CHAPTER ONE
INTRODUCTION

1.1. General Overview

Nanotechnology involves research and technology development at the atomic, molecular or macromolecular levels in the range of approximately 1-100 Nanometers to provide fundamental understanding of phenomena and materials at the Nano-scale. The nanometer scale is about a billionth of a meter. In comparison, a human hair is about 10,000 nanometers in diameter. Basically nanotechnology is used to create structures, devices and systems that have novel properties and functions because of their minute size. Actually, the matter shows unusual physical and chemical properties due to increase in surface area compared to volume as particles get smaller in size & this is called quantum size effect. This means the bulk properties of materials at Nano-scale can be very different from those at larger scale. Taking the advantage of these characteristic of material, scientist designs and produces devices by manipulating the shape and size at Nano- scale with wide-range of implications which could include medicine, electronics, military applications, computing, space science and many more(Arya et al., 2008, Shinde et al., 2012).

1.2. The Importance of Nano-scale

The Greek word "Nano" (meaning dwarf) refers to a reduction of size, or time, 10^{-9}fold, which is one thousand times smaller than a micron(Mansoori and Soelaiman, 2005).One nanometer (nm) is one billionth of a meter, and it is also equivalent to ten Angstroms. As such, a nanometer is 10^{-9} meter, and it is 10 000 times smaller than the diameter of a human hair. A human hair diameter is about 50 microns (i.e., 50×10^{-6} meter) in size, meaning that a 50 nanometer object is about 1/1000th of the thickness of a hair. One cubic nanometer (nm^3) is roughly 20 times the volume of an individual atom. Nano-element compares to a basketball like a basketball compares to the size of the earth. It is obvious that Nano-science, Nano-engineering, and nanotechnology all deal with very small sized objects and systems. Nano-scale is a magical point on the dimensional scale; structures in Nano-scale (called nanostructures) are considered at the borderline of the smallest of human-made devices and the largest molecules of living systems. Our ability to control and manipulate nanostructures will make it possible to exploit new physical, biological, and chemical properties of systems that are intermediate in size, between single atoms, molecules, and bulk materials. There are many specific reasons why Nano-scale has become so important, including the following:

- The quantum mechanical (wavelike) properties of electrons inside matter are influenced by variations on the Nano-scale. By Nano-scale design of materials, it is possible to vary their micro and macroscopic properties, such as charge capacity, magnetization, and melting temperature, without changing their chemical composition.
- A key feature of biological entities is the systematic organization of matter on the Nano-scale. Developments in Nano-science and nanotechnology would allow us to place man-made Nano-scale things inside living cells. It would also make it possible to make new materials using the self-assembly features of nature. This certainly will be a powerful combination of biology with materials science.
- Nano-scale components have very high surface to volume ratio, making them ideal for use in composite materials, reacting systems, drug delivery, and chemical energy storage (such as hydrogen and natural gas).
- Macroscopic systems made up of nanostructures can have much higher density than those made up of microstructures. They can also be better conductors of electricity(Nouailhat, 2010, Hornyak et al., 2008, Mansoori, 2017).

1.3. Synthesis method of Nano-particles:

In the synthesis of Nano-particles, which can be natural or synthetic origin and exhibit unique properties at the Nano-scale, two basic approaches that include various preparation methods and are known from early times are used. The first approach is the "top-down" method which calls for breaking down of solid materials into small pieces by applying external force. In this approach, many physical, chemical and thermal techniques are used to provide the necessary energy for Nano-particle formation. The second approach, known as "bottom-up", is based on gathering and combining gas or liquid atoms or molecules. These two approaches have advantages and disadvantages relative to each other. In the up-down approach, which is costlier to implement, it is impossible to obtain perfect surfaces and edges due to cavities and roughness that can occur in Nano-particles; whereas excellent Nano-particle synthesis results can be obtained by bottom-up approach. In addition, with the bottom up approach, no waste materials that need to be removed are formed, and Nano-particles having smaller size can be obtained thanks to the better control of sizes of the Nano-particles(Nadaroglu et al., 2017). The classification of synthesis methods of Nano-particles is given in Fig.1.1:

