Understanding the role of *Tecoma Stans* Phytochemicals in the green synthesis of Zn-Ag Nanoparticles

A Dissertations

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IN

BIOTECHNOLOGY

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CANDIDATE'S DECLARATION

I, NIKITA SHAW hereby certify that the thesis presented named " **understanding the role of Tecoma Stans phytochemicals in the green synthesis Ag-Zn synthesis nanoparticles"** which is submitted by me to the department of Biotechnology, Delhi Technological University, Delhi in the partial fulfilment of the required for the degree of Master of Science is original thesis done by me under the supervision of Prof. Jai Gopal Sharma.

This thesis with the plant has not previously done and do not form the basis of award of any degree to the candidate or to anybody else from any university

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CERTIFICATE BY THE SUPERVISOR

I hereby certify that the dissertation titled "**Understanding the role of *Tecoma Stans* phytochemicals in green synthesis of Zn-Ag nanoparticles"** submitted by Nikita Shaw (23/MSCBIO/32) to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of master of science, is a record of the project carried out by the student under my Supervision. To the best of my knowledge this Thesis contains original work and has not been submitted or form the basis of award of any other degree to the candidate or to anybody else from this or any university.

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NIKITA SHAW

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Understanding the role of *Tecoma Stans* Phytochemicals in the Grenn synthesis of Ag/Zn Nanoparticles

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ABSTRACT

This experimental research study report the use of *TECOMA STANS* leaf extract assist the synthesis of Silver Nitrate ($AgNO_3$) and zinc Sulphate heptahydrate. *TECOMA STANS* plant already has other benefits like anti- inflammatory and wound healing properties. The nanoparticles are synthesized are confirmed by UV-Vis spectrophotometry with absorption peak were obtained in the range of 480-600nm and characterized by FTIR and TEM. The biological activities of the produced nanoparticle like chemical test and antimicrobial activities was evaluated. Silver nitrate nanoparticle have got more attention due to its natural properties like anti-septic, anti-bacterial and cauterizing agent. The bimetallic nanoparticles have greater potential than monometallic. The zone of inhibition of bimetallic NPs is more than monometallic against various pathogen.

The synthesis was done at different concentration and was monitored. $AgNO_3$ and zinc sulphate heptahydarate has anti-microbial activities as it was confirmed when the NPs were tested against azotobacter by disc diffusion method. In conclusion the current scenario of environment pollution green synthesis method is sustainable, environment friendly, cost-effective and less hazardous than chemical synthesis and has wealthy application in biomedical field

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LIST OF ABBREVIATION

NPs – Nanoparticles

TS- *Tecoma Stans*

A.agilis - Azotobacter agilis

BMNPs- bimetallic Nanoparticles

ZOI – Zone of inhibition

FTIR: Fourier transform infrared spectroscopy

HRTS - High- resolution transmission electron micrscpopy

Ag nanoparticles: AgNP

ZnO NPs – Zinc oxide nanoparticles

SPR – Surface plasmon resonance

TPP – Tripolyphophate

ICP-OES- inductively coupled plasma optical emission spectroscopy

EDAX – Energy dispersive absorption x-ray spectroscopy

INTRODUCTION

1.1 General introduction

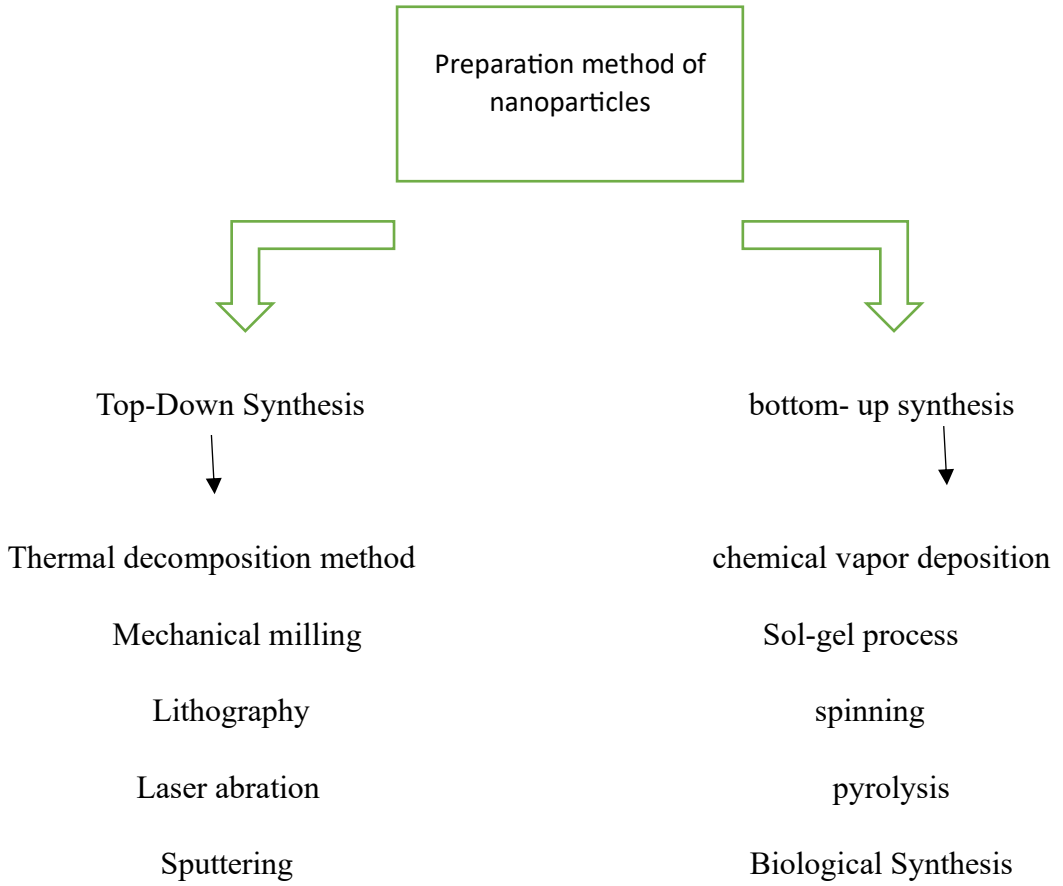
Nanotechnology is interdisciplinary field gaining enormous attention and has reached unpredictable scale as a new branch of research it vow quantum leaps in the field of electronics to pharmaceutical science as it synthesis particles of dimension 1-100 nm. to generate particle that exhibit new properties because it has high surface area to volume ratio that leads to characteristic and property modification that turn them to be more responsive and active. This area of nanoscience has application in bioscience as it has capability to penetrate deep and engaged at molecular level in our biological activities has a potential impact in health sector. There are two amalgamation to produce nanoparticles that is “top-down approach” and bottom up approach. The former method include vapor deposition, aerosol process, laser pyrolysis etc and latter include physical method like mechanical milling, sputtering, etching. Solvent and reducing operators has vast effect on its shape and size. In physical approach metal is reduce by microwave radiation, sonochemical method while in chemical approach reducing agents like sodium borohydrate, tollens reagents are used. Utilizing this build up procedure many NPs like Ag, Au, Cu has been reported. (1)

