GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM PLANT EXTRACT OF MEDICINAL PLANTS *TRICHODESMA INDICUM* AND *OXALIS CORNICULATA*: CHRACTERIZATION AND ANTIMICROBIAL ACTIVITY

Dissertation submitted in partial fulfillment for the degree of

Master of Science in Biotechnology

Submitted By

Juhi Kumari

Registration No.:16530050265

Roll No.: 1661041

School of Biotechnology, Campus-XI

Kiit deemed to be university

Bhubaneswar, Odisha, India

Under the Supervision of

Dr. BIRENDRA KUMAR BINDHANI

Assistant Professor

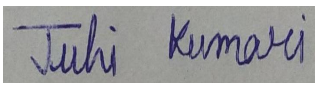
School of Biotechnology,

kiit deemed to be university

DECLARATION

I, **Ms. Juhi Kumari**, a student of **M. Sc. Biotechnology** bearing **Roll No.: 1661041 & Registration No.: 16530050265**, School of Biotechnology, KIIT deemed to be university, Bhubaneswar, Odisha, do here by declare that the project entitled **"Green Synthesis of silver nanoparticles from plant extract of medicinal Plant *Trichodesma Indicum* and *Oxalis corniculata*: Characterization and antimicrobial activity"** is the original work carried out by me in Plant Biotechnology Laboratory, School of Biotechnology, kiit deemed to be university Bhubaneswar, Odisha for the partial fulfilment of the requirement for the award of Master of Science in Biotechnology from School of Biotechnology, KIIT deemed to be university.

I, also hereby declare that this project is the result of my sincere effort and hard work. The research work or any part of it has not been submitted for any other degree or any equivalent qualification.



Date: 16.5.18 **Ms. Juhi Kumari**

School of Biotechnology
Kalinga Institute of Industrial Technology (KIIT)
Deemed to be University
(Established U/S 3 of UGC Act, 1956)
Bhubaneswar, Odisha, India

CERTIFICATE

This is to certify the dissertation entitled **"Green Synthesis of silver nanoparticles from plant extract of medicinal Plant *Trichodesma Indicum* and *Oxalis corniculata*: Characterization and antimicrobial activity"** is Submitted by **Ms. Juhi Kumari** in partial fulfillment of the requirement for the degree of Master of Science in Biotechnology, School of Biotechnology, kiit deemed to be university, Bhubaneswar bearing **Roll No.: 16601041 & Registration No.: 16530050265** is a bonafide research work carried out by her under my guidance and supervision from **28th Nov, 2017** to **15th May, 2018.**

Date: 16.5.2018
Place: Bhubaneswar

Dr. B. K. Bindhani
Assistant Professor-II
School of Biotechnology
kiit deemed to be University
Bhubaneswar, Odisha, India

School of Biotechnology
Kalinga Institute of Industrial Technology (KIIT)
Deemed to be University
(Established U/S 3 of UGC Act, 1956)
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This is also to certify that the above said work has not previously submitted for the award of any degree, diploma, fellowship in any Indian or foreign University.

Date: 16.5.2018
Place: Bhubaneswar

Dr. B. K. Bindhani
Assistant Professor-II
School of Biotechnology
KIIT deemed to be University
Bhubaneswar, Odisha, India

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Juhi Kumari

Date: 16.5.18 **Ms. Juhi Kumari**

Place: KSBT, Bhubaneswar [M. Sc. Biotechnology]

Roll No.: 1661041

Registration No.: 16530050265

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ABSTRACT

Recent advancements in nanoscience and nanotechnology frequently changed the way of diagnosing, treating, and preventing various diseases in all aspects of human life and research. Nanotechnology deals with the nanoparticle having a size of 1-100nm in one dimension. Nanoparticles are used significantly in medical chemistry, atomic physics, and all other known field, due to its small size scale, immense surface area per unit volume, a high proportion of atoms in the surface and near surface layers, and the ability to exhibit quantum effects. These particles can be prepared easily by different chemical, physical, and biological approaches. But the biological approach is the most emerging approach of preparation, as it is easier than other methods, eco-friendly and less time consuming. The green synthesis was done by using the aqueous solution of ***Trichodesma indicum*** plant & ***Oxalis corniculata*** extract and $AgNO_3$. Silver was of particular interest for this process due to its evocative physical and chemical properties. A fixed ratio of plant extract to metal ion was prepared and observed for the color change, which proved the formation of nanoparticle. The nanoparticles were characterized by UV-Vis spectroscopy, FTIR and application of this nanoparticle is shown in gram positive (*Staphylococcus aureus)* and gram negative (*Escherichia coli).*

OBJECTIVES

1. **Green synthesis of Silver nanoparticles using plant extract of different medicinal plant.**
   - ***1.1.*** **Synthesis of AgNPs using aqueous plant extracts of *Trichodesma indicum.***
   - ***1.2.*** **Synthesis of AgNPs using aqueous plant extracts of *Oxalis corniculata.***
2. **To characterize the nanoparticles formed by using Ultraviolet-Visible (UV-Vis) spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, DLS and Zeta potential.**
3. **To check the antibacterial activity of synthesized nanoparticle against gram positive and gram negative bacteria.**

ABBREVIATION

UV/vis	: Ultra violet visible
SEM	: Scanning electron microscopy
DLS	: Dynamic light scattering
FTIR	: Fourier transform infrared
AFM	: Atomic force microscopy
SPR	: Surface plasmon resonance
$AgNO_3$	: Silver nanoparticle
AuNP	: Gold nanoparticle
E.Coli	: *Escherichia coli*
S. Aureus	: *Staphylococcus aureus*
SNP	: Silver nanoparticle
NPs	: Nanoparticles
T.Indicum	: *Trichodesma indicum*
O.corniculata	: *Oxalis corniculata*
µg	: Microgram
ml	: Milliliter
^{o}C	: Degree Celsius
et al.	: ET alia (Latin): and others
mV	: Mega volt

CHAPTER-I

Introduction

1.1. Introduction

Nanotechnology is a fastest growing field of science due to the production of nanoproducts and NPs (NPs) that can have novel or unique and size-related physicochemical properties along with significant difference from large matters. The novel qualities of NPs have been misused and exploited in a wide range of potential applications in medicine, cosmetics, renewable energies, environmental remediation and biomedical devices [1-3]. Because to swift urbanization, our lovely environment is undergoing huge violent break and a large amount of toxic and harmful chemical, gases or substances are released and so from now, it is our responsibility to learn about the secrets behind nature and its useful products which helps in the growth and development of NPs synthesis. Nanotechnology is very suitable for biological molecules, because of their entire properties. The biological molecules display highly controlled assembly for the synthesis of suitable and appropriate metal NPs, which was found to be good and eco-friendly [4]. Metal and semiconductor have a very wide area of research because of its potential application which was implemented and used in the development of the novel technologies [5]. Nanotechnology is a filed which is one of the recent upcoming areas, having completely new or improved properties, such as size, a different type of distribution and morphology of the particles etc. NPs and nanomaterials are fastest growing filed because of its novel properties and different in various filed [6].

NPs of metal have a high specific synthesis area and a high fraction of surface atoms. Due to its unique and different physicochemical characteristic, including catalytic activity and optional properties [7-10], they are gaining a lot of interest in the field of scientific research for their unique or novel methods of synthesis. Over the past several years, the synthesis of metal NPs is an important and emerging topic of research in modern material science technology. Nano-crystalline silver particles show a variety of application in the field of high sensitivity bio-molecules detection, diagnostics, antimicrobial, therapeutics, catalysis, and microelectronics. But there is still need for a method which is economic commercially viable at the same time environmentally friendly clean synthesis method for the synthesis of route silver NPs. Silver is very well known for its possessing an inhibitory effect toward many bacterial strains and microorganisms usually present in medical and industrial processes [11].

NPs can be synthesized by using different methods are a chemical, physical, and biological method. However chemical approach of synthesis requires a very short period of time for the synthesis of huge quantity of NPs, this approach requires capping agent for the size stabilization of the NPs. In chemical methods, a chemical which is used for the synthesis of NPs are highly toxic and comes up as environmental pollutants. So, there is need of a method which is an environment-friendly and non-toxic and this expectation leads to the synthesis of NPs with the help of biological method which is free from the use of toxic and environment pollutants chemical as byproducts. Therefore, the demand for "green nanotechnology" [12] is increasing day by day due to its useful properties. By using biological method NPs can be synthesized by using bacteria, fungi, and plants [13-14]. Plants give a suitable platform for the synthesis of NPs as they are free from toxic and different chemicals. Besides, use of plant extracts can also reduce the cost of isolation of microorganism and culture media enhancing the cost as compared to the microorganism [12].

Sometimes, synthesis of NPs by different plants and their extracts are advantageous as compare to another biological method which involves a complex method to maintain microbial cultures [15-16]. Different type of experiments already started for the synthesis of metal NPs using fungi *Fusarium oxyporum* [17], *Penicillum sp.*[18] and bacteria such as *Bacillus subtilis* [19-20] etc. However, synthesis of the nanoparticle using plant extract is most used method due to its non-toxic, environmentally friendly property and also for its large distribution, easily available, safe to handle and act an as good source of several metabolites [21].

Nowadays, silver NPs can be synthesized by naturally occurring sources and the plant products are such as green tea (*Camellia sinensis*), Neem (*Azadirachra indica*), Leguminous shrub (*Sebania drummondii)*, various leaf broth, natural rubber, starch, Aloe Vera plant extract[22], lemongrass leaves extract, etc. In microbes, the silver nanoparticle gets attached to the cell wall of the microbe, so it disturbs the permeability of cell wall and respiration of cell. NPs have the ability to penetrate deep inside the cell wall, so it causes damage of the cellular compounds by interacting with phosphorous and sulfur-containing compounds such as DNA and also protein, present inside the cell. The property of silver NPs, that it releases silver ions from the bacterial cell, which has antimicrobial effects [23]. Antibacterial effect of silver nanoparticle depends on the size of NPs like smaller particle has high antimicrobial property as compared to the larger particle [24]. Synthesis of nano-sized material in research is of significant interest because of their unique properties like optoelectronics, magnetic, and mechanical, which differs from bulk [25].

CHAPTER-II

A Short Review on Green Synthesis of Silver Nanoparticles and its Biomedical Applications

2.1. Nanotechnology

Nanotechnology is rapidly growing field which has the ability to produce nanoproducts and NPs that have varies novel and size related physicochemical properties significantly different from large matter. The novel or unique properties of NPs can be used in the various field and has a wide range of potential applications which is used in varies field like medicine, cosmetics, renewable energies, environmental remediation and biomedical devices. Nanotechnology is a field which is concerned with development and utilization of structure and devices with organization features at the intermediate scale between individual molecules and about 100nm where novel properties occur as compared to bulk materials. It has the capability to buildup tailored nano-structures and devices for given functions by controlling the atomic and molecular levels. Nanotechnology is recognized as a most emerging enabling technology for the 21st century, in addition to the already established areas of information technology and biotechnology. This is due to its scientific convergence of physics, chemistry, biology materials and engineering at the nanoscale, on almost all technologies. NPs manufacturing is an essential and important component of nanotechnology because the specific properties are realized at the NPs, nanocrystal or nanolayer level and assembling of precursor particle and related structure is the most generic route to generate nanostructured materials [2]. Synthesis of nanosized material in research is of significant interest because of their unique properties like optoelectronics, magnetic, and mechanical, which differs from bulk [25].