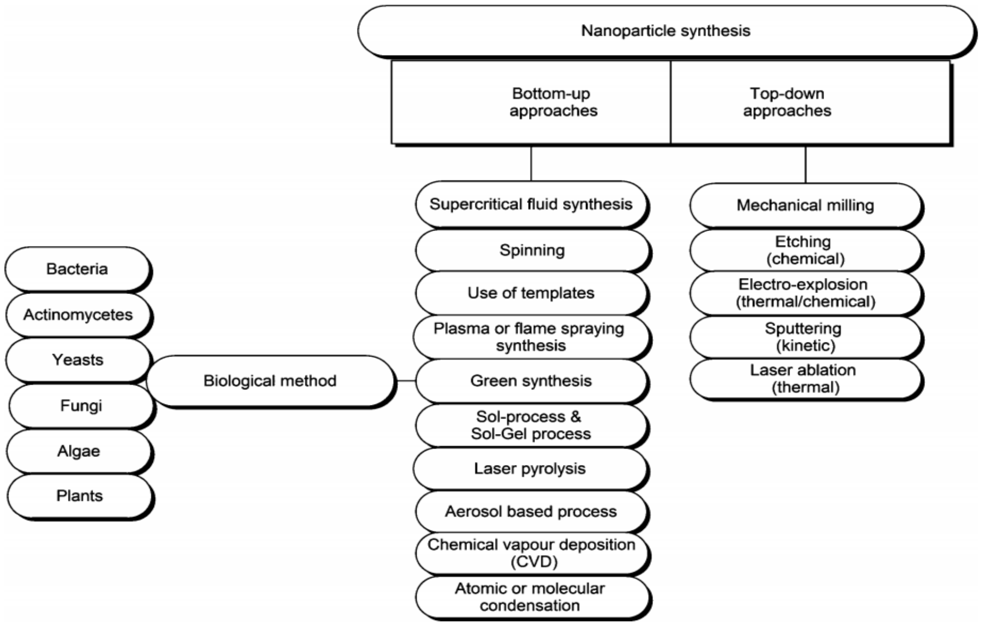


Fig.1.1classification of synthesis methods of Nano-particles

1.4. Green synthesis methods:

Traditional methods are used from past many years but researches have proved that the green methods are more effective for the generation of NPs with the advantage of less chances of failure, low cost and ease of characterization(Abdelghany et al., 2018). Physical and chemical approaches of synthesizing NPs have posed several stresses on environment due to their toxic metabolites. Plant-based synthesis of NPs is certainly not a troublesome procedure, a metal salt is synthesized with plant extract and the response is completed in minutes to couple of hours at typical room temperature. This strategy has attracted much more attention amid the most recent decade particularly for silver (Ag) and gold (Au) NPs, which are more secure as contrasted with other metallic NPs. Generation of NPs from green techniques can be scaled up effortlessly and they are fiscally smart too. In light of their exceptional properties the greenly orchestrated NPs are currently favored over the traditionally delivered NPs. Use of more chemicals, which are harmful and toxic for human health and environment, could increase the particle reactivity and toxicity and might cause unwanted adverse effects on health because of their lack of assurance and uncertainty of composition(Hussain et al., 2016). Green methods of synthesis are significantly attractive because of their potential to reduce the toxicity of NPs. Accordingly, the use of vitamins, amino acids, plants extracts is being greatly popularized nowadays(Baruwati et al., 2009, Gour and Jain, 2019).

1.5. Problem statement:

Synthesis of Nano-material with the desired quality and properties is one of the key issues in current Nano-technology. Today, the green synthesis of metallic Nano-particles has received increasing attention due to the development of eco-friendly technologies in materials science. Use of natural plant extracts in the preparation of Nano-particles by greener route provides advancement over chemical and physical method as it is cost effective, environment friendly.

1.6. Objectives:

- To Synthesis Silver Nano-particles at low cost by using extract of Fenugreek as reducing and stabilizing agent.
- To Characterization of Silver NPs using SEM.
- To Use Silver NPS as antifungal.

CHAPTER TWO

LITRERATURE REVIEW

2.1. Historical Background

Nano-particles and structures have been used by humans in fourth century AD, by the Roman, which demonstrated one of the most interesting examples of nanotechnology in the ancient world. The American physicist and Nobel Prize laureate Richard Feynman introduce the concept of Nano-technology in 1959(Bayda et al., 2020, Komal, 2021).

Feynman made the hypothesis “Why can’t we write the entire 24volumes of the Encyclopedia Britannica on the head of a pin?”, and described a vision of using machines to construct smaller machines and down to the molecular level (. This new idea demonstrated that Feynman's hypotheses have been proven correct, and for these reasons, he is considered the father of modern nanotechnology. After fifteen years, Norio Taniguchi, a Japanese scientist was the first to use and define the term “nanotechnology” in 1974 as: “nanotechnology mainly consists of the processing of separation, consolidation, and deformation of materials by one atom or one molecule”(Bayda et al., 2020).In 1986, Binnig and Rohrer received the Nobel Prize in Physics “for their design of the STM” This invention led to the development of the atomic force microscope (AFM) and scanning probe microscopes (SPM), which are the instruments of choice for nanotechnology researchers today(Komal, 2021).

In1990, the scientists analyzed the Lycurgus cup using a transmission electron microscopy (TEM) to explain the phenomenon of dichroism. The observed dichroism (two colors) is due to the presence of Nano-particles with 50–100 nm in diameter. The Lycurgus cup from the ancient glass industry describes two different types of glass which change colors in certain lighting conditions. The progress of Nano-science and nanotechnology in deferent fields of science has expanded indifferent directions, to observe things from micro to Nano, to even smaller scale sizes by deferent microscopes in physics, from micro size bulk matter to small size carbon dots in chemistry, from room size computers to mobile slim size laptops in computer science.