The general method for the synthesis of the nanoparticles is divided into two main classes

1. The top-down synthesis
2. The bottom-up synthesis

   The top-down approach larger molecule are disintegrated into smaller molecule and then transform to nanoparticles common methods are milling, Laser abration. Aggregation of metal from smaller level

Bottom-up Approach is work on principle of nucleating atomic-sized materials into the nanoparticles. Some common methods are Sol gel process , biological synthesis. Microorganism, yeast, plant extract are used



Physical synthesis

Nanoparticles that are synthesis by physical method which is depended on the use of high energy and physical forces. It involve the numerous energy consumption for the need to maintain high-Pressure and temperature. Top- down method are usually applied in the production of NPs. It involved the presence of Tube furnace and pressure. It is a traditional method for production of NPs. Microwave radiation are also used for the production. For these method proper experts are required to perform. Complex equipment are use , though it produces high quality NPs. This process result in production harmful waste product. It has less harmful effect than chemical synthesis because of use of toxic solvents. The production rate is very low, while cost of production is high. Hence unsuitable for large use

Chemical synthesis

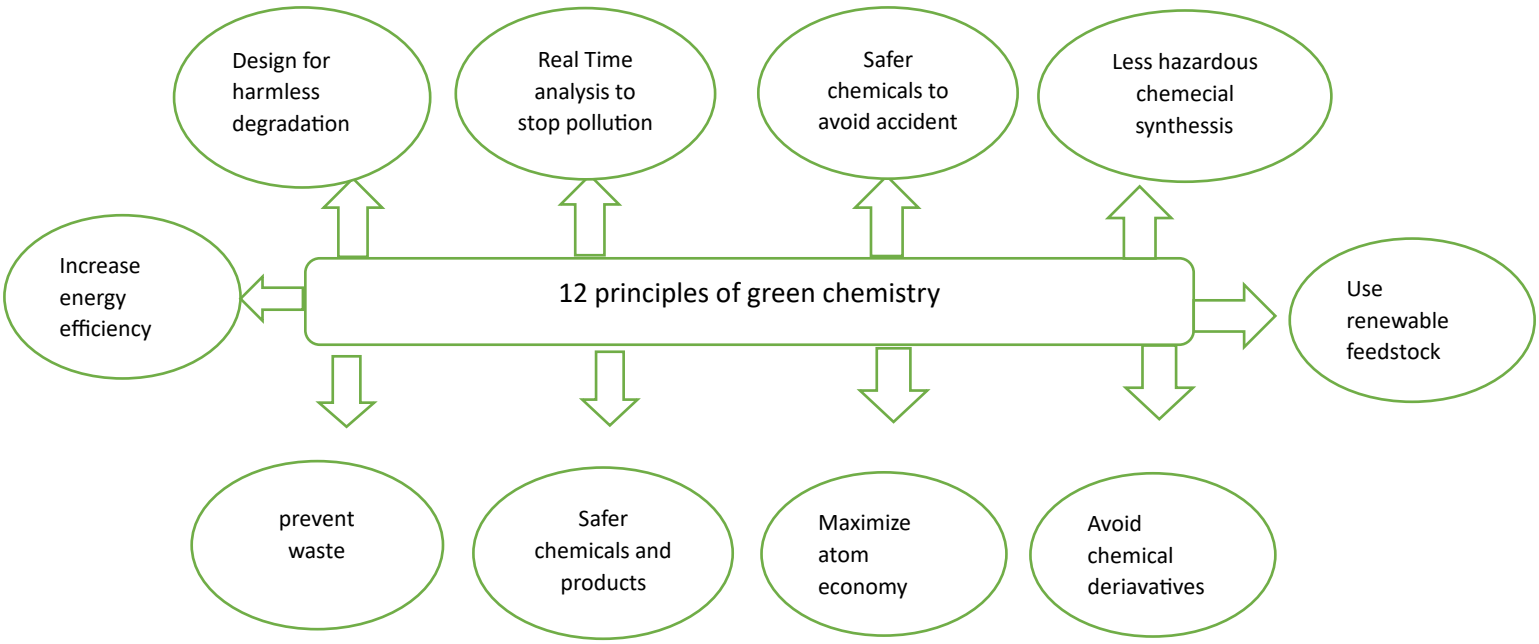
Chemical synthesis is the most widespread and most numerous while not requiring the involvement of heavy apparatus. but at the same time it create heavy burden on environment

by the requirement the use of toxic chemicals for nanoparticles stabilization Thus, making it highly favourable. In addition, with chemicals it is often observe the contamination of the final nanoparticles and significant amount of hazardous by-products are produced. Hence, unsafe for human use.

