Bauhinia variegata leaves mediated green synthesis of Zinc Oxide Nanoparticles and its antimicrobial activity

Abhina V.T

(16PCH001)

Thesis Submitted to

Avinashilingam Institute for Home Science and Higher Education for Women,

Coimbatore-641 043

In Partial Fulfilment of the Requirements for the Degree of

Master of Science in Chemistry

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R. Rav 10/4/18

Signature of the Supervisor

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Signature of the Head of the Department

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ZnO	Zinc Oxide
ZnO NPs	Zinc Oxide Nanoparticles
KOH	Pottasium hydroxide
BVL	Bauhinia variegata leaf extract
NaOH	Sodium hydroxide
ZnONP-C	Zinc Oxide Nanoparticle chemically synthesized
ZnONP-BVL	Zinc Oxide Nanoparticle Bauhinia variegata mediated synthesized
SPR	Surface Plasmon Resonance

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INTRODUCTION

Zinc is classed as a transition metal. It has been known since ancient times. It is an essential element for good health. It is a bluish silver metal that tarnishes in moist air producing a layer of carbonate. It is somewhat brittle at room temperature but malleable it is fair conductor of electricity, and burns in air with a bright bluish green flame. It reacts with both acids and alkalies. Zinc is used in many areas, it is used to galvanize iron to inhibit corrosion, zinc forms so many alloys they are used in die-casting for the car industry. Zinc is an essential trace element for animals and plants; it is used in sun block, cosmetics and in ointments like calamine lotion also used in rubber industry, concrete manufacturing and plants.

1.1 Zinc Oxide

Zinc oxide is an inorganic compound with formula ZnO. It is a white powder that is insoluble in water .It is present in the earth's crust as the mineral zincite, that being said, most ZnO used commercially is synthetic. ZnO is commonly found in medical oinments in more recents times zinc oxide has use in semiconductors, concrete use, ceramic and glass composition. It is an n-type semiconducting metal oxide, had large band gap (3.37 eV) and high excit on binding energy (60meV) so it exhibit a large number of semiconducting properties like high catalytic activity, optic, UV filtering properties, anti-inflammatory, wound healing **(Nurul ain samat *et al*., 2012)**. Zinc oxide NP has drawn interest in past two–three years due to its wide range of applicability in the field of electronics, optics, and biomedical systems. Many of the metal oxides like Fe_3O_4, TiO_2, CuO are used for nano particle synthesis of all these metal oxides, ZnO NPs is of maximum interest because they are easy to produce, Low production cost, safe and can be prepared easily . US FDA has enlisted ZnO as GRAS (generally recognized as safe) metal oxide. These properties had its own applications also, due to its UV filtering properties; it has been extensively used in cosmetics like sunscreen lotions. It has a wide range of biomedical applications like Drug Delivery, Anti Cancer, Antidiabetic, Antibacterial, Antifungal and Agricultural properties. Although ZnO is used for targeted drug delivery, it still has the limitation of cytotoxicity which is yet to be resolved. ZnO NPs have a very strong antibacterial effect at a very low concentration of gram negative and gram positive bacteria as confirmed by the studies, they have shown strong antibacterial effect than the ZnO NPs synthesized chemically. They have also been employed for rubber manufacturing, paint, for removing sulphur and arsenic from water, protein adsorption properties, and dental applications. ZnO NPs have piezoelectric and pyroelectric properties. They are used for disposal of aquatic weed which is resistant to all type of eradication techniques like physical, chemical and mechanical means. They are used for the elimination of toxic chemicals like arsenic, sulphur from water sources **(Happy Agarwal *et al.*, 2017).** Metal oxide nanoparticles have environmental applications as it can act as catalyst which is helpful in reduction or elimination of the toxic hazardous chemicals from environment.

Fig 1.1: Image of zincite

Zinc oxide (ZnO) nanoparticles have received considerable attention due to their antimicrobial, UV blocking, high catalytic and photochemical activities. It is reported that ZnO nanoparticles possess antibacterial and antifungal activities even at lower concentrations hence suitable for thin coating applications. Further antifungal activity of ZnO nanoparticles does not affect soil fertility compared to the conventional antifungal agents. Zinc oxide has unique optical and as well as excellent thermal and chemical stability. ZnO nanoparticles have gathered the increasing interest of the scientific and industrial community due to diverse application in solar energy conversion, sensors, catalysis, cosmetics, paints, fibers, drug-delivery antibacterial and luminescence properties **(Senthilkumar *et al*., 2014)**

Zinc oxide nanoparticles (ZnO NPs) are considered as a bio safe material for biological species. Earlier studies have shown the potential of ZnO NPs in stimulation of seed germination and plant growth as well as disease suppression and plant Protection by its antimicrobial activity Zinc (Zn) is the only metal found in all six enzyme classes, viz. Oxidoreductases, Lyases, Isomerases, Transferases, Hydrolases and Ligases. Zinc being essential micronutrient plays important role in many integral metabolic processes. Zn can also help in increase the biosynthesis of chlorophylls and carotenoids and enhance the photosynthetic apparatus of the plant. Significant optoelectrical, physical and antimicrobial properties of zinc oxide (ZnO) NPs offers great potential to boost agriculture productivity. There are some studies which report strong absorption abilities for a series of organic compounds, heavy metals, ZnO and its NPs. ZnO NPs have a large surface area-to-volume ratio that results in a significant increase in the effectiveness in blocking UV radiation as compared to the bulk material **(Ajey Singh *et al.*, 2017)** ZnO NP were used due to their wide applications starting from antimicrobial properties to semiconducting (n-type) and piezoelectric properties. Its antibacterial property has been already utilized in paint, textile industry, cosmetic products, used as biosensor, in drug delivery and Nanomedicine **(Deependra Kumar Ban *etal*., 2014).**