2.2. Application of Nanotechnology

The nanotechnology is not only scientific filed but it is also related and collaborated to chemistry, physics, material science and biology to develop new novel technologies[26]. The advantage of NPs is its small size which gives large surface area for the particle and its effectiveness. The nanosize also increases the penetration power of the particles which gives NPs another advantage to use it in better way. NPs have ability invade the circulatory system and can also cross blood-brain- brain barrier in human system [27]. Nanotechnologies development gives rise lead in the scientific views in various areas is disease diagnosis, treatment and prevention. The use of this technology is limitless such as it can use in nanomachines, nanofibers, self-assembled polymeric nanoconstructs, nano-membrane and nano-sized silicon chip for drug, protein, nucleic acid or peptide delivery and their release, biosensors, laboratory diagnostics [28].

1. MEDICINE: The nanosized particles are designed in a manner to interact with cells and tissues to carry out biologically specific functions. They are used in different fields like as delivering the drug, peptides/proteins, and genes and in the biomedical area including cancer therapy and vaccination. Nanoparticle can also utilized in different filed like in the field of administrative routes such as oral, nasal, parental or intraocular, which gives effective as compare to the other methods [29].

2. DRUG DELIVERY: Major problem in the delivery of therapeutic compound is not delivering to the target site and this problem can overcome by the help of this technology by controlling drug delivery. In this method drugs are delivered to the place of action on vital tissue and control the adverse effect. In the assembly of increasing therapeutic compounds at the targeted site, the concentration of drug decreases. In this technology, specific design of carriers such as liposomes, polymers, dendrimers, silicon or carbon materials and magnetic NPs are attached to a drug to accomplish a cell-specific target in drug delivery systems [30].

3. DIAGNOSIS: The biological application of NPs has raised new application and possibilities in the field of diagnosis and in the treatment of diseases. The main objective is to improve pathologies and patho-physiological principles of various diseases and treatment. For example; in cancer diagnosis fluorescent NPs used for the multiplex profiling tumor biomarkers and for the detection of multiple genes. The uses of conjugated NPs in future will at-least give ability to detect ten different kind cancer related protein. The super magnetic NPs have been used as contrast agent for the detection of cancer *in-vivo* and monitor their response towards the treatment. Therefore, by using the NPs tumors targeting and drug delivery can be done to achieve therapeutic goal [31-32].

4. TISSUE ENGINEERING: NPs also helpful in the repairing mechanism of tissue and organ substitutes by manipulating their cellular properties. In organ transplantation, there is chance of rejection of tissues by the body but NPs helps to reduce the chance of the rejection during transplantation as well as it also stimulate growth factors [33].

5. BIOIMAGING: For imaging of biological specimens, in vitro and in vivo different molecular imaging techniques are reported but advance development in the luminescent and magnetic NPs have got lots of attention for its bioimaging technologies. The luminescent nanoprobes for optical imaging and magnetic NPs for magnetic resonance imaging have been used for imaging purpose. This paved a way for the design of biologically targeted NPs for medical applications. The bioimaging using quantum dots show their utility in lymph nodes and blood vessels imaging. In one of the studies an antibody (Ab) conjugated magnetic poly-(D, L -lactide-co-glycolide) (PLGA) NPs with doxorubicin were synthesized and used for the simultaneous targeted detection and treatment of breast cancer [34].

6. INFECTIOUS DISEASE: The infection of different disease can be treated with the help of significant drugs but there is huge possibility that the pathogen become resistant to the drug by using different mechanism, where the drug does not able to play the significant role. The Problem can be overcome by the help of NPs and can to increase the drug efficacy. Therefore, NPs are used as antibacterial agents against different pathogens like bacteria, virus, fungi or parasites, multi-drug resistant strains, and biofilms; as targeted vectors towards specific tissues; as a theranostic system and as vaccines. Different types of NPs and its characteristics manifest in delivering drugs to the target and can also used to be addressed in clinical trials [34].

7. AGRICULTURE: The nanotechnology brings new inventions in agriculture. The new inventions approach, i.e., Agrinanotechnology had a great perspective way to survive with food production global challenges, climate changes, and sustainability. This technology helps to agricultural productivity at low cost and energy. The integrity of NPs provides a wide range of applications in varies agriculture sector are nano-fertilizers, nano-pesticides, nano herbicides, nanosensors and smart delivery systems for controlled release of agrochemicals. Apart from this, they are also used in other agriculture field like for plant breeding and genetic engineering purposes. Nanotechnology approaches help to encourage crop productivity and disease management [35].

8. WASTEWATER TREATMENT: The nanotechnology helps to deal with wastewater treatment. Now day's different types of nanoparticle have been designed to detect and remove the chemical and biological substances from water. Primarily, there are four types of nanomaterials are metal-containing NPs, carbonaceous nanomaterials, zeolites, and dendrimers. Nanomaterials had shown suitable amount of result than other techniques. The NPs are also designed and synthesized in such a way that it acts as either separation or reaction media for pollutants [36]

9. ELECTROCHEMICAL SENSORS & BIOSENSORS: The metal NPs has excellent catalytic properties and these can decrease the over potentials of many analytically important electrochemical reactions. As a result of this it gives highly sensitive sensor system. But, the important functions of NPs include the immobilization of biomolecules, enhancement of electron transfer between electrode surfaces and protein, the catalysis of electrochemical reactions, labeling of biomolecules and can also acts as reactant [37].

10. PROTEIN DETECTION: Protein microarrays depend on use of the fluorescent molecular dye but they have certain limitations with respects to parallel multiplexing and rotating of analytes. To perform multiplexed screening of protein, small molecules interactions and protein interactions a chemically designed Raman dye functionalized NPs probe having binding affinities and then coupled with surface-enhanced Raman scattering (SERS) spectroscopy was developed [38].

2.3. Nanoparticles

NPs are an important field in modern research because it deals with design synthesis and manipulation of particle structure and manipulation of particles structure ranging from approximately 1-100nm in one dimension. NPs are a wide category of materials that include particulate substances, which have one Dimension less than 100nm at-least [39]. Depending on the overall shape of NPs it is 0D, 1D, 2D, 3D [40]. Size of NPs can influence the physio-chemical properties of a substance (e.g. optical property). NPs are not simple molecule because it is made up of three-layer such as (a) surface layer, (b) core layer and shell layer. Surface layer is made up of a functionalized variety of small molecules, metal ions, surfactant and polymer. Shell layer is chemically different material from the core in all aspects, and the core is an essentially central portion of the nanoparticle [41].

2.4. Types of NPs

Depending on their morphology, size and chemical properties NPs (NPs) can be broadly divided into various categories.

A. Carbon-based nanoparticle: - Major class of carbon-based NPs is Fullerens and carbon tubes [CNTs]. Fullerences contain nonmaterial that is made of globular hollow cage such as in the form of carbon allotropic [42-43].

B. Metal NPs: - Metal NPs are mainly made of the metals precursors because of its well-known localized surface plasmon resonance [LSPR] characteristics; this NPs possess unique and different opto-electrical properties [44].

C. Ceramics NPs: - ceramics NPs which is made up of are inorganic non-metallic solids can be synthesized via heat and successive cooling dense, porous hollow forms [45].

D. Semiconductor materials: - Materials possess properties between metals and non-metals are known as a semiconductor material and therefore they found various applications in the literature due to this property [46]. They are very important and useful materials in photo catalysis, and electronics [47].

E. Polymeric NPs: - These are mostly nano-spheres or nano-capsular shaped they are normally organic based NPs and in the literature a special term polymer NPs collective used for it. [48].

F) **Lipid-based NPs**: - They contain lipid moieties and effectively using in varies biomedical application. Characteristically a lipid nanoparticle is spherical with the diameter ranging from 10-100nm. Same as polymeric NPs lipid NPs posses a solid core made of lipid and matrix contains soluble lipophilic molecules [49]

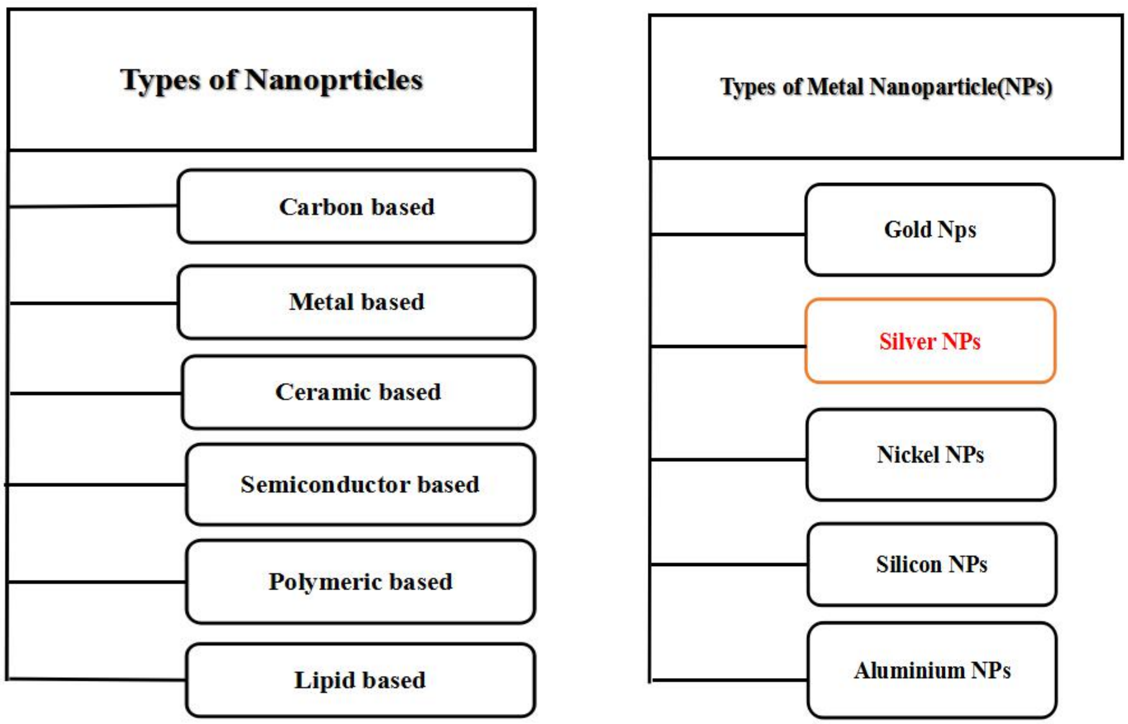
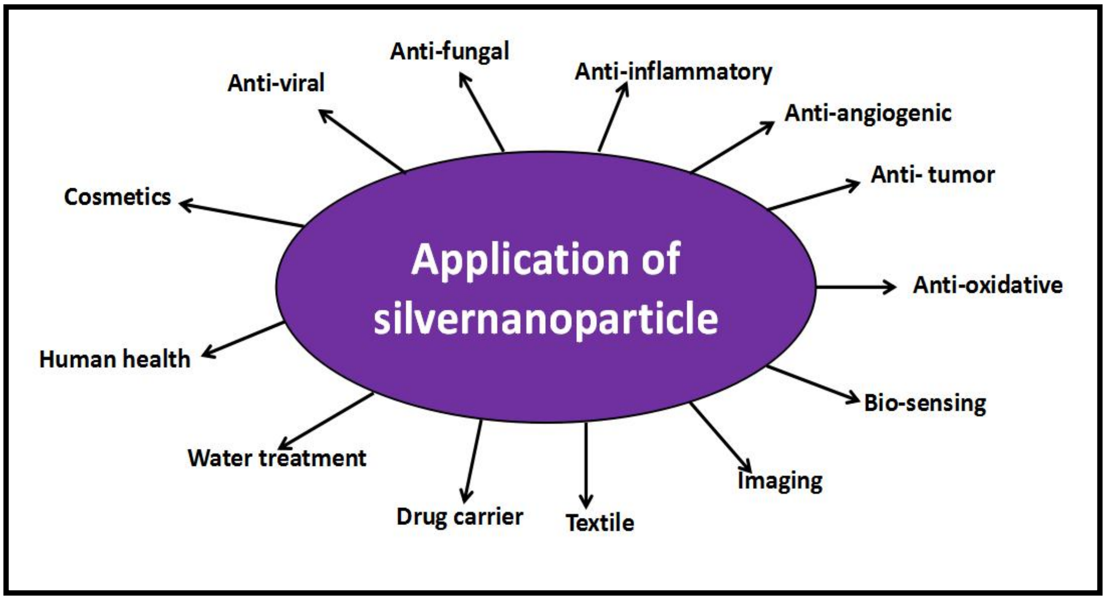


Figure: 2.1. Different approaches for the synthesis of silver nanoparticles.