As the names implies the use of chemical as a reducing reagent for the Substrate use in the process. The main chemical method is synthesis of metal Nanoparticles is the reduction of metal ions ion the solution carries out in organic solvent. The leftover amount of highly reactive agents after the completion of the process is significant which is a great challenge to eliminate it. The reducing agent use commonly use are Sodium borohydride, oxalic acid, oleyl amine, hydrogen peroxide, formaldehyde etc.

1.2 Green synthesis a novel pathway

Rapid industrialization and urbanization has already developed lot of burden in our environment. Biological synthesis of NPs by use of plant, algae is a sustainable approach and has introduced alternative path for ecofriendly, safe method, less hazardous and cost effective method to synthesize. Synthesis NPs has various properties like anti-inflammatory, anti-cancer and drug delivery. (2)Since it is free of toxic chemicals contamination so it makes them safe to use in human contact. Green synthesis is bottom-up approach biological entities contain phytochemicals and secondary metabolites such as alkaloids, tannins, terpenoids act as a reducing agents of metals used for the NPs. Nature of this entities influence the morphology of the NPs . Green route attract researcher due to its wide distribution and easy availability. The concept of “Green Chemistry” is applied for the development of the NPs, unicellular bacteria, multicellular organisms like fungi, yeast , plant are used though of them all scientist prefer plants to be best agents as their waste products are eco friendly. Unlike some microbial extract. Various plant part such barks, leaves . flower are used. Some plants like Brassica Juncea (Indian mustard), willow, cannabis sativa etc shows phytoremediation properties like phytostabilization, phytoextraction, phytodegradation etc. has gain considerable attention due to outstanding potential in heavy pollutants detoxification.(3)



Objectives: metallic nanoparticles can be mono, bi, trimtallic. the main object of my thesis work to synthesis and characterize bimetallic nanoparticles which is produced by the leaf extract of the plant TECOMA STANS as it reduces zinc acetate and silver nitrate. Silver nitrate has been used since long time in medical field Such as in burn treatment, disinfectant, It explores new insight in combination effect of silver and zinc nanoparticle. BMNPs possess enhanced catalytic activity and selectivity compared to their single metallic counterparts. BMNPs have improved thermal, optical, magnetic and biological properties. the research will monitor the antimicrobial activities of the NPs and outcome of my experimental research will determine the practical and environmental benefits of the green synthesis of NPs.

The main objective of my dissertation project is to Green synthesis of silver and zinc NPs and study their characteristic of anti-microbial activity(4).

Nanoparticles can enter in air water soil it has potential to disrupt ecosystem and wildlife. Government and organization should provide safety measures and guideline for the usage. Nanotoxicology deals with the adverse effect of nanoparticles.

Safer nanoproducts are preferred for human use as it is found in our everyday day to day life. Because of its nano size it comes with unpredictable risk

It can enter in environment through natural processed and human activities. So caution should be taken before its disposable. However, with regulation we can harness its potential for our environment for the bioremediation of the pollutants

CHAPTER 3 :

LITERATURE REVIEW

Nanoparticles synthesis have been an active area of research since it is a developing branch because of it unique properties and nanosize particles it becomes more responsive towards its target. It has application in various field of agriculture , food industries, pharmaceutical, semiconductor, memory cards etc. because high toxic waste and hazardous chemical use in the physical and chemical method, green synthesis is gaining importance because of cost effective process, biodegradable waste, less hazardous etc.

Biosynthesized nanoparticles has wide acceptance in nanotechnology and it is a environmental friendly approach and safer NPs produced for the direct human use in biomedicine. Use of more chemical in chemical synthesis make its toxic for human health and can have adverse affect on health.

Classification of the Nanoparticles

The number of dimension use in classification of NPs like nd

1) zero dimension (0-D) Eg: quantum dots. are particle-like structures that do not possess any dimension. Generally They are spherical in shape however single crystalline and polycrystalline are also found
2) one dimension (1D) e.g: nanofilms. have one dimension that is much larger than the other two dimensions. It has needle like shape nanomaterial
3) two dimension(2D) e.g: Nanofibres, nanocoatings. have two dimensions that are significantly larger than other dimension. With Graphene as a classic example
4) three dimension(3D): have similar dimensions in all three directions. Ex: carbon fullerenes. It has all dimension larger than 100nm. It is a bulk nanomaterials with multiple arrangement of nanoparticles in different orientations. (5)

Nano Scale effects on Properties of nanoparticles

1. **Catalytic Efficiency**:
   due to high surface area to volume ratio its catalytic efficiency is increased and bio availability increases. Required dose reduces and specific ligand can be targeted easily.

2. **Mechanical properties**:

   improved hardness and roughness of metal and alloys, Ductility and superplasticity is also affected. The temperature and method of process also has effect on ts mechanical properties. as when ultrasonic wave exposure increases the density of NPs increases with high particle surface binding hence enhancing mechanical strength and flexural strength

   Optical properties:

   on decreasing the size the electrons get confine in the particles due to small size it can developed the ban gap in nanomaterials increase in band gap energy and band energy gets quantized and important phenomenon like Surface Plasmon Resonance, Quantum confinement effects etc. comes into play. which alters the optical properties. hence, band gap shifts to higher energies the absorption spectra shows blue shifts. Due to less mobility of free electrons, nanoparticles react differently with light like by changing the size and composition of NPs the emission wavelength can be changed widely.

3. **Electrical Properties**:

   increased in electrical conductivity and increase in resistance of metal. NPs such as nanotubes exhibit some of the highest electrical conductivities. Though the conduction differ in different dimension like in 0D no delocalization of electrons occurs metals behave as an insulators.

   Quantum size effect and quantum confinement are common phenomenon in nanosize crystals and because of quantization of electron energy the conductivity of metal can change dramatically. Nanosizing has reverse effect on conductor and insulating materials such as gold and copper turns into insulators while SiO_2 can become conductive

4. **Biological Properties**:

   increased permeability through biological barriers. specific drug delivery and transportation and controlled release, stability of drugs is increased and solubility is enhanced. Used as fluorescent labelling of biomolecules, targeting protein and helps in cellular imaging.(7)(8)

It has improved bio availability of the pharmaceutical agents by enhancing the drug delivery inside the cell where large particle are excluded.