We have chosen the ZnO nanoparticles (NPs) since it has a wide area of applications in various fields such as active laser medium and luminescence for fluorescent bulbs as well as in antimicrobial activities. The ZnO NP was one of the semiconducting materials in which its structure belongs to some particular group. ZnO NP has high transparency in the visible range as well as good light trapping property. Also, it has various other advantages such as non-toxicity, natural abundance and good photocatalysis. ZnO NP plays a significant role in toxicity mechanisms by inhibiting immune cells **(Gunabalan Madhumitha *et al.*, 2015**).

Among the inorganic NPs, ZnO nanoparticles are of particular interest because they can be prepared easily inexpensive and safe material for human beings and animals. Indeed, zinc oxide is non-toxic and chemically stable under high exposure to high temperature and is capable of high photo-catalytic oxidation. The antibacterial activity of ZnO was tested and the effect was more pronounced with the gram-positive than the gram-negative bacteria and also ZnO NPs exhibited a preferential ability to kill cancerous HL-60 cells as compared with normal peripheral blood mononuclear cells **(Anila P. Ashokan *et al* ., 2016).**

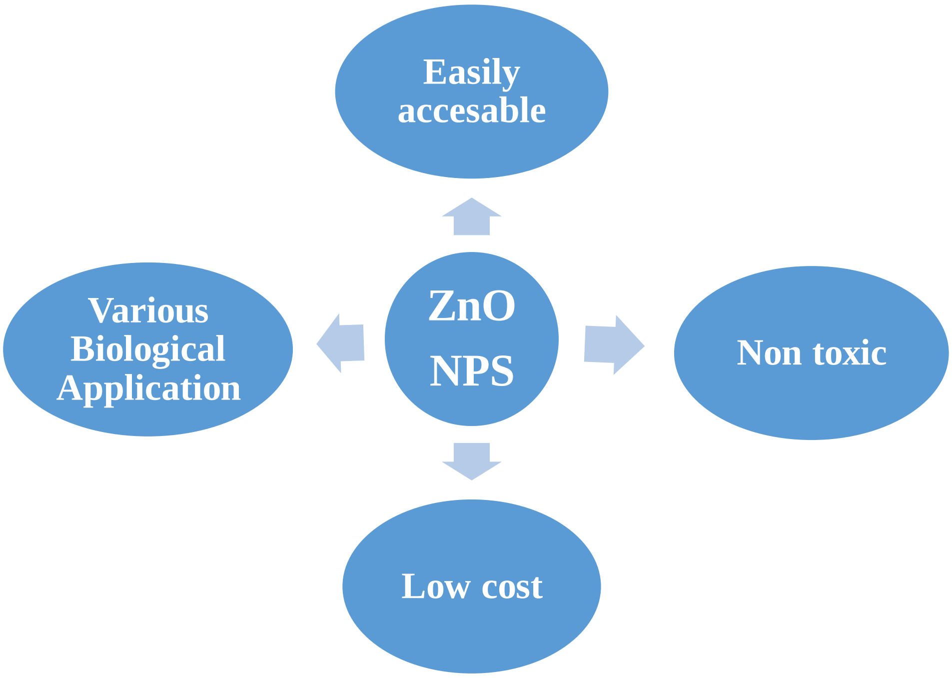


Fig: 1.2 Applications of zinc oxide nanoparticles (Gunabalan Madhumitha *et al*., 2015)

1.2 Nanotechnology

Nanotechnology is a main developing branch of science, in current senario of science and chemistry nano is a developing vast area, so many researches are going through it. The word nano usually used as a prefix (nano is the greek word of **“dwarf”**) it means one billion. One nano meter is one billionth or 10^{-9} of a meter, it is a small unit for measurement. The smallest things visible to the eye are the order of 10,000 nm diameter, the fact that a human hair measures 50,000 - 80,000 nm across give an idea of how small the nanoscale is comparing the nano meter to the meter is comparing the size of a marble (small, spherical glossy thing that children used to play) to that of earth.

Nanotechnology can be seen as an extension of the existing sciences into the nanoscale. It is an interdisciplinary area where researches include different subjects like Chemistry, Physics, Biosciences, Material science, Computational engineering, Colloidal science and even Mechanical and Electrical engineering.