2.2. Previous Studies:

Silver Nano-particles with different sizes (7, 29, and 89 nm mean values) were synthesized using Gallic acid in an aqueous chemical reduction method. The sizes of the silver Nano-particles were controlled by varying some experimental conditions. It was found that the antibacterial activity of the Nano-particles varies when their size diminishes(Panáček et al., 2009).

Silver Nano-particles were synthesized and their antifungal effects on fungal pathogens of the skin were investigated. AgNPs showed potent activity against clinical isolates and ATCC strains of Trichophytonmentagrophytes and candida species(IC80,1-7µg/ml). The activity of AgNPs was comparable to that of amphotericin B, but superior to that of fluconazole (amphotericin B IC80,1-5µg/ml; fluconazole IC80,10-30µg/ml). Additionally, we investigated their effects on the dimorphism of Candida Albicans. The results showed Ag NPs exerted activity on the mycelia. Thus, the present study indicates Ag NPs may have considerable antifungal activity, deserving further investigation for clinical applications(Kim et al., 2008).

2.3. Applications of Nanotechnology:

Nanotechnology represents the ''key technology'' of the 21st century and the expectancy for innovations in the environmental sector is significantly high. The word Nanotechnology includes a wide range of technologies performed on a nanometer scale for widespread applications. The main difference between a Nano-material and a larger-scale-material is the significantly larger specific surface area which grants an increase in the chemical reactivity and/or a change to the physical properties of the material (Yang et al., 2013). The new properties showed by this new class of materials have already been used in the manufacture industry for decades; the large and rapid increase of applications of nanotechnology is due to the convergence of scientific disciplines as chemistry, physics, biology and engineering (Liu et al., 2012). Subsequently several Nano-materials were introduced in medicine, in biotechnology, in computer science, in space exploration, in chemical industry(Bavasso et al., 2016). The Nanotechnology has wide applications such as:

2.3.1. In Medicine:

Current modalities of diagnosis and treatment of various diseases, especially cancer have major limitations such as poor sensitivity or specificity and drug toxicities respectively. Newer and improved methods of cancer detection based on Nano-particles are being developed. They are used as contrast agents, fluorescent materials, molecular research tools and drugs with targeting antibodies. Paramagnetic Nano-particles, quantum dots, Nano-shells and Nano-some are few of the Nano-particles used for diagnostic purposes. Drugs with high toxic potential like cancer chemotherapeutic drugs can be given with a better safety profile with the utility of nanotechnology. These can be made to act specifically at the target tissue by active as well as passive means. Other modalities of therapy such as heat induced ablation of cancer cells by Nano-shells and gene therapy are also being developed. This review discusses the various platforms of nanotechnology being used in different aspects of medicine like diagnostics and therapeutics. The potential toxicities of the Nano-particles are also described in addition to hypothetical designs such as reciprocates and microbivores. The safety of Nano -medicine is not yet fully defined. However, it is possible that Nano-medicine in future would play a crucial role in the treatment of human diseases and also in enhancement of normal human physiology(Bavasso et al., 2016).

2.3.2. In agriculture and food production

With the growing limitation in arable land and water resources, the development of agriculture sector is only possible by increasing resources use efficiency with the minimum damage to agro ecology through effective use of modern technologies. Among these, Nano-technology has the potential to revolutionize agricultural systems, biomedicine, environmental engineering, safety and security, water resources, energy conversion, and numerous other areas(Manjunatha et al., 2016).Nano-technology is working with the smallest possible particles which raise hopes for improving agricultural productivity through encountering problems unsolved conventionally. In the management aspects, efforts are made to increase the efficiency of applied fertilizer with the help of Nano clays and zealots and restoration of soil fertility by releasing fixed nutrients. Research on smart seeds programmed to germinate under favorable conditions with Nano polymer coating are encouraging. In the controlled environment agriculture and precision farming input requirement of crops are diagnosed based on needs and required quantities are delivered in right time at right place with the help of Nano biosensor and satellite system. Nano-herbicides are being developed to address the problems in perennial weed management and exhausting weed seed bank. Nano structured formulation through mechanisms such as targeted delivery or slow/controlled release mechanisms and conditional release, could release their active ingredients in response to environmental triggers and biological demands more precisely(Manjunatha et al., 2019).

Table2.1. Applications of Nanotechnology in agriculture and food production

Rank	Applications	Examples
1	Energy storage, production and conversion	CNT storage of H
2	Agricultural productivity enhancement	Herbicide delivery
3	Water treatment & remediation	Nano-membranes

4	Disease diagnosis & screening	Lab-on-Chip
5	Drug delivery systems	Nano-capsules
6	Food processing & storage	Coating/packaging
7	Air pollution & remediation	Nano-catalysts
8	Construction	Durability
9	Health monitoring	Sensors
10	Vector & pest detection/control	Sensors and pesticides

2.3.3. In Engineering

Nano-fluids are the dilute suspensions of Nano-materials with distinctive and enhanced features. Nano-fluids can be used in a variety of industrial applications because of improved thermo physical properties. Stability of Nano-fluids is the only quandary factor which decreases the efficiency of such smart fluids in engineering applications. The information and studies on interaction of Nano-materials with the liquid have significant importance toward their usage in industrial applications.