Chemicals used in preparation of nanoparticles

1. Silver Nitrate: silver nitrate of 1M solution is used for the preparation of the NPs. It is colourless solution and highly soluble in water hence forms silver ions easily when mixed forms a colourless solution. It is a versatile agent and has enormous uses in medical treatment Silver nitrate has been used since long for disinfectant and burn treatment. It can be use in surgical, cosmetics and water purifiers.

2. Zinc Acetate Heptahydrate: It is a salt of zinc and acetic acid in form of white crystalline solid. It is soluble in water. It is used to treat zinc deficiency and given as Oral supplement. It is in therapeutics modality for decades. Its an active ingredients in dermatological ointment

Though both Ag and Zn are consider as biocidal agents. It is effective against broad spectrum microbes

Here, in this experiment leaf extract of Tecoma Stans is used as a reducing agent for the reduction of $Zn(CH_3COOH)_2.7H2O$ and AgNO3.

To affirm that the synthesis has led to the right track, particle characterization is of utmost importance, because the physicochemical properties can have a notable impact on their biological properties. The characteristic attributes of nanomaterials such as shape, size, surface area, size distribution, solubility, aggregation, etc. need to be analysed before checking toxicity or biocompatibility. Evaluation of the nanomaterial's synthesized, includes different analytical techniques such as Ultraviolet Visible Spectroscopy (UV-vis spectroscopy), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Fourier Transform Infrared Spectroscopy (FTIR), Energy Dispersive X-ray Spectroscopy(EDX), X-ray Photoelectron Spectroscopy (XPS), X-ray diffractometer (XRD), Dynamic light Scattering (DLS), Atomic Force Microscopy (AFM), and so on.(6)

Classification and Description of *the TECOMA STANS*

Kingdom: PLANTAE
Phylum: Trachiobionta- vascular plants
Clade: Angiosperms, Eudicots, Asterids
Division: Mangnoliophyta
Class: magnoliopsida-dicotyledons
Order: Scrophulariales
Family: Bignoniaceae
Genus: Tecoma
Species: *TECOMA STANS*

Fig1: *TECOMA STANS* plants

Tecoma Stans is commonly names as **yellow trumpetbush**, **yellow bells**, **yellow elder**. It belongs to Bignoniaceae family. The funnel shape yellow flower It can grow in dry region**.** It is an perennial plant It has many medicinal properties like helps in treating type 2 diabetes and stomach ulcer and intestine disorder also exhibit anti cancer properties. The leaf that has been use in our experiment has antisyphilis properties. it has significant therapeutics potential. It has been long use in traditional medicine

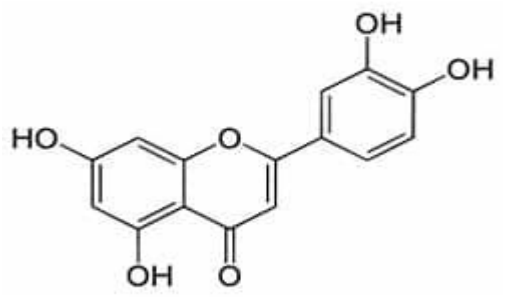
A recent report prove that Paulownin is responsible for its cytotoxic activity. Kaempferol isolated from Tecoma Stans has been a potential inhibitor of protease that are highly conserved in virus like SARS-CoV-2. The biochemical constituent of the extract has anti fungal, analgesic and neuro protective properties. Proteases secreted by Candida species has an anti fungal effect with high toxicity against human cell, but the anionic protein isolated from T.stans is a strong antifungal agent without being toxic towards human cells.(7)

Plant shows the presence of many phytochemicals component such as flavonoids, tannins, terpenoids, saponin, caffeic acid,

Tecostatin:

Caffeic acid: it is a derivative of hydrocinnamic acid, naturally occurs in Plant including coffee, herbs etc. It contribute to maintain a healthy cardiovascular health, it can also reduce inflammation. It is a phenolic compound produced through secondary metabolism of fruits and vegetables and has free phenolic hydroxyl which is associated with its anti-oxidant properties by preventing the production ROS (reactive oxygen species).(8)

Luteolin: is a type of flavonoid, heat-stable, commonly found in fruits, vegetables and flowers. It is a strong anti-oxidant and also exhibit anti cancerous activity, it show potential effects on neurogenerative diseases. In cancerous cells it interfere with cell signalling mechanism and also induce apoptosis by activating Caspese 3. Due to its immense effects in medical science further research are going on to fully understand in true potential .(9)



Chemical structure of Luteolin

Quercetin: bioactive agent that is typically found in the form of quercetin glycoside, anti-inflammatory, chelator of heavy metals, and antioxidant properties.

Tecomine and chlorogenic acid present in leaves have shown ability of lowering Glucose

Further research study on this plant reported that extract of *T.Stans* has anti diabetic activity it reduces hyperglycemic peak and also lower triglycerides and cholesterols. The "paper disc method" determine its Anti microbial activity. It has also has anti plasmodic activity which occurred without generating NO, and without implying opoid or adrenoreceptors. This spasmolytic appear might caused by the calcium channels.