Nanotechnology provides a great possibility of novel application in the fields of biotechnology and agricultural industries. It is the branch of technology that deals especially the manipulation of discrete atoms and molecules. Nanotechnology is considered as the application of nano particles (NPs) which can be defined as a small object that behaves as a whole unit in terms of its transport and properties. Nanotechnology has a great potential to provide an opportunity for the researchers to develop new tools for incorporation of NPs into plants that could supplement existing functions and add new ones. Interaction of NPs with plants causes several morphological and physiological changes, depending on the properties of NPs. The efficiency of NPs is determined by different factors like their chemical composition, size, surface covering, reactivity and most importantly the extent to which they are effective

The synthesis of nanoparticles by conventional physical and chemical methods has some adverse effects like, critical conditions of particles has gained significant importance in recent years and has become the one of the most preferred methods. Green synthesis procedures have several merits such as, simple, inexpensive, good stability of nanoparticles, less time consumption, non-toxic by products and large-scale synthesis

1.3 Synthesis of ZnO NPs

Various chemical methods have been proposed for the synthesis of nanoparticles (ZnO NPs), such as chemical method, sol gel method vapour transport, hydrothermal synthesis, precipitation method, chemical microemulsion, wet chemical, electrochemical depositions, microwave assisted combustion, co-precipitation and sonication etc., and all of these methods have their own advantages and drawbacks. For example in chemical methods, the synthesis can be carried out in large scale but use of toxic chemicals poses major environmental and biological problems. To avoid the use of toxic chemical and harsh reaction conditions for the preparation of nanoparticles, biosynthesis has proved to be a useful tool. Recently, green synthesis of nanoparticles has gained much attention due to its inexpensive, simplicity and eco-friendly procedure.

Two basic approaches have been suggested for nanoparticle synthesis:

- Bottom up approach
- Top down approach.

The top–down approach involves milling or attrition of large macroscopic particle. It involves synthesizing large-scale patterns initially and then reducing it to nanoscale level through plastic deformation. This technique cannot be employed for large scale production of nanoparticles because it is a costly and slow process. Interferometric Lithographic (IL) is the most common technique which employs the role of top–down approach for nanomaterial synthesis. This technique involves the synthesis of nanoparticles from already miniaturized atomic components through self-assembly. This includes formation through physical and chemical means. It is a comparatively cheap approach [6]. It is based on kinetic and thermodynamic equilibrium approach. The kinetic approach involves MBE (Molecular beam epitaxy) **(Happy Agarwal *et al*., 2017)**

Top down approach refers to slicing or successive cutting of a bulk material to get nano particle. It implies that the nanostructures are synthesised by etching out crystal planes. Which are already present on the substrate. A top down approach can thus be viewed as an approach where the building block is removed from the substrate to form the nanostructure. Microelectronics is fabricated using this approach

A bottom up method implies that the nanostructures are synthesised onto the substrate by stacking atoms onto each other, which give rise to crystal planes, crystal planes further stack onto each other, resulting in the synthesis of the a nanostructure. A bottom –up approach can thus be viewed as a synthesis approach where the building blocks are added onto the substrate from the nanostructures.

These are two different approaches used in nanofabrication. The bottom up approach is more advantageous than the top down approach because the former has a better chance of producing nanostructures with less defects, more homogenous chemical composition, and better short and long range ordering. Low cost, scalability and in general better uniformity of the product. Top down typically provides better control, but is limited to countable number of structures, though this number may be billions and billions.