Agglomeration among particles is a common issue due to interactive forces, which effects the dispersion, theology, and overall performance of Nano-suspensions. Characterization of Nano-fluids plays an important role to evaluate the stability of Nano-fluids. The effect of agglomeration on the stability of Nano-fluids can be reduced by introducing different mechanical and chemical techniques to prolong dispersion of suspended particles in liquids(Korada and Hamid, 2017, Keidar and Beilis, 2013, Mirzaei, 2020).

2.4. Metal Nano-particles

Metal Nano-particles (NPs) are a subject of global interest in research community due to their diverse applications in various fields of science. The stabilization of these metal NPs is of great concern in order to avoid their agglomeration during their applications(Manojkumar et al., 2016). The term metal Nano-particle informally used to described Nano-sized metals with dimensions(length, width or thickness)within the nanometer size range1–100 nm(Khan et al., 2016b, Khan et al., 2016a). Metal Nano-particles have attracted much attention over the last decade owing to their unique properties as compared to their bulk metal equivalents, including a large surface-to-volume ratio and tunable shapes. To control the properties of Nano-particles with particular respect to shape, size and disparity is imperative, as these will determine the activity in the desired application(Khan et al., 2016a).

2.5. Silver Nano-particles:

Silver Nano-particles (AgNPs) are of particular interest due to their remarkable antimicrobial and localized surface Plasmon resonance properties, which render them unique properties such as broad-spectrum antimicrobial, surface-enhanced Raman spectroscopy (SERS), chemical /biological sensors and biomedicine materials [6-8], biomarker and on. Silver Nano-particles are usually ranging from 1 to 100nm in size(Lee and Jun, 2019).They have unique optical, electrical, and thermal properties and are incorporated into industrial application of electronics, catalysis, and photonics. In recent years, with higher integrated density of electronic components, there are growing demands for the thickness or the width of printed electronic circuits due to considering the space between these circuits. Therefore, the synthesis of AgNPs becomes an important issue in the electronic industry(Natsuki et al., 2015). Silver Nano-particles are one of the most attractive Nano-materials for commercialization applications. They have been used extensively as electronic products in the industry, anti-bacterial agents in the health industry, food storage, textile coatings and a number of environmental applications. As anti-bacterial agents, silver Nano-particles were used for a wide range of applications from disinfecting medical devices and home appliances to water treatment(Abbasi et al., 2016).

2.6. Methods for the synthesis of silver Nano-particles:

2.6.1. Physical Approach

The physical synthesis of AgNPs usually utilizes the physical energies to produce AgNPs with nearly narrow size distribution. The physical approach can permit producing large quantities of AgNPs samples in a single process. This is also the most useful method to produce AgNPs powder. However, primary costs for investment of equipment should be considered.

2.6.2. Photochemical Approach

The photo-induced synthetic strategies have also been developed. For example, Huang and Yang synthesized AgNPs via photo reduction of $AgNO_3$ in layered inorganic clay suspensions, which serves as stabilizing agent that prevent Nano-particles from aggregation. Irradiation disintegrated the AgNPs into smaller size with a single mode distribution until a relatively stable size and diameter distribution were achieved (Huang and Yang, 2008). However, in this method, the equipments with high cost and experimental environment are required.

2.6.3. Chemical Approach

Chemical reduction is the most common method because of its convenience and simple equipment. Control over the growth of metal Nano-particles is required to obtain Nano-particles of small size with a spherical shape and narrow distribution in diameter. It is well known that silver Nano-particles can be produced by chemical reaction at low cost and in high yield(Huang and Yang, 2008). In this review we describe savories chemical synthesis methods to prepare the silver Nano-particles mainly.

2.6.4. Biological Approach (Green method)

Recently, biosynthetic methods using naturally reducing agents such as polysaccharides, biological microorganism such as bacteria and fungus or plants extract, i.e. green chemistry, have emerged as a simple and viable alternative to more complex chemical synthetic procedures to obtain AgNPs. Bacteria are known to produce inorganic materials either intra- or extracellular. This makes them potential bio-factories for the synthesis of Nano-particles like gold and silver. Particularly, silver is well known for its bio-ticalproperties. A. R. Vilchis-Nestor et al. used green tea extract as reducing and stabilizing agent to produce gold silver Nano-particles in aqueous solution at ambient conditions.(Vilchis-Nestor et al., 2008) Moreover, K. Kalishwaralal et al. reported the synthesis of AgNPs by reduction of aqueous Ag+ ions with the culture supernatant of Bacillus licheniformis .(Kalishwaralal et al., 2008).The synthesized AgNPs are highly stable and this method has advantages over other methods as the organism used here is nonpathogenic bacterium. The biological method provides wide range of resources for the synthesis of AgNPs, and this method can be considered as a method of Nano-particles synthesis with advantages over conventional chemical routes of synthesis and as an environmentally friendly approach as well as a low cost technique. However, it is not easy to obtain a large quantity of AgNPs by using biological synthesis(Natsuki et al., 2015).