2.5. Silver NPs (AgNPs)

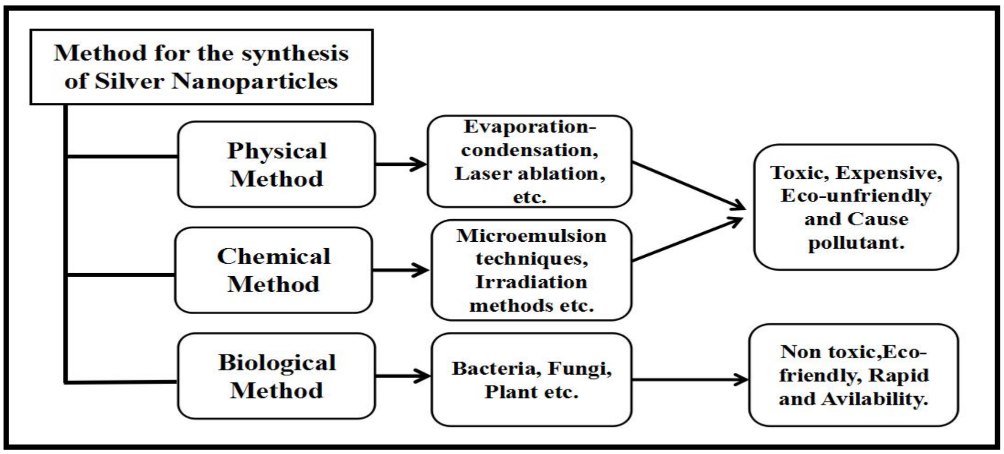
Silver NPs are NPs of silver of between 1 nm and 100 nm in size. While frequently described as being 'silver' some are composed of a large percentage of silver oxide due to their large ratio of surface-to-bulk silver atoms. Numerous shapes of NPs can be constructed depending on the application. Commonly used are spherical silver NPs but diamond, octagonal and thin sheets are also popular. Their extremely large surface area permits the coordination of a vast number of ligands. Silver Nitrate ($AgNO_3$) is mostly used for its antimicrobial effect, but when silver is used in the form of NPs, the availability of surface area increases and so the exposure to the microbes. Silver NPs can



be used in varies antibacterial applications, the action of this metal on microbes is not well known. As the action of metal is not well known it has been believed that silver NPs can cause cell disruption or inhibit cell transduction. There is a large number of the mechanism involved in cell lysis and growth inhibition of silver NPs (AgNPs) can be used in different filed for different purposes like medical, food, healthcare, and consumer, industrial purposes, due to its unique physical and chemical properties. These include optical, electrical, and thermal, high electrical conductivity, and biological properties [50-52]. Due to its another peculiar property, AgNPs are used for varies industrial applications, including development of antibacterial agents, coating household appliances, healthcare related products, in consumer products, medical devices like coatings, optical sensors, and cosmetics in the pharmaceutical industry. Apart from these application of AgNPs have been reported in the field of diagnostics, orthopedics, drug delivery, cancer biology as an anticancer agent and or by combining with other existing drugs [19]. Recently, AgNPs showed useful application used in textiles, keyboards, wound dressings, and biomedical devices [49- 54].

2.6. Method for the synthesis of Silver NPs

There are mainly three different approaches for the synthesis of silver NPs namely, physical, chemical and biological.



2.6.1. Physical approaches

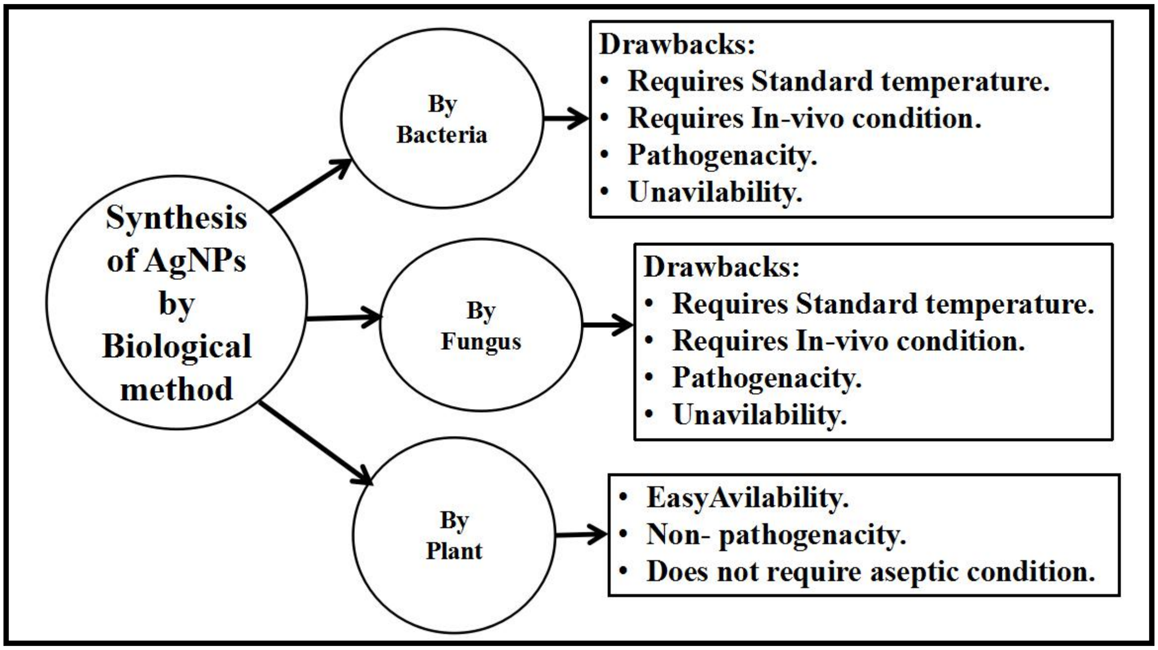
In the physical method, NPs are prepared by a different method such as evaporation-condensation using a tube furnace at atmospheric pressure and laser ablation [55-57]. Conventional physical method includes spark discharging and pyrolysis for the synthesis of AgNPs [58-59]. The advantage of physical methods includes; its speed of synthesis, radiation as reducing agent, and its safety (no hazardous chemical involved) but the downsides are low yield, high energy consumption, solvent contamination, and lack of uniform distribution [60-64]

2.6.2. Chemical approaches

There are various chemical methods, such as Micro emulsion techniques, Irradiation methods, polyol method [65], radiolytic process [66] etc. Chemical approach is the simplest method involved in the chemical reduction of the metal salt $AgNO_3$ by sodium borohydrate ($NaBH_4$) or Sodium citrate in water [67]. Chemical methods use water or organic solvents to prepare the silver NPs. This process usually employs three main components, such as metal precursors, reducing agents, and stabilizing/ capping agents. Basically, the reduction of silver salts involves two stages (a) nucleation and (b) subsequent growth [68-69].

2.6.3. Biological approaches

The use of plants as production assembly of silver NPs has drawn attention, because of its rapid, eco- friendly, non-pathogenic, economical protocol and providing a single step technique for the biosynthetic processes. The reduction and stabilization of silver ions by a combination of bio-molecules such as protein, amino acids, enzymes, polysaccharides, alkaloids, tannis, phenolics, saponics, terpinoids and vitamins which are already established in the plant extract having medicinal values and are environmental friendly [70]. Chemical and physical methods cause environmental pollution, the release of toxic chemicals and expensive as compared to the biological method. It is a fact which is unavoidable that silver nanoparticle synthesized have to be handled by humans and must be available at cheaper rates for their effective utilization. Thus, there is a need of a method which will be environmentally friendly and economically feasible way to synthesize these NPs. The search for such a method has led to the need for biomimetic production of silver nanoparticle whereby biological methods are used to synthesize the silver NPs. Physical and chemical method cause environmental pollution and are very expensive so the growing need of silver NPs can fulfill by developing environmentally friendly and economically feasible technologies for the synthesis of AgNPs [71]. Different kind of literature has been shown that use of microorganisms such as bacteria, fungi, and plants used for synthesis of AgNPs due to their antioxidant or reducing properties which is responsible for the reduction of metal compounds in their respective NPs. Although among the various biological method of silver NPs synthesis, microbe mediated synthesis is generally not always feasible everywhere due to the requirement of highly aseptic conditions and their maintenance. Therefore, the use of plant extract for this purpose is potentially advantageous over microorganisms due to the availability, ease of improvement, less bio-hazardous [72].



2.6.3.1. Synthesis of the silver nanoparticle by bacteria

Synthesis of silver nanoparticle was first reported by using bacteria *Pseudomonas stutzeri* AG259 that was isolated from silver mine [73]. There are some kinds of microorganism that can grow undermine condition, and this is due to their resistance to that metal which gives them the ability to grow undermining condition. The mechanism which is involved in the resistance are efflux systems, alteration of solubility and toxicity via reduction or oxidation, biosorption, bioaccumulation, extracellular complex formation or precipitation of metals, and lack of specific metal transport systems [74]. There is also another mechanism through which organism can grow at the lower concentration, their exposure to higher concentrations of metal ions can induce. A wide range of research has focused largely on prokaryotes because synthesizing silver NPs from prokaryotes has the ability to adapt to extreme conditions that is why bacteria are a good choice for study. They can also inexpensive, easy to grow and easy to manipulate. Growth conditions require such as temperature, oxygenation and incubation time can be easily controlled [75]. Controlling the environment is very important, because varies size of NPs requires for different application such as optics, catalysts or anti-microbial.