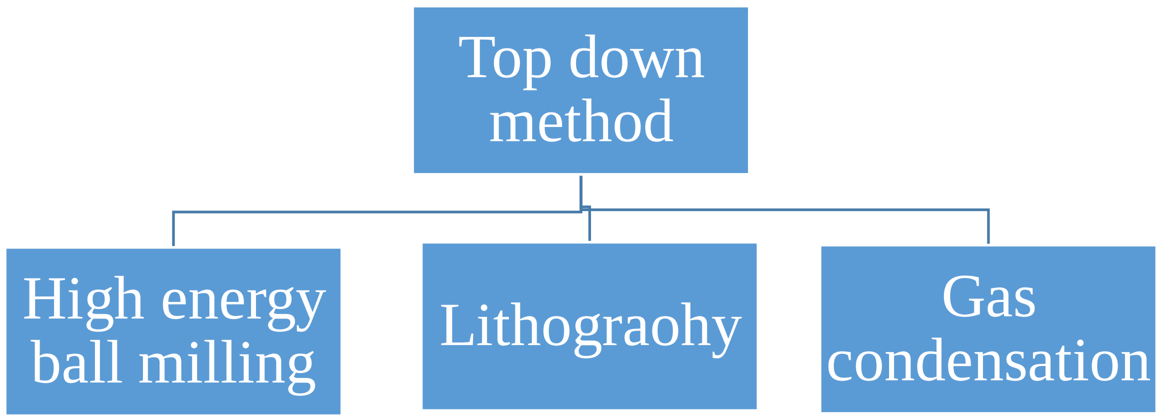


Fig 1.3: Synthesis of nano materials through top down method

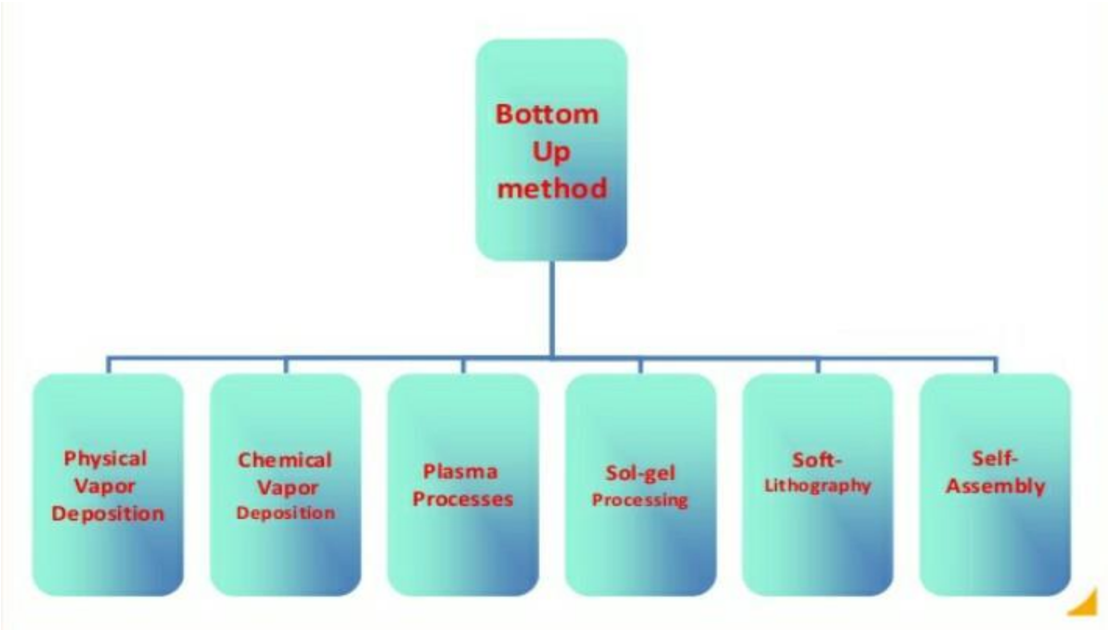


Fig 1.4: Synthesis of nanoparticles through bottom up method

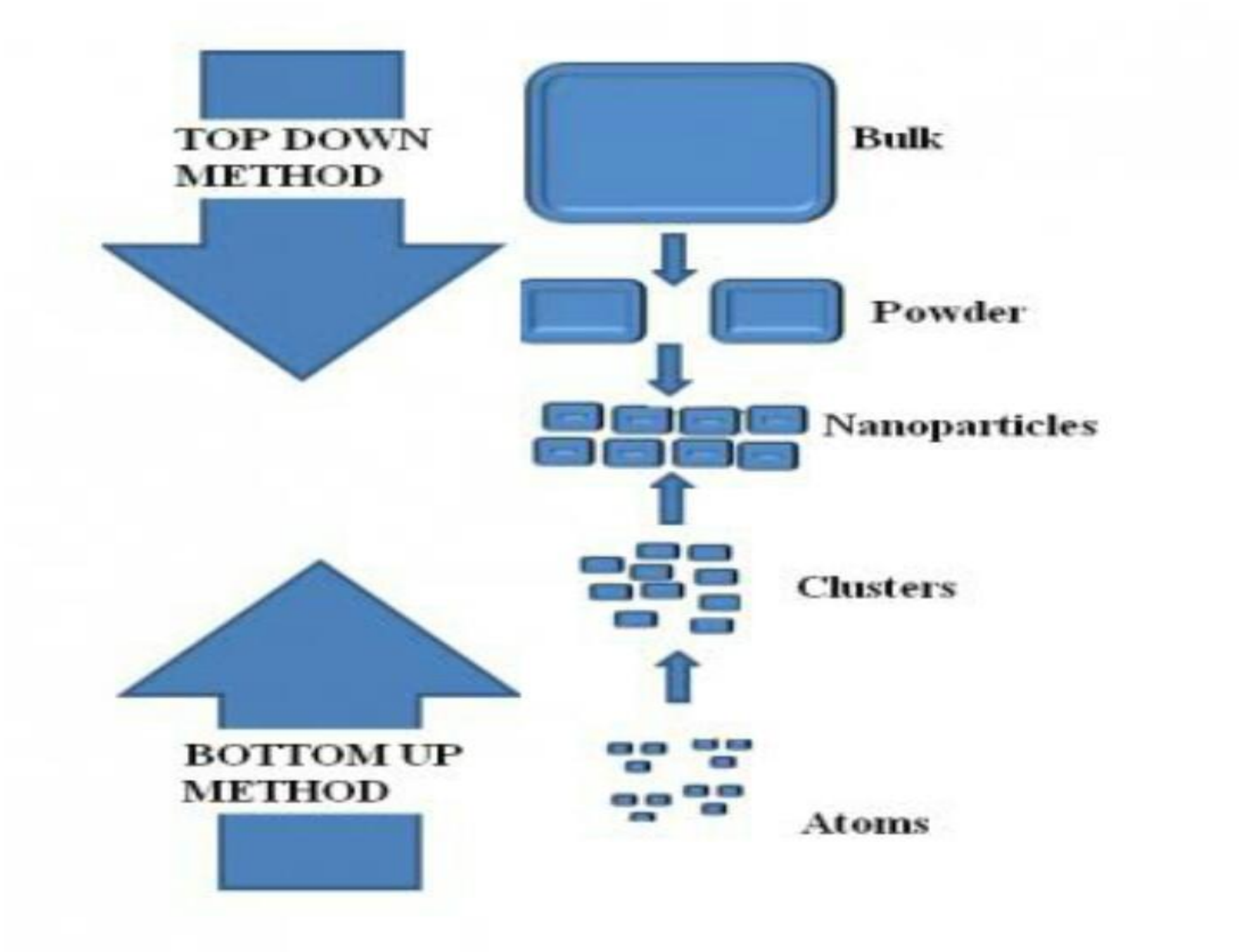


Fig 1.5: Synthesis of Nano particles through chemical methods

Methods of synthesis

Chemical method, sol gel method, precipitation method and hydro thermal method are some of the major chemical methods for the preparation of zinc oxide nano particles.