2.7. Green methods of synthesis using fenugreek seeds:

2.7.1. Physical properties of fenugreek seeds

Fenugreek is an annual herb of leguminosae, is being used as spice with its seeds and as vegetable with its leaves. It has a long history as both a culinary and medicinal herb in the ancient world. Fenugreek is known as Greek hay. Its seeds have a strong aroma and somewhat bitter in taste. Fenugreek is native to Southern Europe, the Mediterranean region and Western Asia. It is cultivated from Western Europe to China for the aromatic seeds and is still grown for fodder in parts of Europe and Northern Africa. The seeds are very hard, and difficult to grind. Seed extract is used in imitation vanilla, butterscotch and rum flavoring and is the main flavoring in imitation maple syrup. Rich in vitamins and minerals, and because it is a seed and a legume, it is high in protein.

2.7.2. Fenugreek chemical constituents.

The chemical composition of Fenugreek seeds and defatted Fenugreek seeds is given in Table-1. These seeds are a rich source of fiber and protein. The fiber maybe further classed as gum (gel fiber) and neutral detergent fiber. Whole Fenugreek seeds also contain 4.8% Saponins. Fenugreek seed Saponins are of steroidal nature (type FurostanolSaponins) with Diosgenin as the principal steroid alSaponin.

Table 2.2. Proximate compositions % of Fenugreek seeds

Component	Whole Seeds	Defatted Seeds
Moisture	9.0	9.0
Ash	3.0	3.5
Lipids	8.0	Negligible
Protein	26.0	28.3
Starch	6.0	6.5
Total Fiber	48.0	51.7
Gum	20.0	19.2
Neutral Detergent Fiber	28.0	32.5

2.7.3. Antimicrobial

fenugreek seeds have traditionally and commonly been used to treat diabetes, coughs, congestion, bronchitis, fever, high blood pressure, headache, migraines, diarrhea, flatulence, anemia, irregular menstrual cycles and arthritis, to ease labor pains and menstruation pain, and as an appetite stimulant(Alkofahi et al., 1996, Alkofahi et al., 1997). Fenugreek has also been used as an external poultice to control inflammation and dandruff. Modern medicine is beginning to provide confirmation of many of the traditional medicinal applications of fenugreek seeds(Puri, 1998, Billaud, 2001, Billaud and Adrian, 2001, Ahmad et al., 2016). To the best of our knowledge, no previous studies have been reported for antimicrobial activity of fenugreek. Thus, this study was aimed to evaluate the antimicrobial activity of fenugreek seeds in order to be used in some infectious diseases(Ahmad et al., 2016).

2.7.4. Candida Albicans

Candida Albicans is the best studied and most prevalent of the human fungal pathogens. Candida species are fungi that grow as yeasts and that are 'imperfect', meaning they apparently lack a complete sexual cycle; etc. Albicans and several related species clearly have the potential to engage in 'parasex' (described below). C. Albicans, thought to bean obligate diploid, can form true filamentous hyphae in addition to the budding yeast and pseudo hyphal (elongated yeast) cells seen in other Candida species and in the model yeast Saccharomyces cerevisiae.

2.8. Applications of Silver NPs

Due to their unique properties, AgNPs have been used extensively in house-hold utensils, the health care industry, and in food storage, environmental, and biomedical applications. Applications of AgNPs in various biological and biomedical applications, such as antibacterial, antifungal, antiviral, anti-inflammatory, anti-cancer, and anti-antigenic. Silver Nano-particles are one of the most attractive Nano-materials for commercialization applications. They have been used extensively as electronic products in the industry, anti-bacterial agents in the health industry, food storage, textile coatings and a number of environmental applications(Verma and Maheshwari, 2019).

As anti-bacterial agents, silver Nano-particles were used for a wide range of applications from disinfecting medical devices and home appliances to water treatment (Bosetti et al., 2002, Cho et al., 2005). Moreover, this encouraged the textile industry to use AgNPs in different fabrics. In this direction, silver Nano-composite fibers were prepared containing AgNPs incorporated inside the fabric. The cotton fibers containing AgNPs exhibited high anti- bacterial act to catalyze the chemi-luminescence from luminol–hydrogen peroxide system with catalytic activity better than Au and Pt colloid(Natsuki et al., 2015).

Recently, inkjet technology has been used to produce flexible electronic circuits at low cost, and many studies regarding this application have been reported in recent years. To fabricate flexible electronic displays via inkjet printing, it is necessary to develop suitable inks. Nano-sized metal particles such as Au or Ag are useful for producing electronic circuits because of the uniformity of the small metal particles dispersed in the inks and their high electrical conductivity. For example, using our methods described above, AgNPs with small size and uniform can be prepared easily, and have high electrical conductivity, indicating that they are useful for producing electronic circuits. In the manufacture of electronic circuits, Nano-particles must be sintered to obtain high electrical conductivity. It is preferable to perform sintering at the lowest temperature possible. However, the use of polymeric materials as dispersing agents means that a high temperature is required for sintering.