2.6.3.2. Synthesis of silver NPs by fungi

Synthesis of metallic silver NPs by using fungi shows significant attention as they offer advantages over bacteria for silver NPs synthesis. For large scale preparation and its down-stream processing fungi have been preferred over bacteria due to the economic feasibility and the presence of *mycelia* which offers an increased surface area for synthesis of silver NPs [76]. According to Mukherjee et al. fungi secrete higher amounts of protein than bacteria, this would enhance the NPs synthesis process and its yield.

The fungus *Fusarium oxysporum* has been used in a large number of times to study and to create metallic NPs, especially AgNPs. Pure AgNPs can synthesize at a size range of 5-15 nm and it is capped because capped AgNPs is more stable. But exact mechanism involved in the synthesis of silver NPs production by fungi is not fully known, so it is believed that the above-mentioned phenomenon is responsible for the reduction process. On the other hand this process is very slow compared to plant mediated nanoparticle synthesis. Hence, the use of plant extracts to synthesize silver NPs becomes an option that is feasible and suitable [77].

The biosynthesis of NPs by using green method has been proposed as a cost-effective and environmentally friendly as compared to chemical and physical methods. Plants-mediated synthesis of NPs is green chemistry approach that connects nanotechnology with plants. Novel methods for synthesizing NPs are required for ambient temperatures, neutral pH, low cost and environmentally friendly fashion. Plants are nature's "chemical factories". They are cost effective and require low maintenance. [78]. Green synthesis provides advancements over chemical and physical methods, as it is cost-effective, environment-friendly, loss toxic, easily scale up for large-scale synthesis, and in this method no need of high pressure, high temperature, and toxic chemicals.

2.6.3.3. Synthesis of silver NPs by plants

In past few years synthesis of metallic silver NPs by bacteria and fungi have been studied in large number. However, there has been less focus on plants in this matter. Recently the synthesis of silver NPs by using plant and plant extract is increasing day by day in the field of nano-research. This area is not explored in past but it offers promising results for the field of nanotechnology. A very important advantage of using plants rather than bacteria and fungi for the production of NP is its lack of pathogen city [79-81]. Various study have been reported for the synthesis of AgNPs by using plant extracts. Bar et al. illustrated a simple green synthesis route for AgNPs from $AgNO_3$ salts using the extract from *Jatropha curcas*. The results were the production of fairly homogenous (10-20nm) AgNPs in 4 hours. Another study using *Acalypha indica* leaf extracts showed that AgNPs synthesis using this plant is possible. The size of AgNPs was found to be 20-30 nm, which was again significantly homogenous in its size[82][83]. In a study by Kasthuri et al. Phyllanthin was extracted from the plant *Phyllanthus amarus* and subsequently used for the production of AgNPs and AuNPs. This is a unique study because single constituents of a plant extract were used for the synthesis of the metallic nanoparticle; as compared to the other studies mentioned earlier in which whole plants or extract were used [84].

An extensive study was done by Song et al. for the production of AgNPs from the number of different plant leaf extracts. They examined the use of *Pine, Persimmon, Ginkgo, Magnolia* and *Platanus* extracts and compared their ability to produce AgNPs [85]. In this study, the NP synthesis duration was long, over two weeks for NP extraction. So, in order to synthesize nanoparticle using biological process and making it commercially feasible, reducing time for production, non-toxicity and eco-friendly plant would be an emerging source for nanoparticle synthesis.

So, we have synthesized novel AgNPs using *Trichodesma indicum* and *Oxalis corniculata* plant extracts.

2.7. Mechanism of action of silver NPs on microbes

The exact mechanism by which silver NPs employ to cause antimicrobial effect is not clearly known and is a debated topic. There are however various theories on the action of silver nanoparticle on microbes to induce the anti-microbial effect. Silver NPs have the ability to anchor to the bacterial cell wall and subsequently penetrate through it, thereby causing structural changes in the cell membrane and ultimately death of the microbial cell. Application of AgNPs causes formation of 'pits' on and there is an accumulation of the NPs on the cell surface occurs. The formation of free radicals by the silver NPs may be considered to be another mechanism by which the cells die. Varies studies of electron spin resonance spectroscopy (ESRS) suggested that silver NPs cause the formation of free radicals when it comes in contact with bacteria and the free radical which are a form of silver NPs damage the cell membrane and increases its porosity, which cause the death of the cell. It has also been suggested that there can be the release of silver ions inside the cell and these ions can interact with the thiol groups (-SH) of many vital enzymes and inactive them. The bacterial cells in contact with AgNPs take in silver ions, which inhibit several functions in the cell and damage the cells. Then, there is the generation of reactive oxygen species (ROS), which are produced possibly through the inhibition of respiratory enzymes by silver ions and attack the cell itself. Silver is a soft acid, and there is a natural tendency of an acid to react with a base. The cells are majorly made up of sulfur and phosphorous which are soft bases. The action of these NPs on the cell can cause the reaction to take place and subsequently lead to cell death. Another fact is that the DNA had sulfur and phosphorous as its major components; the NPs can act as its major components; the NPs can act on these soft bases and destroy the DNA which would definitely lead to cell death. The interaction of the silver NPs with sulfur and phosphorous of the DNA can lead to problems in the DNA replication of the bacteria [86]. **Figure 1.3** diagrammatically explains the mechanism of anti-microbial activity of AgNPs.

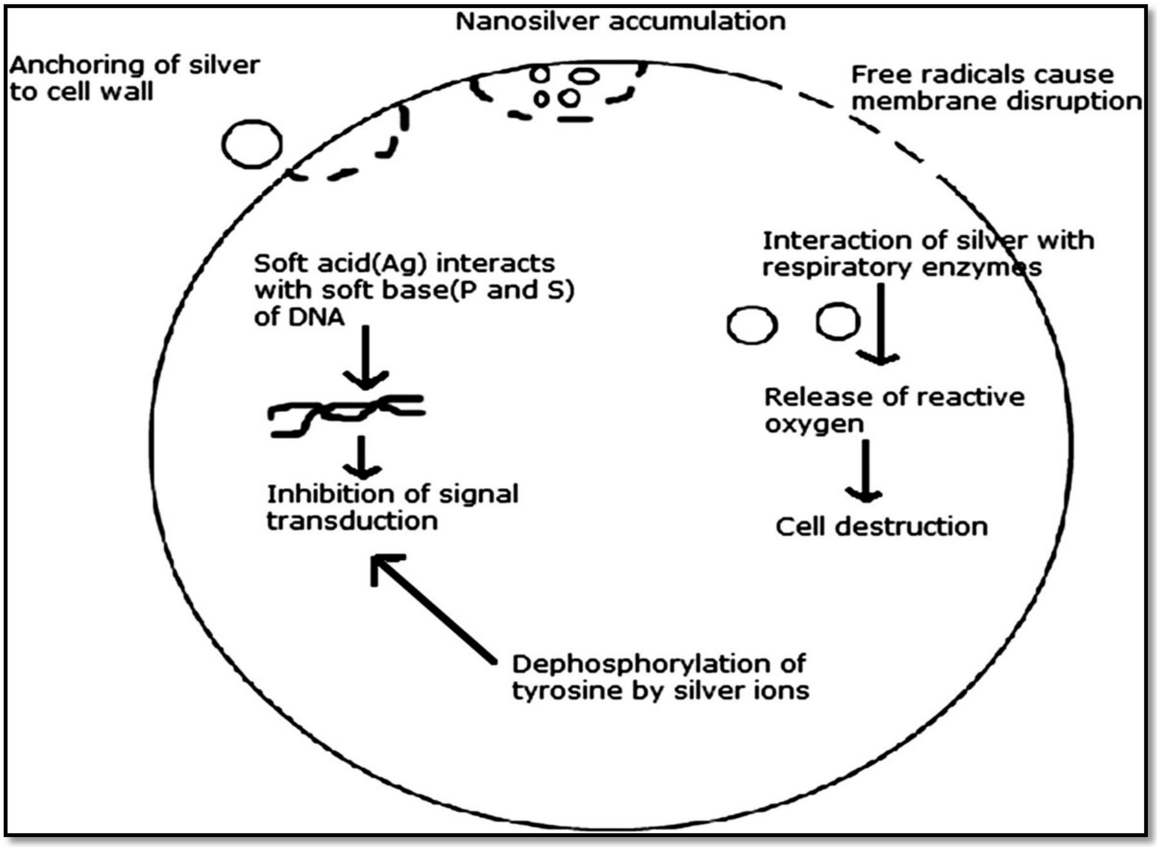


Figure: 2.1: Schematic representation of various mode of action of silver nanoparticle [86].

2.8. Toxicity of the Silver nanoparticle

Nanotechnology has been rapidly growing with utilization in a wide range of commercial products throughout the world. However, there is still a lack of information concerning the increase of humans, animal and ecological exposure to NPs including AgNPs and the potential risks related to their short and long-term toxicity. The unique physical and chemical properties of silver NPs make them excellent candidates for a number of day-to-day activities, and also the antimicrobial and anti-inflammatory properties make them excellent candidates for many purposes in the medical field. However, there is study and report that suggest that nano silver can allegedly cause adverse effects on humans as well as the environment by its overuse.

It is estimated that huge amount of silver are released into the environment causes conversion of silver to free silver ions in the aqueous phase which creates toxicity. The adverse effects of these free silver ions on humans and all living beings include permanent bluish-gray discoloration of the skin (argyria) or the eyes (argyrosis), and exposure to soluble silver compounds may produce toxic effects like liver and kidney damage; eye, skin, respiratory, and intestinal tract irritations; and untoward changes in blood cells [87]. NPs surprisingly enter the environment through water, soil, and air during various human activities [88].

CHAPTER-III

Synthesis and Characterization of silver Nanoparticles using Trichodesma indicum and Oxalis corniculata plant extract

3.1. Introduction

3.1.1. *Trichodesma indicum*

It is a yearly prostrate herb or much known weed, which belongs to the family Boraginaceae. The plant usually grows all over India, with the dry wastelands roadsides. The plant has varied ayurvedic uses in arthritis, anorexia, dysentery, skin diseases, poisoning and also for wound healing. In ayurvedic use, the paste of plant roots and leaves can be applied to swelling of joints in the wound for three to four times for 3-4 days. The aerial part of the plant has a noteworthy diuretic activity which may be useful in the therapy of acute pulmonary, edema, chronic heart failure, ascites, nephrotic syndrome and renal failure. [90]

Figure 3.1: Picture of *Trichoderma indicum*

The plant also shows diverse medicinal and useful effect and this effect are reported by modern lab studies are Alexeteric: counteracts on infection or toxin, Anodyne: relives pain without causing loss of consciousness, Antiode :counteracts a poison, anti-dysenteric : reducing inflammation by acting on body mechanism, Carminative:

preventing the formation or causing the expulsion of flatulence, Constipating: to cause constipation, Dieretic: promoting excretion of urine agent that increase the amount of urine excreted, Deparative: purifying agent, Emollient: soothing and softening effect on the skin or an the skin or an irritated internal surface, Antipyretic/Antifebrile/febrifuge: effective against fever, Termogenic: heating, ophthalmic: pertaining to the eye, Wound healing: heal the wounds.[89-93].