This study delves with plant-based synthesis of NPs an alternative for the traditional method . synthesis of bimetallic NPs of Zn and Ag . the reaction was carried at room temperature and the optical properties of the NPs was detected by UV-Vis, TEM, FTIR was done to examine reducing agent and anti-microbial activity by disc diffusion method(10)(11)(12)

leaves of the Tecoma Stans plant has volatile oils that exhibits efficiency as an antimicrobial agent against pathogens such as azetobacter, E.coli , etc. isolation and analysis of extracts from fresh leaves were done by hydro-distillation and GC/MS technique. Agar plates were prepared and test samples for antimicrobial were applied osn pates by agar diffusion assay which was then kept incubated at 28-37 degree centigrade for 1-2 days. The diameter zone revealed that essential oil present in leaves showed antimicrobial activity (13)

CHAPTER 3: METHODOLOGY

Aim: The main scope behind this thesis was to green synthesize bimetallic Nanoparticles of Zinc and Silver and its characterization

Experimental method

3.1 SYNTHESIS OF NANOPARTICLES

3.1.A MATRIALS REQUIRED

1) Silver Nitrate solution
2) zinc Sulphate Heptahydrate
3) Distilled water
4) beef extract
5) cotton plugs
6) bacterial culture
7) Antibiotics
8) Tissue roll
9) Isopropyl Alcohol
10) Agar

3.1.B GLASSWARE TOOLS AND EQUIPMENT

Beaker, test tubes, Eppendorf, magnetic beads, measuring cylinder, spatula, inoculating loop, spirit lamp, spreader, petri plates, centrifuge tubes, conical flaks, micropipettes.

3.1.C ANALYTICAL INSTRUMENT

1) Weighing balance -it is digital weighing balance use in the labrotary to measure minute components. It is very precise and gives accurate value. The weighing pan is closed inside the glass box with one side operatable to place the sample so that there is no interference from the external environment while measuring the sample. This is known as magnetic mixer. The instrument should be calibrated before every use.(13)

2)Magnetic stirrer: It is a laboratory device that require magnetic field to rotate to cause a magnetic beads totally dipped in the solution to spin very rapidly, hence stirring. It helps to homogenize the liquid content. One of the main advantage of the stirrer is to automate the stirring with varying speed and temperature can also be controlled. The duration can also be managed as timer can be set as per requirement(14)

3) Laminar Flow: is a system in laboratory to provide continuous unidirectional flow of filtered air to maintain the germ free environment inside. It helps to prevent contamination. The HEPA filter is use to remove airborne germs and purifying the air and creating clean air source. Before using UV light is use to kill the germ and to create sterile condition. It is use for Tissue culture, tissue culture and other microbiological processes(15)(16)

4)High speed Centrifuge: It is the device use in labarotary that work on the principle of sedimentation to separate different particles present in the sample of different mass and densities. The high speed centrifugation mainly use subcellular organelle, bacteria, plasmid, nucleic acid etc. it spins at a very high speed. The centrifugal force come into play to seprate it. The spin can be anywhere from 5000 to 20000 RPM. (18)

3.1.D Plant sample collection

Leaves of plant i.e. TECOMA STANS(FM) were gathered from Delhi Technological University (DTU) campus, Delhi. To get all of the dirt off the surface of the leaves, they were cleaned using tap water and continued with distilled water thoroughly.

3.1.E Leaf Extract Preparation

1. 20 grams (total 40 g) of fresh leaves of TECOMA STANS(Yellow Elder) were sundried for 3 days and the dried leaves were crushed by pastel and mortar till it turns into fine powder. Powdered leaves when weighed in weighing balance turn out to 10 grams

Fig 2: powder leaf extract

2. The prepared powder was dissolve in 100 millilitres of distilled water. This resultant solution was kept for boiling for 40 minutes on heating mantle plate at 65^0C With constant stirring with the help of magnetic beads the obtained leaf extract solution was cooled in room temperature before filtration.

Fig 3: dissolved leaf extract in distilled water

3. After one hour, the residue was removed and the leaf extract solution or mixture was filtered in a beaker to get phytoconstituents using Whatmann's Filter paper. Residue is safe to be discarded as it has no toxic chemicals and the filtrate is ready to be use for the further process and can also be stored for future Use.

Fig4: plant extract is filtered Filtrate

Fig 5: TECOMA STANS

3.1.F Preparation of Zinc Acetate and Silver Nitrate Stock solution

1. To prepare 0.5M Silver Nitrate solution weighing 51.92 mg of Silver Nitrate as a metal precursor by weighing balance and mixing it in 200 milliliters of distilled water using a stirrer with magnetic fields.
2. To prepare 0.5M solution zinc Sulphate heptahydrate. weighing 51.92 mg of zinc Sulphate heptahydrate. as a metal precursor by weighing balance and mixing it in 200 milliliters of distilled water using a stirrer with magnetic fields

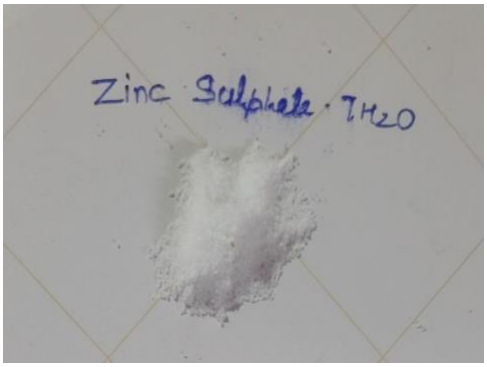


Fig 6. $ZnSO_4.7H2O$

3.1.6 synthesis of Nanoparticles

1. For synthesis of silver nanoparticles, 50 ml of extract was reacted with 0.5 M AgNO3 under constant stirring at room temperature for reduction of Ag+ ions to nanoparticles. Similarly, 50 ml leaf extract was also treated with 0.5 M Zinc Sulphate solution to form ZnO nanoparticles. ZnO-Ag Bimetallic nanoparticles were synthesized
2. For, synthesis of NPs, the extract is heated to 65 Centigrade upon continuous stirring and Zinc Sulphate heptahydrate was added. The $AgNO_3$ solution of 0.5 Molar was mixed after 5 min to the working solution and reduction process reaction was carried for around 2 hours.

