 4.8: % age mortality of silver nanoparticles against brine shrimps at different concentration

TABLE 4.5: % age mortality and IC_{50} of silver nanoparticles and plant extract

Samples	**Percentage scavenging**			
	100 ppm	**50 ppm**	**25 ppm**	**IC_{50}(ppm)**
AgNPs	78	61	58	701
Plant extract	93	86	73	956

TABLE 4.6: Analysis of variance for factors affecting the mortality of brine shrimps

Source of variation	**Df**	**Sum of squares**	**Mean square**	**F-Value**	**P-Value**	**significant**
Interaction	4.0	382.1	95.54	11.02	<0.0001	Yes
Sample Type (AgNPs and Seed Extract)	2.0	4906	2453	283.0	<0.0001	Yes
Concentration	2.0	801.2	400.6	46.22	<0.0001	Yes
Residual	18	48.67156.0	8.667			

Chapter 5

Conclusion And Future Work

Conclusion

The green synthesis of silver nanoparticles using *Trigonella foenum* shows that the presence of bioactive compounds in plant extract play a vital role in the synthesis process and they act as a reducing and capping agent. It was observed that synthesized silver nanoparticles from methi seeds having absorption spectra 390-410 nm, size ranging from 43 ± 4nm, exhibiting standard peaks in the range of 2850-3000 through FTIR analysis which describes the involvement of primary amines in silver nanoparticles synthesis and crystalline nature was confirmed with identified peaks through XRD. These particles were found effective antimicrobial, cytotoxic and antioxidant agents and hence can be explored further as antibiotics and antifungal drugs. It was observed that bioactivities of synthesized nanoparticles was found to be enhanced as compared to the plant extract.

Future Work

As synthesized silver nanoparticles were found to be very effective antimicrobial, cytotoxic and antioxidant agents this makes them potent therapeutic agents which can be used as effective drug delivery agents in in vitro experiments providing us an opportunity to further discover them and unravel their potential as potent antibiotics and anticancer agents. Moreover, the smooth morphological features can also be used as drug carriers in controlled and targeted drug delivery systems in future.

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