3.1.2. *Oxalis corniculata*

It is a plant which belongs to family *Oxidalaceae* and mainly derived from Hawaii and south Europe. However, it is also found in the regions of America. In Asian region, it is found in varies country like India, Pakistan, Afghanistan, Taiwan, and Japan. And as far as India is a concern in it is usually found in open gardens, grasslands, riversides, mountains, wastelands, and road sides. *Oxalis corniculata* (Changeri) is herbaceous and perennial herb (plants which do not grow more than two years and has no wood are considered as a perennial herb) with primary slender roots. Changeri is a different kind of plant which specifically grows in very low amount and it is a kind of herb which has creeping weed generally found in gardens wastelands, hedges, and roadsides. Plant branches of *Oxalis corniculata* lie on the ground and start rooting to form a new plant. The height of this plant grows up to 30 cm with erect and creeping branches; the stem is narrow and creeping with small hairs. It mainly contains three heart shape leaflets that are joined to long small hairs. It contains three heart-shaped leaflets that are joined to along petiole (the stalk that joins the leaf).

Leaves of this plant are about 4-12 mm long and 10-20 mm long. Flowering season of the plant starts from July and end on December. It is extremely effective herb used for treating stomach and liver problems in ayurvedic medicine system. Leaves of this plant are consumable, with tangy taste just like the taste of lemons. This herb is a very good source of vitamin-C, vitamin-B, potassium and oxalic acid. Flowers are pungent in taste and rich in oxalic acid and potassium oxalate. Leaves of this plant are extremely astringent (prevent the skin from bleeding and makes oily skin less oily) and bitter in taste. It has self-pollinating flowers that have the ability to grow fast in open grasslands area[94].

Figure 3.2: Picture of *Oxalis corniculata*

This plant is edible/ consumable and it is also used as salad. *Oxalis corniculata* is antiscorbutic and used in the treatment of scurvy. A most common chemical found in this herb is oxalic acid and vitamin-C. This herb is also rich in water, fat, proteins, calcium (Ca), phosphorous (P_4), iron (Fe), niacin ($C_6H_5NO_2$) and beta-Carotene (C_4OH_{56}). Other chemical compounds found in this herb are flavonoid, phytosterol, phenol, tannin, fatty acids and volatile oils. Leaves are the rich source of flavonoid, isovitexine, and vitexine. It also contains various essential fatty acids like linoleic acid, oleic acid, palmitic acid and stearic acid. The stem is a good source of tartaric, malic acid and citric acid. The plant has varied medicinal uses. This plant is used in the treatment of scurvy because it is rich in vitamin-C, beneficial for the treatment of influenza, paste of the leaves is used an antidote for the poisoning caused by snake bites, dhatura, mercury and arsenic. leaf extract or juice of the plant is used for treating burns, insect bites and various skin eruption. It is also useful for various skin disorders like warts, corns, inflammation and boils. Juice of the leaf is mixed with onion extract to remove warts, decoction (concentrated liquor resulting from boiling of plant extract for medicinal use) of this herb is used for gargles, leaves of plant in liquid is used to cure irritation of eyes and lack of clarity of cornea, this herb is anthelminthic (used to destroy the pathogen) in nature and it is used to destroy the newborn (infants) to get rid of hookworms which are harmful to human and it is also useful for cure diarrhea. Juice of the leaf is used in the treatment of jaundice and it is very effective to cure the symptoms of diabetes i.e., poly-hydra, paste of the leaf herb is applied over forehead externally to relieve for a headache, crushed leaves are used to reduce inflammation, when the leaf extract is mixed with oil and massage locally it gives relives to insomnia. Aqueous extract of this herb is cardio-protective in nature and it is very beneficial or useful for overall health. [94]

3.2. Experimental

3.2.1. Materials

$AgNO_3$, Distilled Water, plant extract of *Trichodesma indicum* and *Oxalis Corniculata*, Measuring cylinder, Magnetic stirrer, Scissor, Bloating paper, Tissue paper, Heating Mantal, Aluminum Foil, Beaker, Conical flask, Burret, Ethanol, Round bottom conical flask, Tray, Whatman filter paper, Centrifuge, Laminar air flow, Falcon, Test tube stand, pipette, petri-plates, *Escherichia coli*, *Staphylococcus aureus,* LB media, Agar-Agar, Autoclave, UV-visible spectroscopy(Aligent cary 60,USA),FTIR (Thermo scientific),DLS.

3.2.2. Methods

3.2.2.1. Preparation of plant extract

3.2.2.1.1. *Trichodesma indicum* plant extract:

Plant extract of *Trichodesma indicum*, were collected from KSBT Bhubaneswar campus, and washed several times with distilled water to remove the dust particles and then sun-dried to remove the residual moisture and stirred on magnetic stirrer for 2 hour in 250 ml conical flask in 200 ml water with sun-dried plant extract and filtered it with whatman filter paper to form aqueous plant extract. Then the solution was used for the reduction of silver ions (Ag^{+2}) to form NPs (Ag^0).

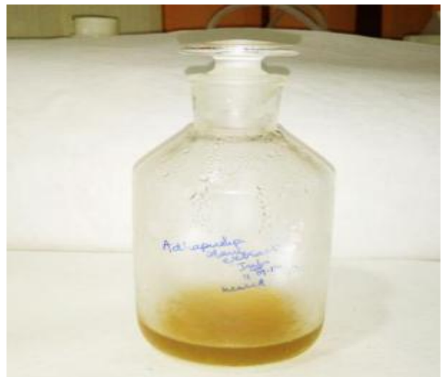


Figure 3.3: Shows the plant extract from *Trichodesma indicum*.

3.2.2.1.2. *Oxalis corniculata* plant extract:

Plant extract of *Oxalis corniculata*, were collected from KSBT Bhubaneswar campus, and washed several times with distilled water to remove the dust particles and then sun-dried to remove the residual moisture and stirred on magnetic stirrer for 2 hour in 250ml conical flask in 200ml water with sun-dried plant extract and filtered it with whatman filter paper to form aqueous plant extract. Then the solution was used for the reduction of silver ions (Ag^{+2}) to form NPs (Ag^0).

Figure 3.4: Shows the plant extract from *Oxalis corniculata* leaves.

3.2.3. Preparation of solution $AgNO_3$

10ml AgNO3 Prepared from 1M $AgNO^3$, molecular weight of $AgNO^3$ is 169.8 gm/mol

So, dissolved 1.698 gm/mol in 10ml distilled water by the formula $C_1V_1=C_2V_2$

Where, $C_1V_1=C_2V_2$

$$1000\times X = 169.8\times 10$$

$$X=169.8\times 10/1000$$

X=1.698gm/mol in 10ml distilled water.

3.2.4. Preparation of 1mM 200ml $AgNO_3$ from 1M $AgNO_3$

1mM AgNO3 (200ml) prepared from 1M AgNO3 by the help of the formula $C_1V_1=C_2V_2$,

$$C_1V_1=C_2V_2$$

$$1000mM \times V_1= 1mM \times 200ml$$

$$V_1= 1mM \times 200ml/1000mM$$

V_1= 0.2ml AgNO3 in 199.8 ml distilled water.

3.2.5. Synthesis of AgNPs by *Trichodesma indicum*

For the preparation of AgNP from *Trichodesma indicum* plant extract, a 50 ml conical flask was taken and fully covered with aluminum foil and then added 35ml, 1mM $AgNO_3$(200ml) in it and left it in magnetic stirrer with heating at 90 ^oC for 30 min. Then, 5ml of plant extract was added through burret drop wise and were mixed with the $AgNO_3$ solution. The solution is kept at room temperature for 24 hour and UV-Visible reading was taken at different time interval for characterization of silver NPs.

Plant extracts of *Trichodesma indicum* + $AgNO_3$ $\longrightarrow Ag^0 + \frac{1}{2}H_2$

3.2.6. Synthesis of AgNPs by *Oxalis corniculata*

For the preparation of AgNPs from *Oxalis corniculata* plant extract, a 50 ml conical flask was taken and fully covered with aluminum foil and then added 35ml, 1mM $AgNO_3$ (200 ml) in it and left it in magnetic stirrer with heating at 90 °C for 30 min. Then, 5ml of plant extract was added through burret drop wise and were mixed with the $AgNO_3$ solution. The solution is kept at room temperature for 24 hour and UV-Visible reading was taken at different time interval for characterization of silver NPs.

Plant extract of *Oxalis corniculata* + $AgNO_3$ $\longrightarrow Ag^0 + \frac{1}{2}H_2$

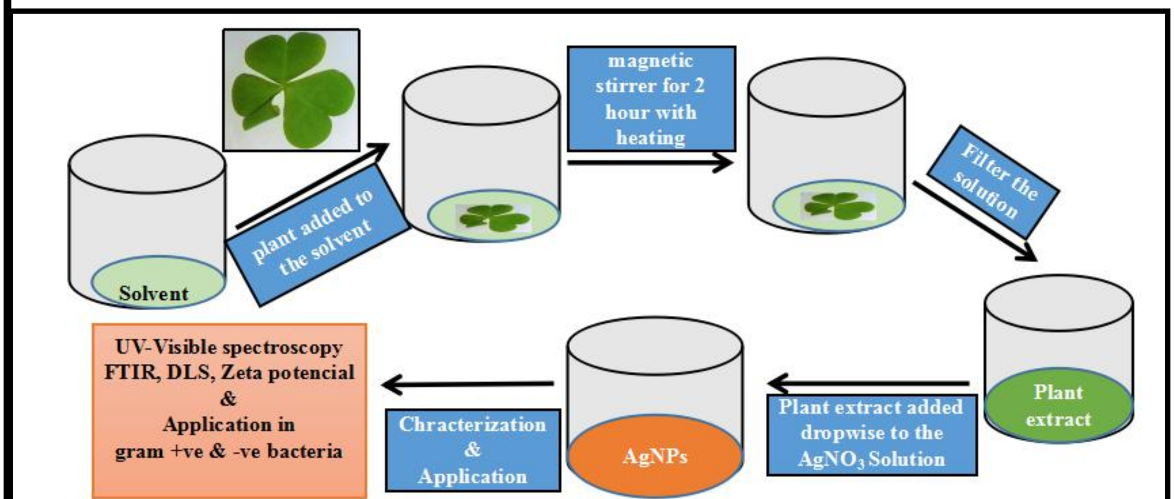


Figure: 3.5: Schematic representation of the synthesis, characterization and application of AgNPs.

3.3. Characterization

3.3.1. UV-Vis spectral analysis

The reduction of pure Ag^{2+} to Ag^0 NPs were monitored by measuring the UV-Vis spectrum the most confirmatory tool for the detection of surface Plasmon Resonance

Property (SPR) of AgNPs, by diluting a small aliquot of the sample in distilled water. UV-Vis spectral analysis was done by using UV-Vis spectroscopy (Aligent technologies cary 60, USA) at the range of 200-700 nm.

3.3.2. Dynamic light scattering (DLS)

It is called as photon correlation spectroscopy (PCS) is a well established and non-invasive technique for measuring the size of particles and molecules generally in submicron regions and with technology less than 1 nanometer. In this techniques, the particles or molecules in suspension undergoes Brownian motion which causes a doppler shift when exposed to monochromatic light. The monochromatic light exposure strikes the moving particle resulting in a change in wavelength of incoming light. This change in wavelength determines the size of the particle and evaluates the size distribution.