- **Chemical method**

  In chemical method we use zinc nitrate or zinc oxide for the synthesis we use starch and sodium hydroxide also .preparation of zinc oxide nanoparticle is simple in this way also, but it use some toxic chemicals and it take one day tie for the preparation.

- **Sol gel method**

This method is somewhat similar to chemical method. Zinc nitrate or zinc oxide is used for this also along with we use sodium hydroxide. Zinc oxide nanoparticles synthesis become the used research area because of the fact that the chemicals used for the preparation is common and the method is simple.

- **Precipitation method**

Zinc oxide nanoparticle is synthesised with the help of zinc nitrate and KOH solution .The KOH solution was slowly added into zinc nitrate solution under vigorous stirring, then the product is centrifuged and washed many times and then dried, zinc oxide nanoparticles were formed.

- **Hydrothermal method**

It is an efficient alternative synthesis method because of the low process temperature. For synthesizing nanoparticles stock solutions of zinc acetate and NaOH is used. Solution made in methanol is added under stirring to get the pH value between 8-12.Then it autoclaved under different temperatures. The resulting product will be washed in methanol and dried.

- **Vapour transport method**

It is common method for synthesis of ZnO NPs .In this process ,zinc and oxygen or oxygen mixture vapours are transported and react with each other resulting in the formation of ZnO nanostructure .There are more than one method to generate Zinc and oxygen ,decomposition of ZnO is easier method.

1.4 Different morphologies of ZnO NPs

ZnO NPs have been reported in different morphologies like Nanoflake, Nanoflower, Nanobelt, Nanorod, Nanowire.

Nanoflake

Nano flakes are a type of semiconductor that has potential for solar energy creation as the product itself is only in the prototype phase, with crystalline structure; the crystals are able to absorb light. In a general meaning nano flake is a flake that is an uneven piece of material with one dimension substantially smaller than the other two with at least one nanometric dimension. It is characterised by a plate - like structure .There are nanoflakes of all sorts of materials.

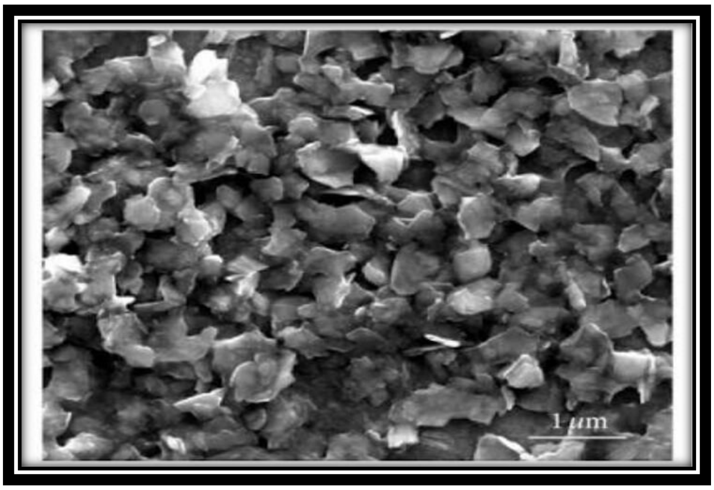


Fig: 1.6 Images of Nanoflake

Nanoflower

A nanoflower refers to a compound of certain elements those results in formations which in microscopic view resemble flowers or in some cases, trees that are called nanobouquets or nanotrees. These formations are nanometers long and thick so they can only be observed using electron microscopy.

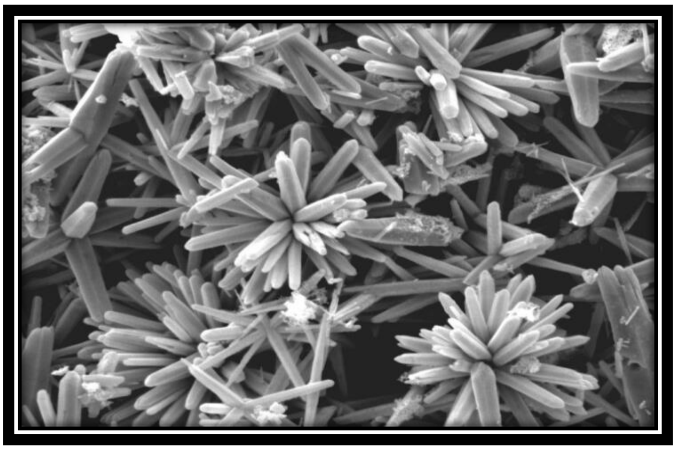


Fig 1.7: Images of Nanoflower

Nanobelt

Nanobelt is a quasi - one dimensional structurally controlled nanomaterial that has well defined chemical composition, crystallographic structure and surfaces like growth direction, side surfaces.