2.9. Characterization of Silver Nano-particles

The physicochemical properties of Nano-particles are important for their behavior, bio-distribution safety, and efficacy. Therefore, characterization of AgNPs is important in order to evaluate the functional aspects of the synthesized particles. Characterization is performed using a variety of analytical techniques, including UV-via spectroscopy, X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), dynamic light scattering (DLS), scattering (DLS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM).

2.10. Scanning electron microscope (SEM)

The scanning electron microscope (SEM) image of the test sample surface is obtained by scanning it with a high energy beam of electrons in a vacuum chamber. When the beam of electrons strikes the surface of the specimen and interacts with atoms of a sample, signals in the form of secondary electrons and backscattered electrons are generated that contain information about the sample’s surface morphology(Usha et al., 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Materials for Synthesis of Silver NPs:

The Fenugreek Seeds were purchased from local market in Sudan, Silver Nitrate Ag No3 from Industrial Consulting Research Center, Khartoum Sudan and used without further purification. Three liter distilled water.

3.2. Methods:

3.2.1. Preparation of Fenugreek Seeds:

The Fenugreek Seeds extract was prepared by these ways: Weighted 5g from plant seeds using sensitive balance and then washed with distilled water to remove any contaminant or dust particles, then put on the sterile container and added 100ml boiled distilled water and maintained for 24h at room temperature. After a soaking period, the extract was filtered using filter paper and the filtrate was used immediately for preparation of the Nano-particles.

3.2.2. Preparation of Silver Solution:

Firstly, sure all glassware cleaned, weighted0.85 g of Silver Nitrate and carefully dissolved in small beaker then poured on volumetric flask and added distilled water to complete the volume to 50 ml, the solution has been diluted by taking (25,5) ml from solution and added distilled water to complete the volume to50ml to prepare different concentration of silver Nitrate (0.1,0.05,0.01).

3.2.3. Biosynthesis of Silver Nano-particles:

Silver Nano-particles were synthesized using the following procedure: The plant extract of Fenugreek Seeds (20ml) was mixed with (10ml) of (0.1, 0.05,0.01M) aqueous Silver Nitrate immediately the color changed to brown. then put in Autoclave at121c,0.2Mpa (Vigneshwaran et al., 2006), for 30mins. the color of the mixture of Silver Nitrate (0.01) and extract of fenugreek seeds changed from brown to black this indicated the formation of Silver Nano-particles. In 45mins the color of the mixtures of Silver Nitrate (0.1,0.05) and extract of fenugreek seeds changed from brown to black. The solution was then centrifuged at1000 rpm for 14min. followed by re-dispersion of the pellet in deionizer water to remove any unwanted biological materials and dried at room temperature for 24hrs to be ready for Characterization by Scanning electron microscope.

Fig.3.1. Autoclave

Fig.3.2. Centrifuge

Fenugreek seeds

⇩

Cleaning and Washing

⇩

Adding 5 of seed in 100ml of boiled distilled water

⇩

Soaked overnight

⇩

The extract was filtered(using filter paper)

⇩

The extract was used for preparation of the Silver nanoparticles (added 20ml of extract to 10ml of Silver nitrate)

⇩

Put in Autoclave(at 121c,0.2Mpa) change in color To black(indicates the formation of Silver nanoparticles)

⇩

Stable silver nanoparticles

Fig.3.3. Process flow chart for biosynthesis of silver NPs.

3.3. Characterization of Silver NPs by Scanning electron microscope:

The scanning electron microscope (SEM) image of the test sample surface is obtained by scanning it with a high energy beam of electrons in a vacuum chamber. When the beam of electrons strikes the surface of the specimen and interacts with atoms of the sample, signals in the form of secondary electrons and backscattered electrons are generated that contain information about the sample's surface morphology. The morphological features of silver Nano-particles were studied using SEM).

Fig.3.4. scanning electron microscope(SEM) device model T. scan republic of check

3.4. Testing of antimicrobial susceptibility

The paper disc diffusion method was used to screen the antimicrobial activity of plant extracts and performed by using Mueller Hinton agar (MHA) and Sabouraud dextrose agar. The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial and fungal suspension were diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred micro-liters of bacterial and fungal suspension were swabbed uniformly on surface of MHA and SDA the inoculums were allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on

the surface of the MHA and SDA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.
According to National Committee for Clinical Laboratory Standards (NCCLS) (1999) and Performance standards for antimicrobial susceptibility testing, the antibacterial activity results were expressed in term of the diameter of zone of inhibition and <9mm zone was considered as inactive; 9-12mm as partially active; while13-18mm as active and >18mm as very active.