3.3.3. Zeta potential

Many NPs or colloidal particles have a surface charge when they are a suspension. When an electric field is applied, the particles move due to the interaction between the charged particle and applied field. The direction and velocity of the motion is a function of particle charge, the suspending medium, and the electric field strength. Particle velocity is then measured by observing the Doppler shift in the scattered light. The particle velocity is proportional to the electrical potential of the particle at the shear plane which is zeta potential. Thus, this optical measurement of the particle motion under an applied field can be used to the determine zeta potential.

3.3.4. Fourier transforming infrared spectroscopy (FTIR): It is used to study the surface chemistry of adsorbed small molecules. With the application of this technique, the functional group of the molecules can be determined on the NPs and gives the information about the surface structure of metallic NPs.

3.4. Anti-bacterial effect by broth dilution method: Two different bacterial culture *E. coli* and *S. aureus* were used to check anti-bacterial activity of plant based AgNPs. Single colony of each strain was inoculated to 5 ml LB media and grown over night at incubator shaker. 10 ml of LB media was added to ten 50 ml falcon tube and inoculated both the grown strains. Then five falcon tube is treated with different concentration of AgNP-1 (i.e., 10µg, 20 µg, 30 µg, 40 µg and 50 µg) and another five falcon tube is treated with different concentration of AgNP-2 (i.e., 10µg, 20 µg, 30 µg, 40 µg and 50 µg) followed by overnight shaking at 37^0C. In another set 10 ml of ten falcon tubes were prepared and inoculated overnight grown *S. aureus* into each falcon tube. Then five falcon tube is treated with different concentration of AgNP-1 (i.e., 10µg, 20 µg, 30 µg, 40 µg and 50 µg) and another five falcon tube is treated with different concentration of AgNP-2 (i.e., 10µg, 20 µg, 30 µg, 40 µg and 50 µg) followed by overnight shaking at 37^0C. Then OD was taken at UV-visible spectroscopy 600 nm.

3.5. Results

3.5.1. Synthesis of silver NPs

The research on the green synthesis of nanomaterials with different biopolymer has brought significant interest in its application, as compared to that of chemical synthesis. In this study, we have synthesized the silver NPs which changes its color from colorless to yellow then red color. The color changes initiated the reduction of silver nitrate to form the silver NPs.

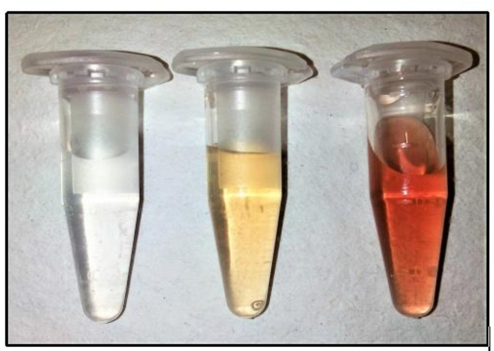


Figure: 3.6: Picture shows the colour change from colorless to pale yellow then red in color confirms nanoparticle formation.

3.5.2. UV-Visible spectroscopy

The synthesized silver NPs were characterized by using UV-Visible spectroscopy (Aligent technologies cary 60, USA) from 200- 700nm range. The synthesized NPs were recorded against the distilled water. Synthesis of silver NPs was confirmed in both plants by taking the reading at a different time interval. There was an increase in both, intensity of absorption peaks after regular intervals of time and the intensity of color with the duration of incubation in both the plants. **Figure 3.7** shows lowest absorption peak at 30 days, which indicates the stability of the AgNPs and the highest absorption peak at 24h, which confirms the synthesis of silver NPs. While **Figure 3.8** shows lowest absorption peak at 30 days, which indicates the stability of the AgNPs and the highest absorption peak at 24h, which confirms the synthesis of silver NPs. It was also observed from **figure 3.7 & 3.8** that the intensity of absorption peaks decrease with the time interval may be due to the aggregation of the silver NPs.

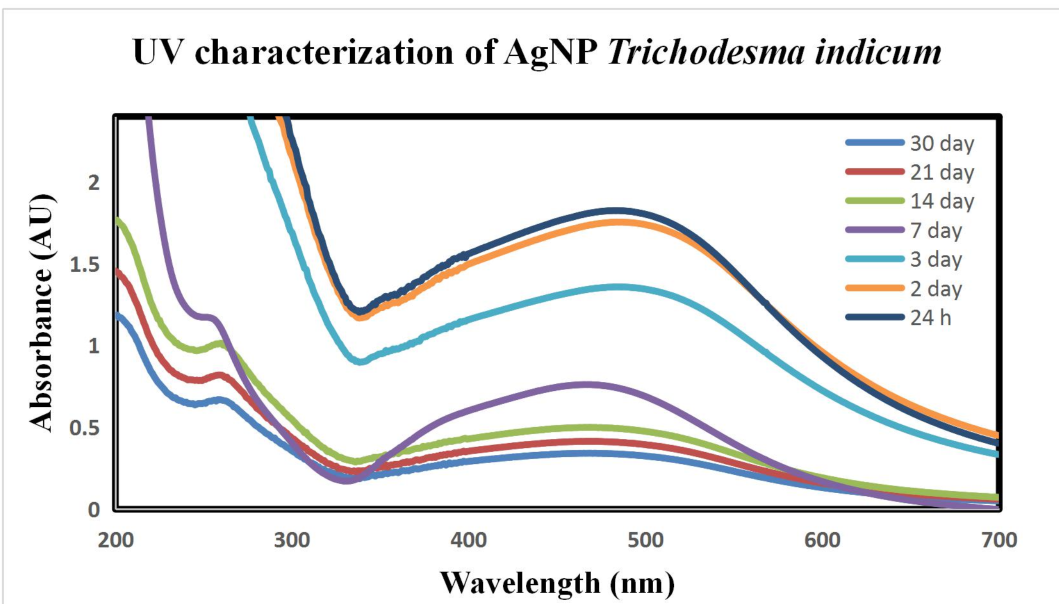


Figure: 3.7. UV-Vis Characterization of AgNPs formed by *Trichodesma Indicum* plant extracts at a different time interval.

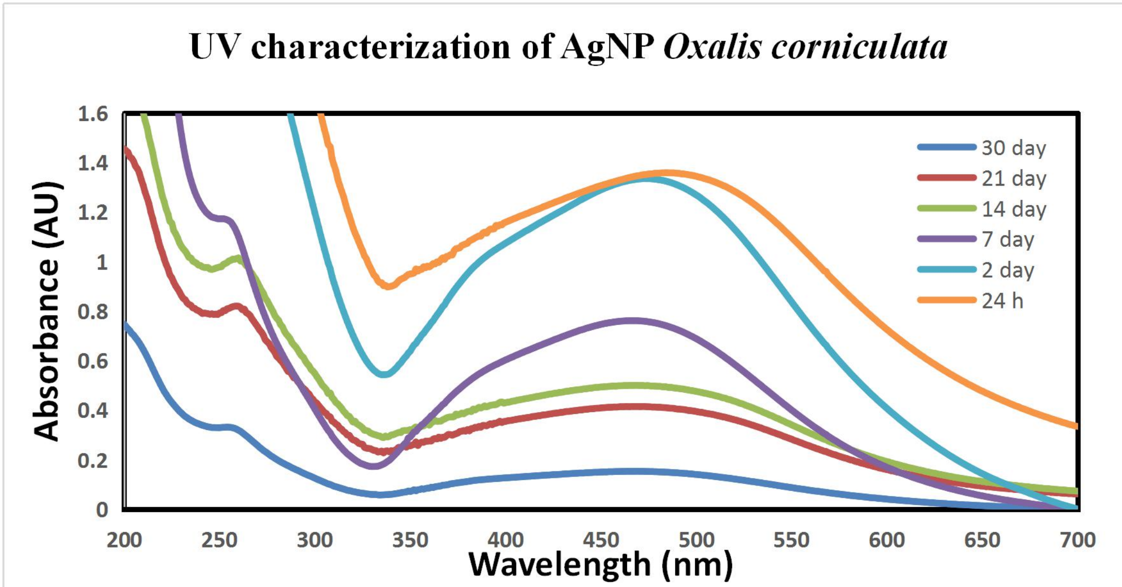


Figure: 3.8: UV-Vis Characterization of AgNPs formed by *Oxalis corniculata* plant extracts at a different time interval

3.5.3. Dynamic Light Scattering (DLS)

The DLS measurement can provide the qualitative information about the NPs and indicates the hydrodynamic volume which represents the size of overall solvent associated with NPs. The DLS analysis was also accomplished to determine the size distribution in a suspension. In DLS analysis average size of the silver NPs in case of *Trichodesma indicum* is found to be 147.3 nm (**Figure- 3.9)** and 167.1 nm (**Figure- 3.10)** for *Oxalis corniculata* respectively.

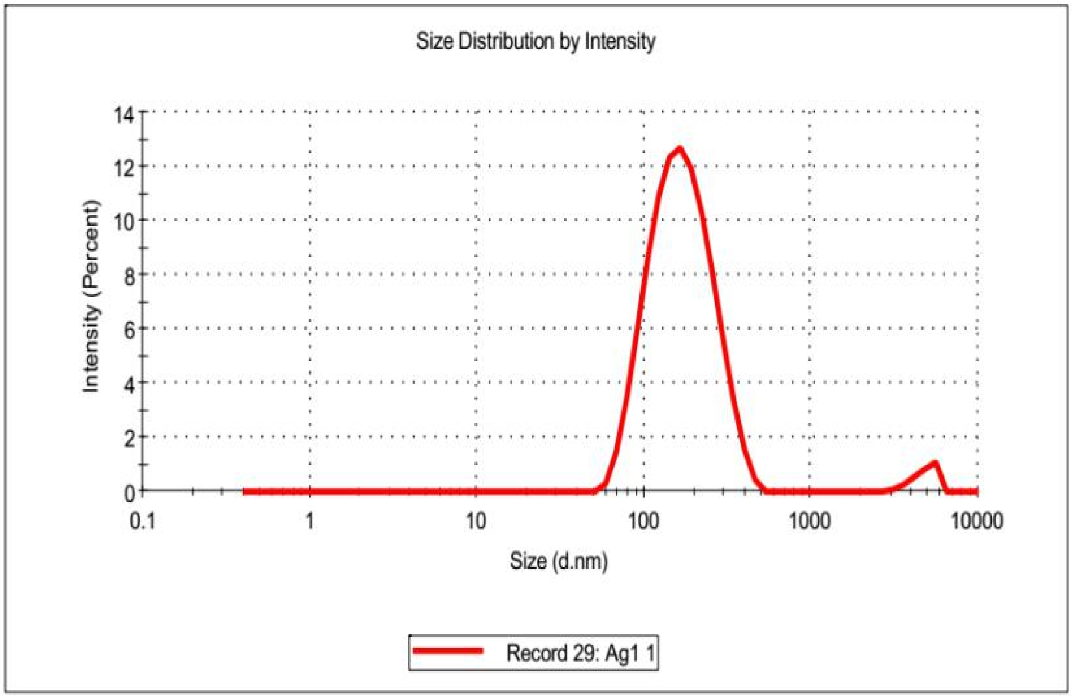


Figure: 3.9: Picture shows **s**ize distribution of silver NPs of *T. Indicum* as studied by DLS.