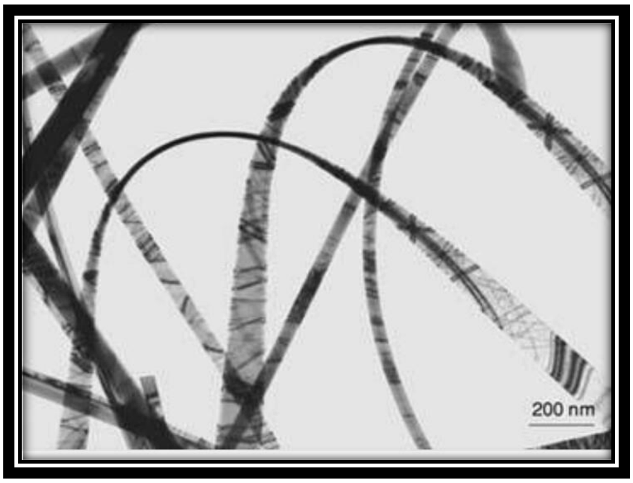


Fig 1.8: Images of Nanobelt

Nanorods

Nanorods are one morphology of nanoscale objects .each of their dimension range from 1-100nm .they may be synthesised from metals or semiconducting materials.

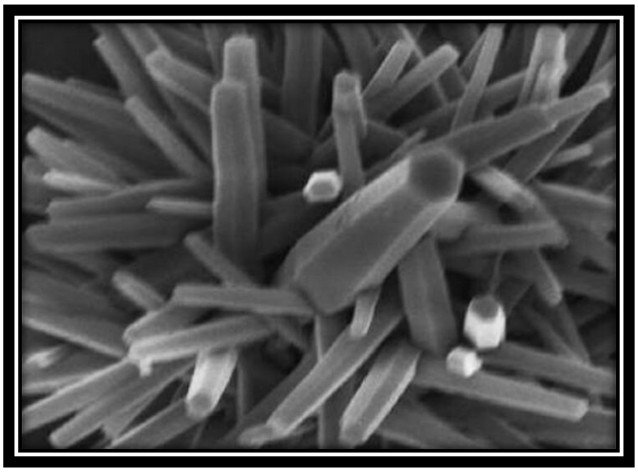


Fig 1.9: Image of Nanorods

Nanowire

A nanowire is a nanostructure with the diameter of the order of a nanometer. It can also be defined as the length to width being greater than 1000.

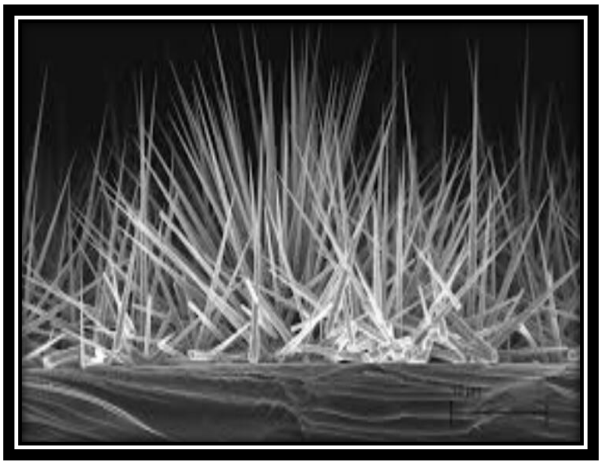


Fig 1.10: Image of Nanowires

Alternatively, nanowires can be defined as structures that have a thickness or diameter constrained to tens of nanometers or less and an unconstrained length. At these scales quantum mechanical effects are important which coined the term quantum wires.

1.5 Applications of ZnO NPs

ZnO NPs has attained the remarkable attention, for its semiconducting properties, unique antifungal, antibacterial, wound healing and UV filtering properties, humidity sensing, photochemical and high catalytic activity Furthermore, ZnO NPs finds wide range of applications in pigments, gas sensors, rubber additive, optical devices and varistors The characteristics and application of ZnO nano powder depend on its size, shape and methods of preparation **[Bappi paul *etal*.,]**

Zinc oxide nanoparticle gets more attention in the metal oxide nanoparticle synthesis because of its simple methods and large number of applications of these nanoparticles. Popularly known calamine lotion is made out of zinc oxide powder. It is also used in a host of other creams and ointments that are used to treat skin diseases. Zinc oxide is used in the manufacture of rubber and cigarattes, it is used as a filter. It is used as an additive in the manufacture of concrete, ceramic industry has a number of uses for zinc oxide powder. It isalso used as an additive in food products such as breakfast cereals. Paints using zinc oxide as a coating agent.

Drug Delivery

ZnO Nanomaterial are used for drug delivery due to their large surface area, versatile surface area chemistry, phototoxic effect .Some in vitro studies shows that ZnO NPs is highly toxic to cancer cells and leukemic T cells. Therefore not only have ZnO nanomaterials have been investigated as drug delivery vehicles; they have also been studied for cancer therapy. ZnO quantum dots with intrinsic blue fluorescence were coated with folate conjugated chitos via electrostatic interaction, which could be loaded with doxorubicin DOX, a widely used chemotherapy drug at approximately 75% efficiency.