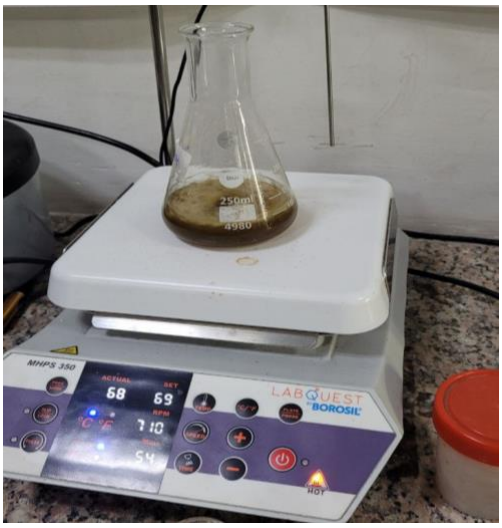
a. 

Fig7: synthesis of nanoparticle working solution

3. The newly formed nanoparticles in our solution has to seprated and collected. So it is centrifuged in high speed of 12,000 RPM for 15 min. which will result to nanoparticles to get settled down in the centrifuge tube. Discard the supernatent
4. washed the pellets few times with distilled water (this is optional)

(A) (B)

Fig 8. Sedimentation of synthesized and centrifuged nanoparticles in pellets in falcon tubes

5. Collect pellets in dried glass petri plates or leave in centrifuge tube and dry them in a hot air oven @ 45 °C overnight.

Fig 9. Dried pellet

6. The dried pellet was scratched away the Petri dish weighed, and gathered in an Eppendorf tube for characterization and biological actvities

3.2 Characterization of Silver and Zinc Oxide NPs

Reduction of metal salts in nanoparticles shown by a shift in the color of the solution throughout the reaction

3.2.1 Optical Characterization via UV-vis Spectroscopy:

it has been reported as an powerful tool to detect the formed nanoparticles it work on the principles of the interaction of incident photon and conduction band electrons and helps to detect the shape ad size of the formed NPs. When the light is passed is pass through the sample it cause the molecules to vibrate then a phenomenon localised surface plasmon resonance comes into effect. LSPR is responsible for forming a specific absorption spectrum peak at one particular wavelength of light (Zambare et al., 2022). and synthesized metal nanoparticles is successfully characterized by UV-vis spectrum. Both quantative and qualitative analysis can be performed(19)(20)(21)

Dissolve 5 mg of synthesized formed NPs in test tubes and add distilled water (5 ml) to test tubes. then it was subjected to a vortex and followed by sonication to separate each particle. Load 2 ml of this mixture in the glass cuvette one after the other to measure the absorbance by using a UV-vis spectrophotometer(22).

3.2.2 Antibacterial Assay of Cu & ZnO Nanoparticles:

The department of Biotechnology at Delhi Technological University has pure Gram negative bacteria *Azeobacter agiliss* which is employed in my current experimental work Here, the disc diffusion technique is employed to measure the zone of inhibition of respective culture plates.(23)

Disc Diffusion method(25)(26)

1. Firstly, the nutrient broth was prepared by using beef extract , it was meausred and dissolved in distilled water and was placed in autoclave for 1 hour.
2. The broth is ready to use

3. The inocolum of the Azetobacter was prepared by incubating it in nutrient broth perfoemed under laminar flow in sterile enviroment to prevent contamination and was kept for 24hours in an incubator shaker
4. Utilising a clean swab stick, spread prepared suspension of bacteria on the surface of nutrient agar plates, uniformly in laminar airflow.
5. Agar plate was then inoculated by bacterial inoculum by spreading 10 micro litre by the way of micropippete with the help of spreader
6. Prepare a test sample of silver and zinc by mixing 10 mg of bimetallic nanoparticles in 500 µL of sterile distilled water in one test tube.
7. Cut the sterile filter paper disc and dipped it in nanoparticles solution and place them in all (three) agar plates with the help of a tweezer and other side antibiotic test samples dipped filter paper onto the sterile discs.
8. Seal culture plates with paraffin and place them in an incubator @34 37°C for 24-48 hrs for bacterial growth.
9. The diameter of inhibitionaround each paper disc was measured in millimeters after the incubation(27)(28)(30)

3.2.3 FTIR analysis:

In FTIR fourier tranfer infrared spectroscopy. Is a yechnique use for characterization of the Nanoparticles. It work as the fact that molecules absorb infrared light at specific wavelength, correspondinf to their natural wavelength and their vibraational frequencies. The instrument used are interferometer generate an interference pattern of infrared light. By detecting vibrational and rotation of the molecules functionl group present in the sample.(29)(31)(35)

It provides surface chemistry of the nanoparticles and the type of chmecial bond present in the sample. But at the same time some limitaions are also there such as high surface sensivity, signal-to-noise ratio i.e presence of other material can affect the result and interpretation of complex spectra of FTIR can bechaalenging(33)

can be identified. For FTIR inspection, the prepared sample was required to examine reducing agents on the surface of NPs, for characterizing the surface chemistry and

organic functional groups. Different peaks were obtained from FTIR analysis. It provides information about the structure and composition of such nanomaterials. It has high notable benefits such high.

If nanoparticles are coated or functionalized (e.g., with polymers, surfactants, drugs), FTIR can confirm successful binding or capping. (36)(37)