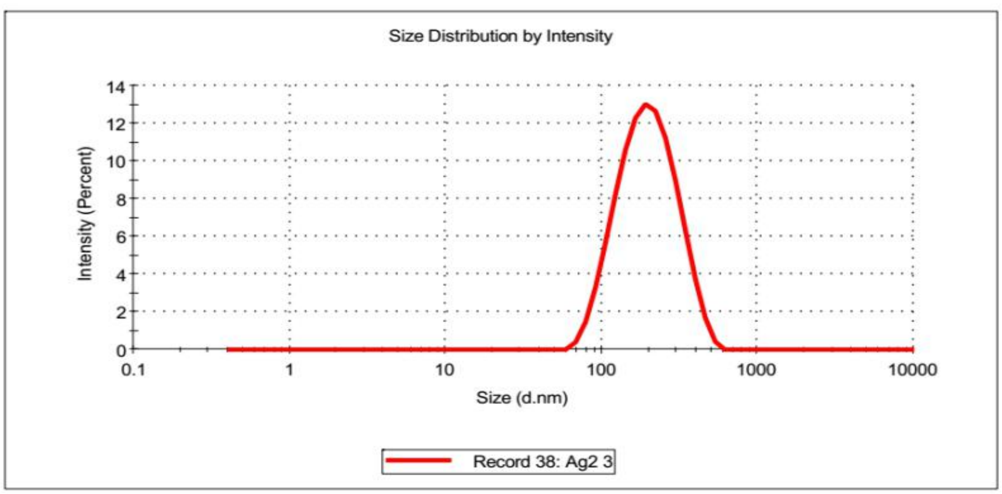


Figure: 3.10: Picture shows size distribution of silver nanoparticle of *O. corniculata* as studied by DLS.

3.5.4: Zeta potential

The zeta potential indicates the stability of the colloidal dispersion. The zeta potential analysis shows at -38.9mV of AgNPs *T. Indium* (**Figure- 3.11.**) and -38.9mV (**Figure- 3.12.)** of AgNPs *O. corniculata* which is moderate stable in nature.

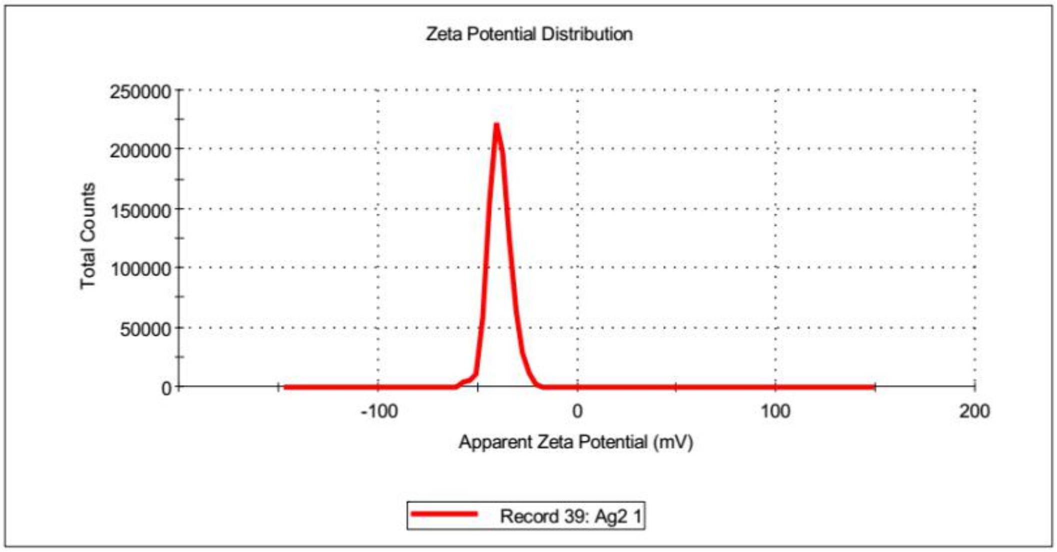


Figure: 3.11: The zeta potential of the silver nanoparticle of *T. indicum* which is measured by DLS showing a value of -38.9 mV, which is within the range of stability.

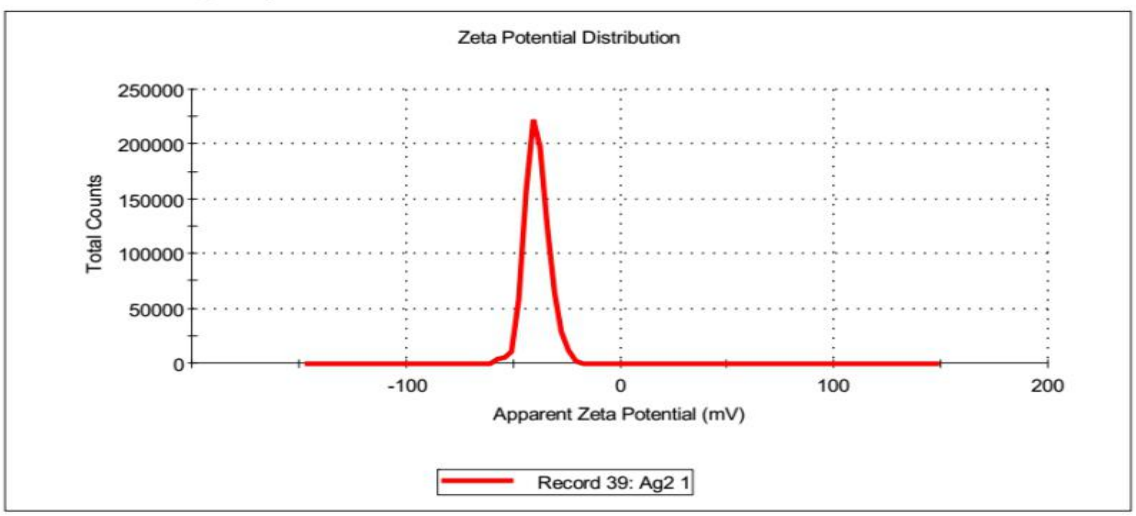


Figure: 3.12: The zeta potential of silver NPs of *O.corniculata* which is measured by DLS showing a value of -38.9 mV, which is within the range of stability.

3.5.5: Fourier transforming infrared spectroscopy (FTIR)

The chemical composition of the synthesized silver NPs of both plants was studied by using FTIR spectrometer (Thermo scientific). FTIR analysis of the synthesized silver NPs. The absorption band shows peaks at 3311 cm^{-1} (O-H stretching), 2885.64 cm^{-1} (C-H stretching), 2170.59 cm^{-1} (C≡C stretching), 1639.04 cm^{-1}(C=C stretching), 1552.09 cm^{-1} (C-H stretching), peak around 1450- 1500 cm^{-1} showed the bond stretch of N-H, whereas the stretch for AgNPs was found around 500-550 cm^{-1}.

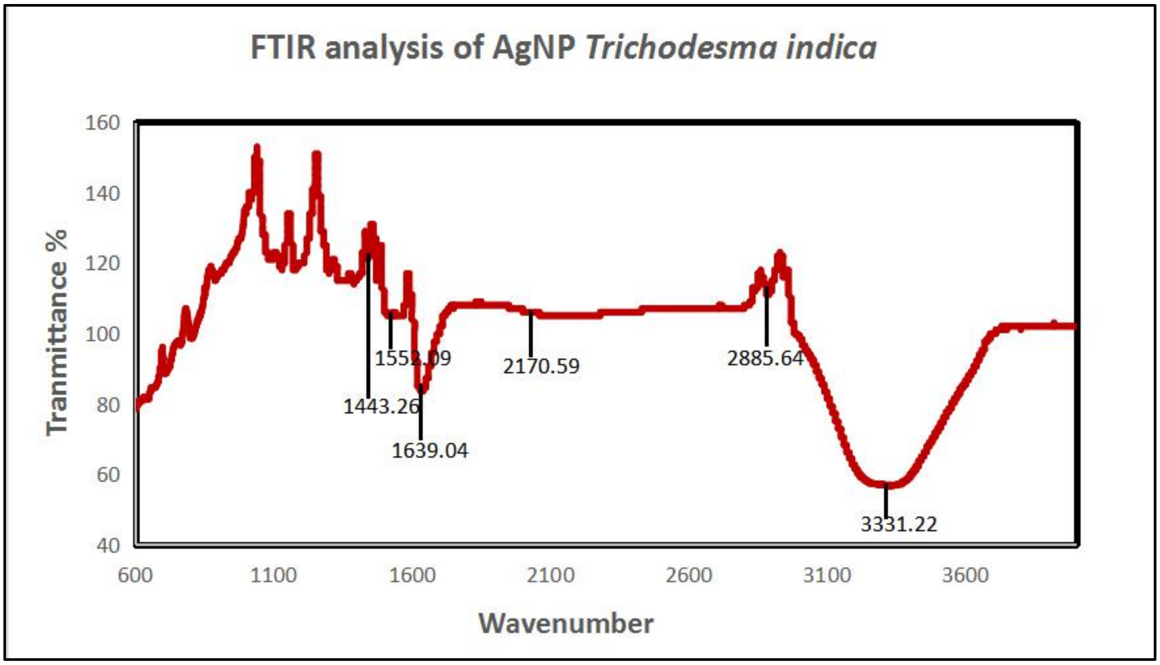


Figure: 3.13. FTIR spectra of the bio-synthesized *T. indicum* silver NPs.

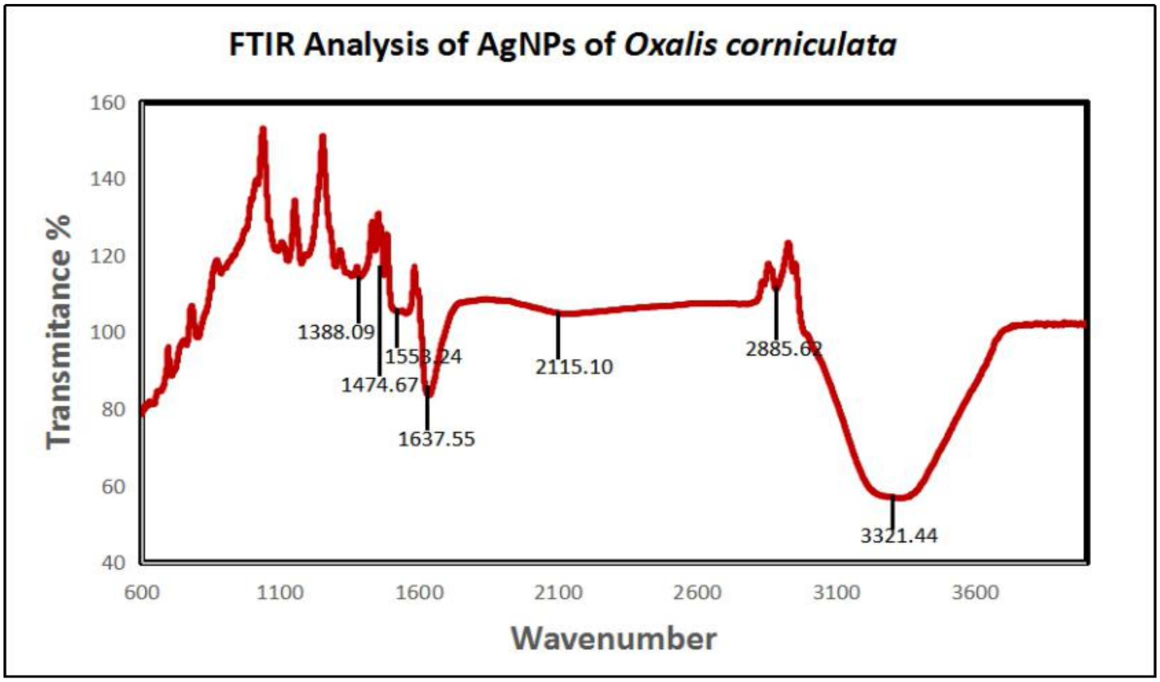


Figure: 3.14. FTIR spectra of the bio-synthesized *O. corniculata* silver NPs.