CHAPTER FOUR
RESULTS AND DISCUSSIONS

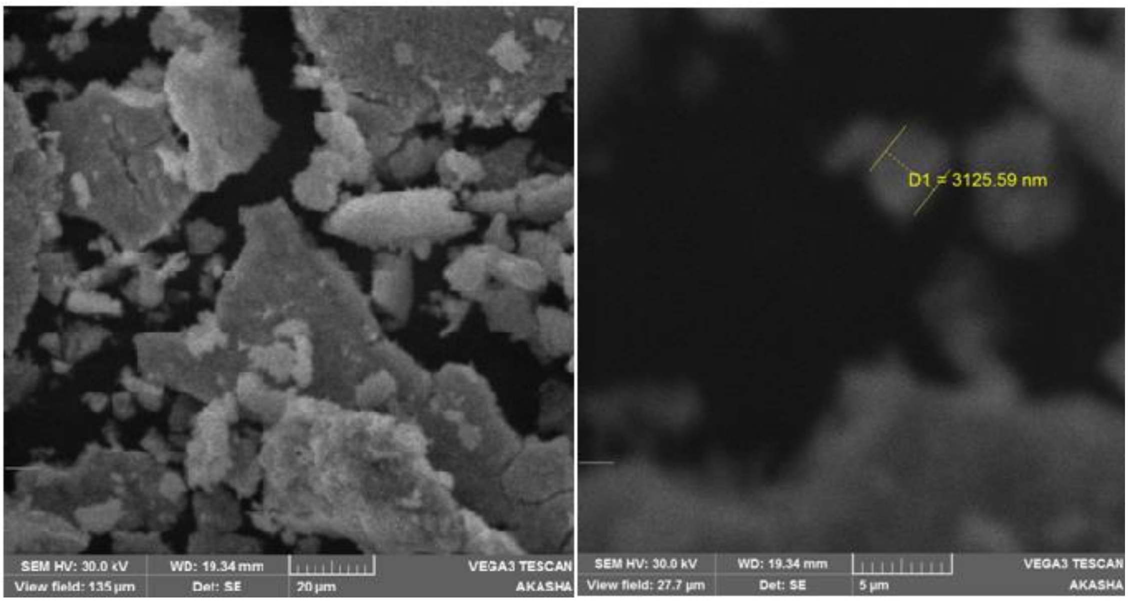
4.1. Visual Observations:

After addition, the plant extract to the Silver solution, the color changed to oily color, and after inserted to autoclave the color changed to black. Shown in Fig

Fig.4.1. Biosynthesis of Silver Nano-Particles (changing in the color)

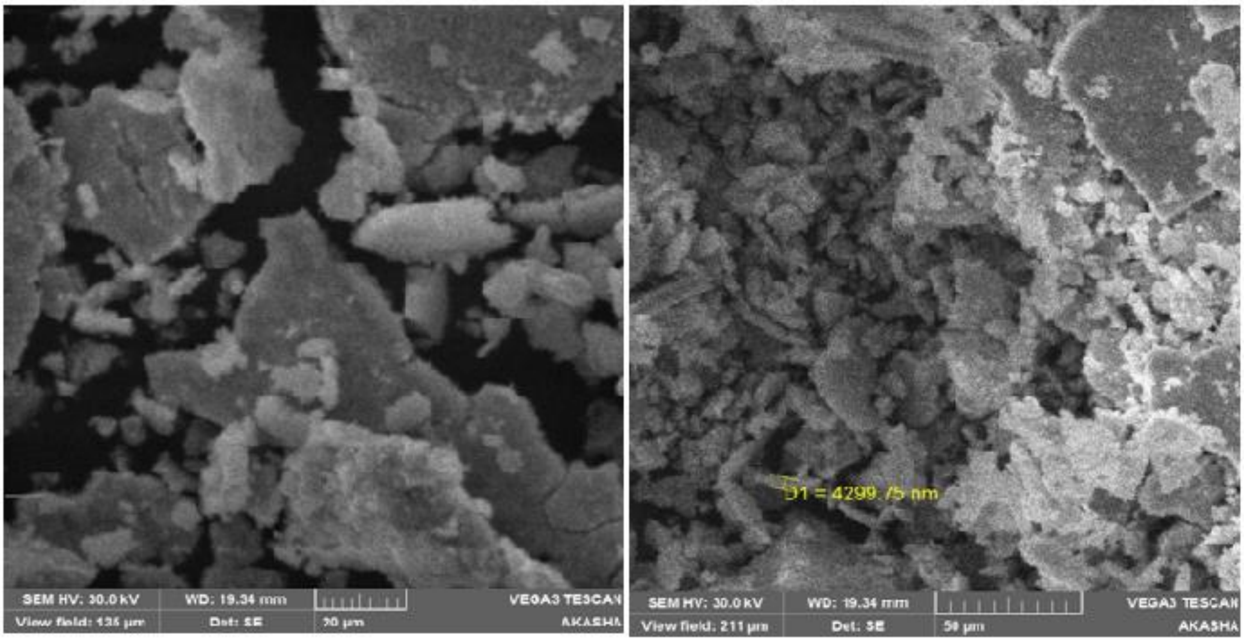
4.2. Characterization of Synthesis Silver Nano-Particles (Ag NPs) by Scanning Electron Microscopy (SEM)

The scanning electron microscopy image of the Nano-particles presented the topography of the particles were showed in fig.