Chapter 4: Results and Discussion

1.UV-VIS SPECTROSCOPY

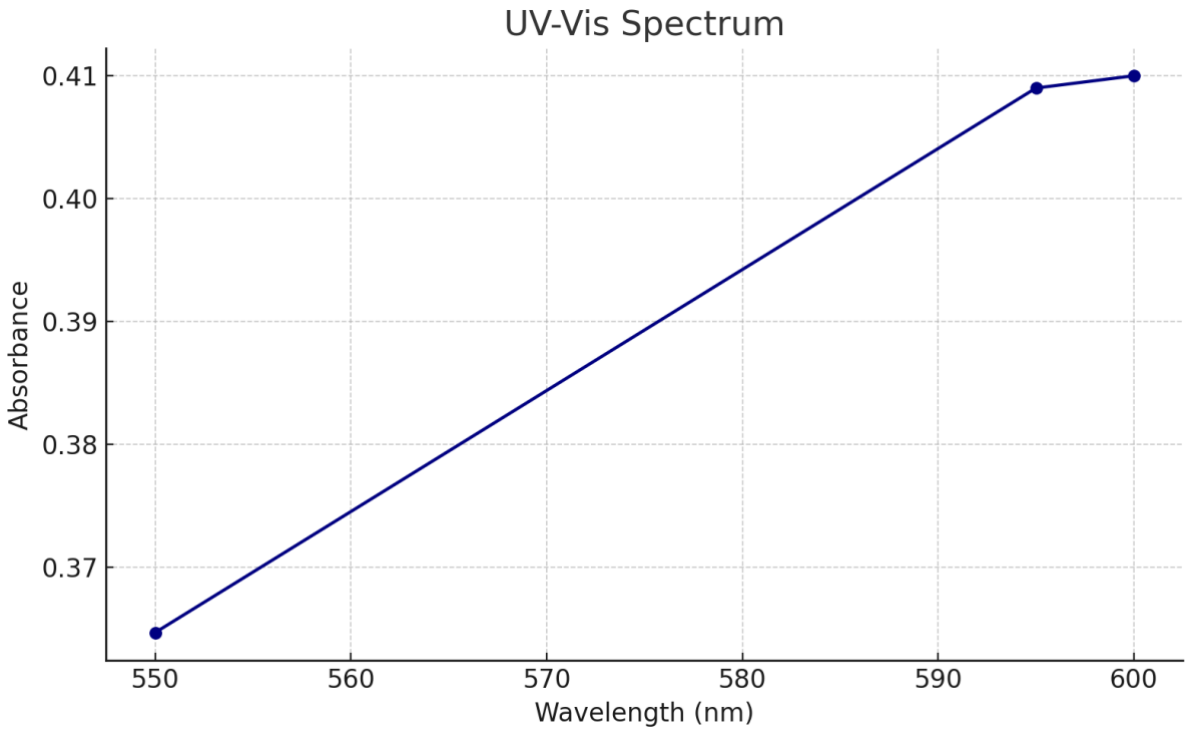


Fig 10:UV-vis spectroscopy Ag/ZnO NPs

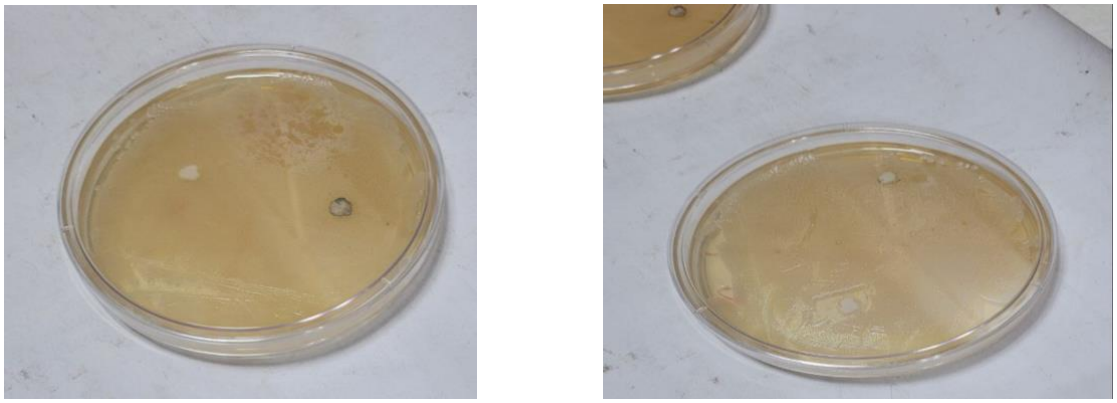
the absorbance increases from 550 nm to 600 nm.The **maximum absorbance (A = 0.410)** occurs at **600 nm**. **λmax** (peak) observe at 600nm with absorbance at 0.410. which lies in the visible region. The compound might have conjugated double bond and chromophores or an organic compound with extended π-system

1. Antibacterial Assay:

   It determine the potency of the antibiotics by measuring ability to inhibit the growth of microorganisms. The zone of inhibition is related to the antibiotic concentration. The Gram negtaive bacteria were calculated for the antibacterial activity of Ag-ZnO bimetallic NPs. The picture of the zone of inhibition against gram negative bacterials shown below n the fig 18. Ag-ZnO bimetallic Nanoparticlea have shown to have moderate effects the size of zone of inhibition around the paper disc which is

approxmately very low 5-6mm reveals bacteria is suspectible and vunerable towards the Ag-ZnO NPs.

The Zone of antibiotics i.e Drotvine-M, can be seen



(A) (B)

Fig 11: Zone of inhibition can be observed

2. FTIR analysis:

Regions at 2500 to 4000 cm corresponds to C-H and O-H stretchings vibration. Peak at 1760 cm corresponds to uniform C=O bond length signifies carbonyl group ester, ketones. Peak at 3700 to 3200 cm sharper peak is observed which corresponds to N-H functional groups it can be of amines and amdes. Peak at 2200-2100 cm corresponds to uniform C-C and C-N stretch presence of alkynes and nitriles. Peak at 400-500 cm^{-1} signify C-halogen bonds, especially C-Cl.

In the region of Region of 400-600cm^{-1} is the area of intrest which is out of plane bending can be noticed which signifies pesence of aromatic or oxide compound

C=C stretch around **1600–1500 cm^{-1}**

=C–H stretch above **3000 cm^{-1}**

Strong peak between 1700–1750 cm^{-1} depending on the specific group:

- Aldehyde: ~1740
- Ketone: ~1715
- Ester: ~1735
- Acid: ~1700 (with O–H stretch)

Identifying functional groups based on peak positions,

- Giving a detailed interpretation,

- Useful for **characterizing nanoparticles, polymers, coatings, composites**, and biological samples.
- Confirms surface functionalization or modification in nanomaterials or drug-delivery system
- Confirm **synthesis and surface capping** (e.g., biomolecules, polymers).
- Examine drug loading or bio interaction in drug delivery.