3.6. Application of silver NPs

3.6.1. Effect of silver NPs on bacteria

To confirm the antimicrobial activity of biological synthesized silver NPs we quantify the minimum inhibitory concentration (MIC) for gram-positive and gram-negative bacteria. In gram-positive bacteria, we have taken *S.aureus* and in gram-negative *E. coli* to see the effect of silver NPs of *Trichodesma indicum* and *Oxalis corniculata*. In MIC we have taken a different concentration of silver NPs to see the effect on bacteria. The minimum inhibitory concentration of AgNPs-1 from *T. indicum* is found to be 30 µg/ml in *Escherichia coli* and 40 µg/ml in *S.aureus*. The minimum inhibitory concentration of AgNPs-2 from *O. corniculata* is 20 µg/ml in *Escherichia coli* and 30 µg/ml *in S.aureus*.

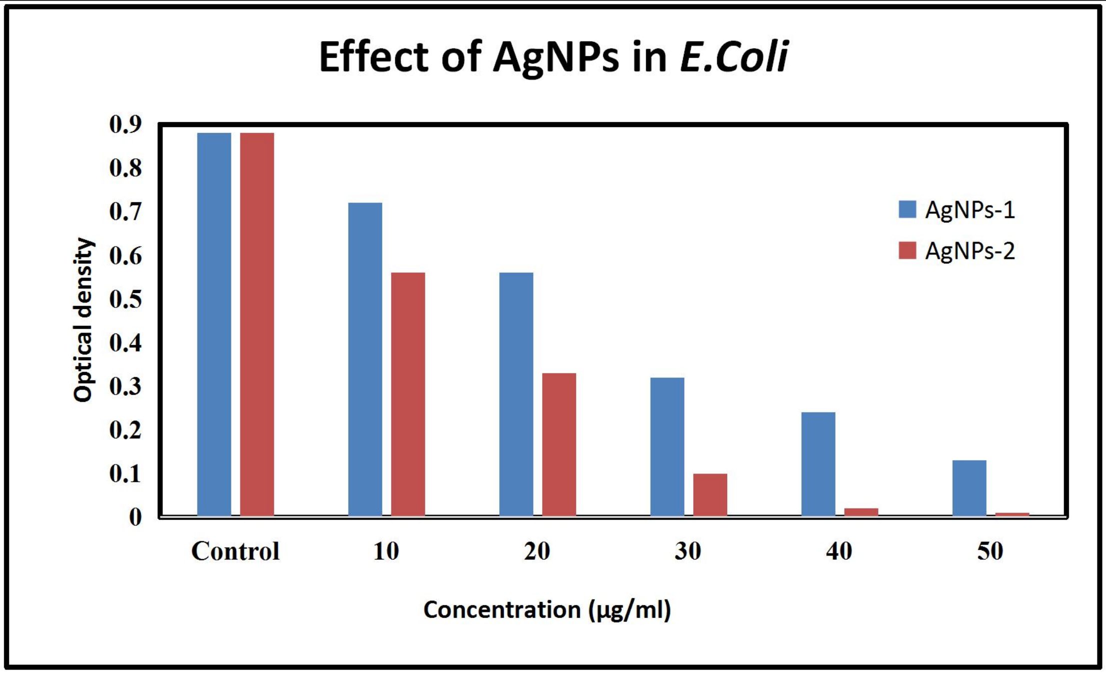


Figure: 3.15. Antimicrobial efficacy of AgNPs-1(*T. Indicum)* And AgNps-2 (*O.Corniculata)* on *E. coli*

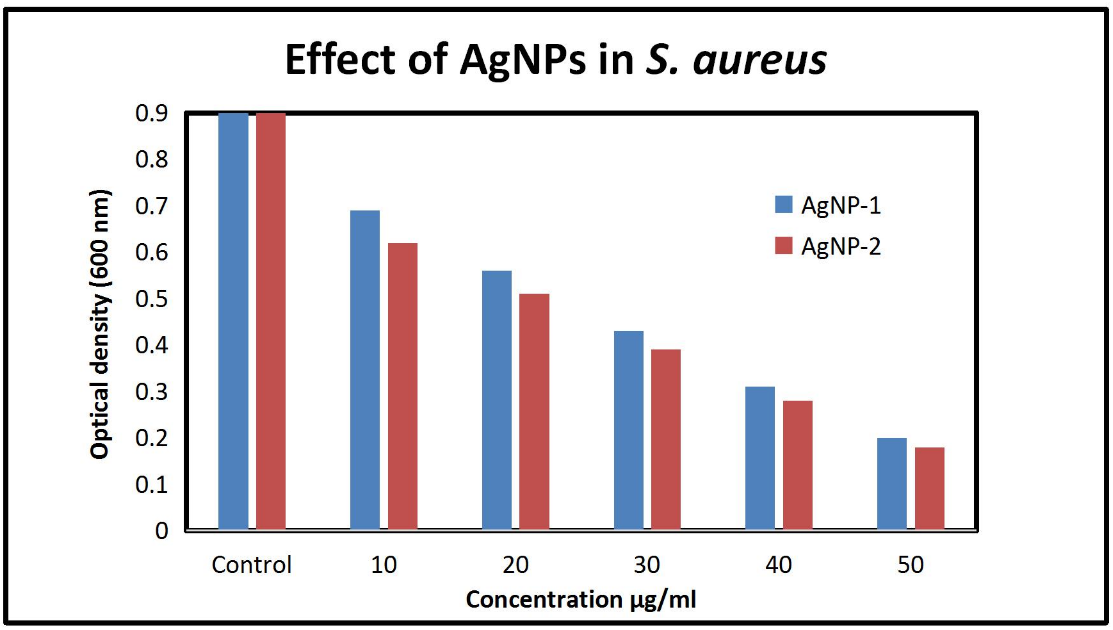


Figure: 3.16. Antimicrobial efficacy of AgNP-1 (*T. Indicum*) and AgNP-2 (*O.Corniculata)* on *S. aureus.*

CHAPTER-IV

Discussion, Conclusion and Future prospects

4.1. Discussion

The synthesis of silver NPs is a well-established method. There is various types of chemical and physical methods, which are not only harsh and toxic, but also ends up with polluting the environment. So to decrease the risk of environment pollution, biological synthesis matter evolved which neither harms the environment nor is of high cost. The present experiment, show the synthesis of SNPs using plant extract of *Trichodesma indicum* and *Oxalis corniculata*. The absorbance spectra of silver NPs range from 400nm-500nm. The synthesis of silver NPs was confirmed by color changing pattern which was monitored by UV-Visible spectroscopy at different time duration. UV-Visible analysis leads to the confirmation of formation of precise AgNP *Trichodesma indicum* (**figure 3.7.**) and AgNPs *Oxalis corniculata* (**figure 3.8.**). Synthesis of silver NPs was confirmed in both plants by taking the reading at a different time interval. There was a decrease in intensity of absorption peaks after regular intervals of time in both the plant due to the aggregation of the silver nanoparticle. In AgNPs *Trichodesma indicum* (**figure 3.7.**) showed lowest absorption peak at 30day and the highest absorption peak at 24h which confirms the synthesis of silver NPs. Similarly, in AgNPs *Oxalis corniculata* (**figure 3.8.**) showed lowest absorption peak at 30 days and the highest absorption peak at 24 hr which also confirms the synthesis of silver NPs. It was also observed from **figure 3.7. & 3.8.** that the intensity of absorption peaks decreases at different time duration due to the aggregation of silver NPs.

In DLS analysis average size of the NPs in case of *Trichodesma indicum* and *Oxalis corniculata* is 147.3nm (**figure- 3.9.**) and 167.1nm (**figure-3.10.**) respectively. The value of zeta potential -38.9mV (**figure- 3.11. & 3.12.**) shows the moderate stability of the silver NPs of both the plants *Trichodesma indicum* and in *oxalis corniculata*.

FTIR analysis of the synthesized silver NPs shows the absorption band peaks at 3311 cm-1 (O-H stretching), 2885.64 cm-1 (C-H stretching), 2170.59 cm-1 (C≡C stretching), 1639.04 cm-1(C=C stretching), 1552.09 cm-1 (C-H stretching) and 1450- 1500 cm-1 (N-H stretching). Whereas, the stretch for AgNPs was found around 500-550 cm-1. It could, therefore, be concluded from **figure 3.13. & 3.14.** that AgNPs formed by the help of reducing sugar present in 1639 cm-1 absorption peak may be due to the presence of glucose, fructose or polysaccharide which helps in the formation of silver NPs. Reducing sugar acts as reducing agent, resulting in the reduction of Ag^{+2} to Ag^{0} , which helps in the formation of silver NPs.

In (**figure- 3.15 & 3.16**) MIC, effect of SNP-1 and SNP-2 is found to be most effective in *Escherichia coli* as compared to *S.aureus,* may be due to the thick peptidoglycan layer of gram-positive bacteria as compared to thin peptidoglycan layer of gram-negative bacteria. SNP-2 is more effective as compare to SNP-1.

4.2. Conclusion

In conclusion, there has been increasing interest in the biological synthesis of AgNPs. In this study, AgNPs were synthesized by an eco-friendly and convenient method using plant extract of *Trichodesma indicum* and leaves extract of *Oxalis corniculata* used as a reducing agent for the synthesis of silver NPs from silver nitrate. Green synthesis of silver NPs is confirmed by the color change which was monitored quantitatively by UV-Vis spectroscopy showing 474nm for *Trichodesma indicum* and 480nm for *Oxalis corniculata*. FTIR gives the respective bands of the synthesized NPs, and information about the stretch of bonds. In DLS analysis, average size of *T. Indium* and *O.Corniculata* AgNPs is 147.3nm and 167.1nm respectively and according to zeta potential analysis, AgNP *Trichodesma indicum* and AgNP O*xalis corniculata* both are moderately stable. We can conclude according to MIC test that both the plants AgNP have antimicrobial activity and SNP-1& SNP-2 have higher effect in *Escherichia coli* as compare to *S. aureus*.

4.3. Future prospective

The further characterization of AgNPs is required to establish the size, shape, and morphology by SEM, TEM, AFM and crystallinity powder- XRD. The SNP on different cell lines has to be studied to bring out the conclusion whether it is effective against different types of cells. The study on the multi-drug resistant microorganism has to be performed to see the antimicrobial activity of SNP. Further study has to be done to see if it is effective for treating various diseases or not. The drug release kinetics on SNP also needs to be studied to find the suitability for drug delivery.

CHAPTER-V

References

5.1 References

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