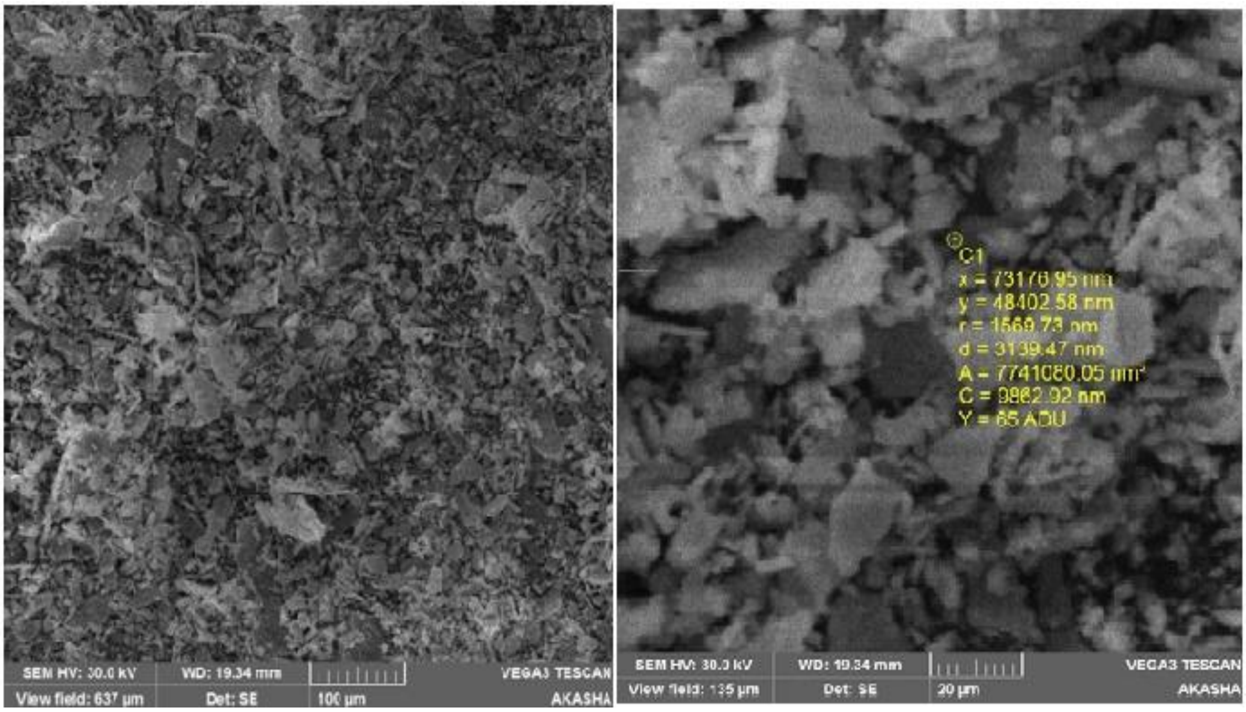
D=3125nm

Fig.4.2. The SEM photographs of the synthesized Silver Nano Particles using extract of Fenugreek (5g), (0.1M).



D=4299nm

Fig.4.3. The SEM photographs of the synthesized Silver Nano Particles using extract of Fenugreek seeds(5g) (0.05M)



D=3139nm

Fig.4.4. The SEM photographs of the synthesized Silver Nano-Particles using extract of Fenugreek *s*eeds (5g) (0.01M)

4.3. Antifungal activity of Silver NPs

Silver Nano-particles have shown antifungal activities against Candida Albicans

Table 5.1.The effects of Silver NPs (different concentration) on the Candida Albicans:

Plant name	Solvent	concentration	C.a	C.a
Nano-silver	Dimethyl sulphoxide	0.1mg\ml	16mm	17mm
Nano-silver	Dimethyl sulphoxide	0.05mg\ml	10mm	10mm
Nano-silver	Dimethyl sulphoxide	0.01mg\ml	13mm	15mm

Candida Albicans (C.a)

The results were expressed in terms of the diameter of the inhibition zone: < 9 mm inactive; 9-12 mm partially active; 13-18 mm active; >18 mm very active.

The antifungal activity of silver Nano-particles increases as the concentration of silver nitrate increase.

The antifungal activity of silver Nano-particles increases as the size of silver Nano-particles decrease.

This means the antifungal activity is directly proportional to the concentration of silver nitrate, but is inversely proportional to the size of silver Nano-particles.

Fig 4.5. Antifungal activity against Candida Albicans

CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

- The green synthesis of silver nanoparticles using plant extract as reducing and capping agent has been done.
- The extract of fenugreek seeds reduced the silver particles to Nano-particles.
- The size and structure of obtained silver nanoparticles were characterized by SEM.
- These silver Nano-particles have shown antifungal activities against Candida Albicans, and can be used in baby diapers.

5.2. Recommendations

- Use a low concentration of Silver Nitrate to reduce cost.
- The heater can be used instead of the autoclave to reduce costs.
- Characterization of Silver NPs using TEM.
- As silver nano-particles showed antifungal activity also they can be examined against different bacteria and microorganisms.

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