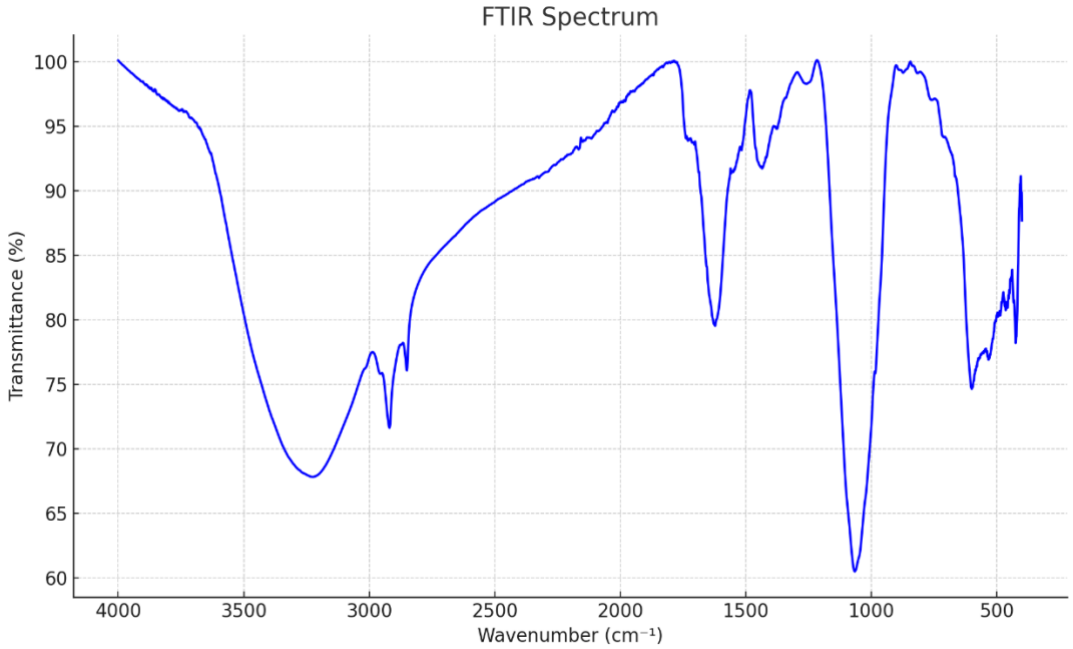


Fig 12: FTIR Graph

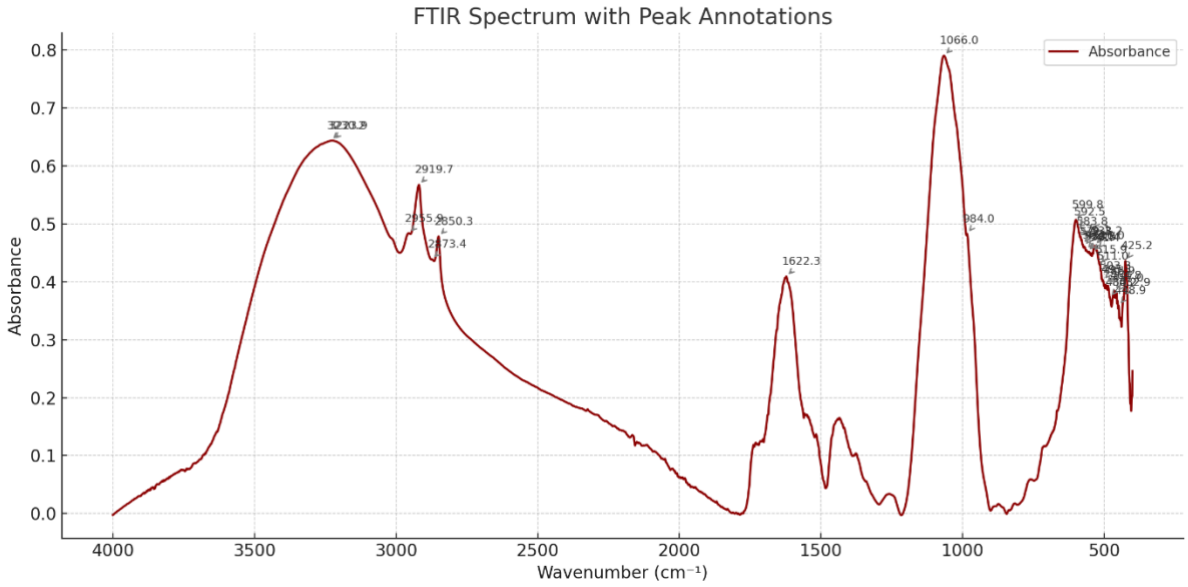


Fig13: graph with peak annotation

CHAPTER 5: Conclusion and prospective Direction

The work demonstrated the sustainable production of Silver and zinc bimetallic Nanoparticles by utilizing the Plant Extract. In this summary the focus is on the biological use of production of NPs. Green synthesises of BMNPs Which is easily avaiable, cost effective and eco friedly approch. The potential applications of flora-mediated nanoparticles in environment protection and in healthcare that is highlighted in this work. Improving functionality and adaptability for practical uses should be the main aim of future research. The UV-Vis spectroscopy characterization and FTIR confirms its potential application in various field. This the silver- zinc nanoparticles formed by grenn synthesis are the better alternative to chemcial synthesis method

The produced BMNPs can be employed as an antibacterial ingredients in cosmtics products and anti septic lotions. The non toxic nature of production make it a healthy choice. The development of metallic NPs is very rapid and the bimetallic has a combined effects of both the metals thus, enhancing its potential. These can be new tools for future therapeutic drug delivery method,. The large scale manufacturing with high biocomptibility should be emphasize.

In cancer treatment and antiviral therapy. Where the specific location is targated and it should be highly precise. Thererfore, ample research in the field of nanotoxicology and pharmacutical espescilly in drug delivery method can be a fascinating topic . The wide range of capibilities of nanotechnology make it a important tool that must be used thoughtfully and responsibly be used to change the world for the good purpose

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