Gene Delivery

Gene therapy has attracted considerable interest over the last several decades for cancer treatment. One major challenge of gene therapy is the development of safe gene vectors which can protect DNA from degradation and enable cellular uptake of DNA with high efficiency. A wide variety of nanomaterials have been investigated for gene delivery and gene therapy applications, including ZnO nanomaterials. In a series of studies, three dimensional tetrapod like ZnO nanostructures were investigated as gene vectors to deliver pEGFPN1 DNA (which contains the gene for gene fluorescent protein) to A375 human melanoma cells .The plasmid DNA was attached to ZnO nanostructures via electrostatic interactions and the three needle shaped legs favored the internalization of the tips within the cells for gene delivery.

1.6. Biosynthesis of zinc oxide nanoparticles

The chemical and physical method of preparation had some disadvantages also. Toxicity is a problem and the high cost and other problems are there .so researches move on to some advanced area, biosynthesis of zinc oxide nanoparticles. Bio synthesis not only applicable to zinc oxide nanoparticles but it is applicable to other metal oxide nanoparticles also.

Biosynthesis of metal or metal oxide NPs using plant extract, bacteria and fungi for is considered to be immense importance owing to modern legislation on clean environment. Therefore, the synthesis of nanoparticles using natural sources is an excellent strategy that could reduce the environmental impact. Such methods are also useful for large-scale synthesis of nanoparticles with well-defined size and shape nanoparticles.

The chemical and physical methods of synthsis involve so many disadvantages like toxicity, high cost and other problems. Green synthsis of nano particles is a new approach and it is very easy, time consuming, low cost, environmental supportive method this include the synthesis of nanoparticles from different parts of a plant. Biosynthesis of nanoparticles is an approach of synthesizing nanoparticles using microorganisms and plants having biomedical applications. This approach is an environment-friendly, cost-effective, biocom patible, safe, green approach. Green synthesis includes synthesis through plants, bacteria, fungi, algae etc. They allow large scale production of ZnO NPs free of additional impurities. NPs synthesized from bio mimetic approach show more catalytic activity and limit the use of expensive and toxic chemicals. These natural strains and plant extract secrete some phyto chemicals that act as both reducing agent and capping or stabilization agent. Plant parts like roots, leaves, stems, seeds, fruits have also been utilized for the NPs synthesis as their extract is rich in phyto chemicals which act as both reducing and stabilization agent

Plant mediated synthesis is pollution free ,environment friendly and according to plants taken it has wide varities of applications also .plant or micro organism based synthesis has applications in many area they have antimicrobial, antifungal, antidiabetic properties.

In this paper, demonstrate a novel biological route for the synthesis of ZnO NPs using aqueous extract of *Bauhinia variegata*. (**Ebrahim shayegan *et al.*, 2017)**

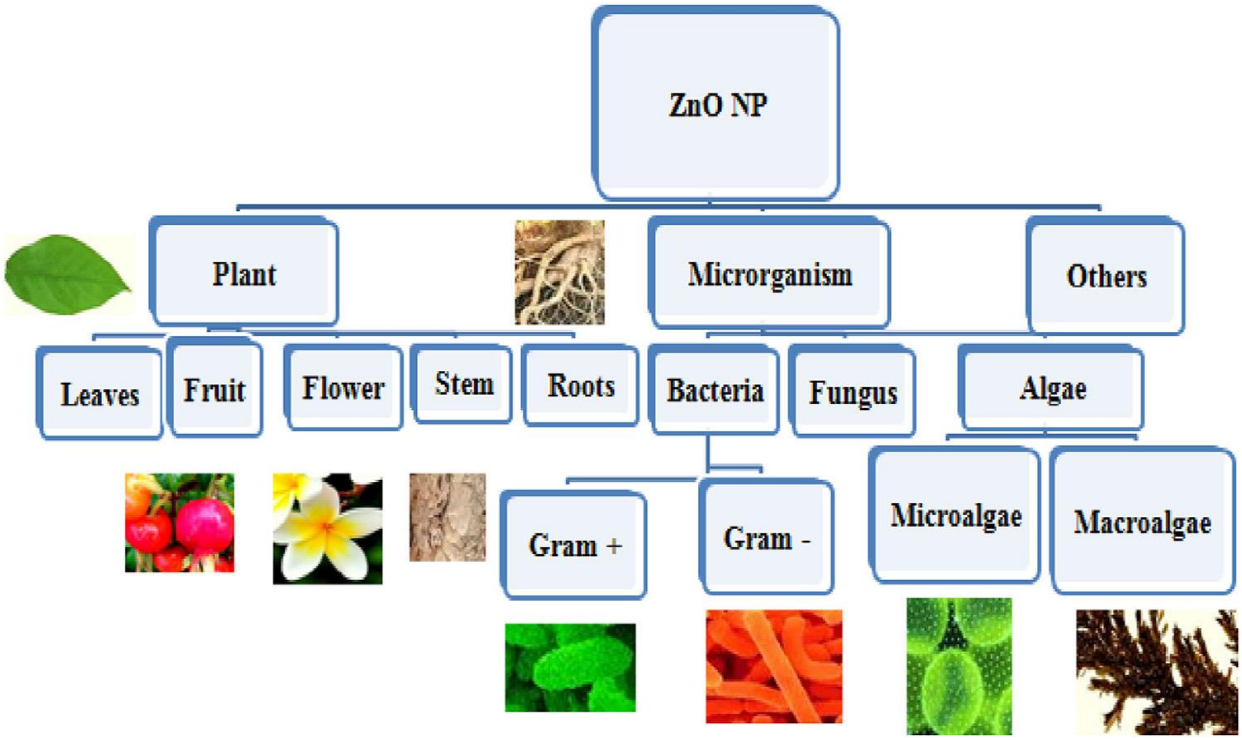


Fig: 1.11 ZnO NPs Synthesis by using different sources. (Happy Agarwal *et al*., 2017)

1.7. Plant description

Bauhinia variegata is a species of flowering plant in the family Fabaceae .It is native to south asia and southeast asia , from southern china ,Burma, India , Nepal , Pakistan, Sri Lanka .Common name include orchid tree, camel's foot tree , Kachnar and mountain ebony. Kachnar is deciduous tree of small to medium sized height upto 15mt with spreading crown and short bole. Twigs of the tree are slender, light, green, angled, hairy and brownish grey in colour. Outer bark is scaly, smooth to slightly fissured and brownish grey in colour and inner bark is pinkish in colour, fibrous and bitter. Leaves are bilobed with minute stipules, ovate to circular and it look like camel's hoof print, had five overlapped and irregular petals of yellow and pinkish purple colour. Cluster of flowers are unbranched at the end of twigs fruit is dehiscent pod; strap shaped contains various flat seeds and about15-30cm long Kachnar is closely related to peacock flower.

Fig 1.11: Images of *Bauhinia variegata*

Scientific classification of *Bauhinia variegata*

Common name	Kachnar
Kingdom	Plantae - Angiosperm
Order	Fabales
Family	Fabaceae
Genus	*Bauhinia*
Species	*B. varigata*

OBJECTIVES

1. To provided a simple one step method to prepare zinc oxide nanoparticles from locally available plant resources.
2. To characterise the synthesised zinc oxide nanoparticles using UV-Visible spectroscopical analysis,FT-IR analysis ,3D Optical profiler ,TGA ,SEM analyses.
3. To investigate which natural resources are available for the preparation of zinc oxide nanoparticles.
4. Study the anti-microbial activity of the synthesised zinc oxide nanoparticles.

2. REVIEW OF LITERATURE

- **Elizabeth Varghesev *et al*., (2015)** Prepared Nano sized ZnO particles from plant leaf extract of ***Aloe Vera. Aloe Vera*** is a medicinal plant and which have therapeutic properties. The structural, optical, thermal, photo catalytical properties of zinc oxide Nano particles have been evaluated. In this study we mainly focussed on anti-bacterial properties of zinc oxide Nano particles. Photo catalytic activity of the Nano particles was studied with fluorescein dye. Several techniques like XRD, SEM, FTIR, solid UV-Vis and TGA were used for structural and morphological analysis. Synthesis of ZnO Nano particles through plant extract is simple and cost effective.
- **Happy Agarwal *et al*., (2017)** were proposed different sources for green synthesis of zinc oxide Nano particles. ZnO Nano particles found in application in anti-bacterial, anti-fungal, anti-diabetic, anti-oxidant, wound healing and optic properties. ZnO Nano particles have been prepared by physical, chemical methods .Here we discuss about production of ZnO Nano particles from different parts of a plant .Plants mediated synthesised Nano particles have huge application in different area.
- **Santhoshkumar *et al*., (2017)** zinc oxide Nano particles are known to be one of the most multifunctional inorganic Nano particles, in this study they were prepared from ***Passiflora caerulea,*** which is a medicinal plant and study its applications in treatment of urinary tract infection. Its morphological and structural characterization is done with SEM, XRD, FTIR, EDAX and UV-VIS techniques .presence of phytochemicals in the leaf extract is helping in the eco-friendly synthesis of zinc oxide Nano particles.
- **Senthilkumar *et al*., (2014)** from the aqueous extract of green tea (***Camellia sinensis***) ZnO nano particles were synthesised and explain about its hexagonal wurtzite structure anti-microbial activities ,anti-bacterial activities ,anti-fungal activities of zinc oxide nano particles were explained in this study . Characterization is carried out with different techniques SEM, EDAX, FTIR, XRD. Found that gram negative bacteria are sensitive to the ZnO nano particles and also effective in inhibition of the fungi.
- **Ebrahim shayegan mehr *et al*., (2017**) highly pure crystalline zinc oxide nano particles were prepared with ***Ferulago angulata*** (schlecht), along with ZnO, CuO nano particles were studied using Rhodamine B. From the study it is clear that photo catalytic degradation capacity of the ZnO nano particles was higher than that of copper oxide nano particles.
- **Ajey Singh *et al*., (2017)** this paper is a review on the application of zinc oxide Nano particles in the field of biotechnology, zinc oxide nano particles were considered as a bio

safe material for biological species .We can understood the applications of ZnO nano particles as Nano fertilizers.

- **Gunabalan Madhumitha *et al*., (2015),** this study is an overview on various type of synthesis and applications of zinc oxide nano particles. And this review they pointed out the drawbacks of physical and chemical methods (it includes hydrothermal process, sonochemical process) and advantages of green synthesis .In new research works the eco-friendly approach is dominating because of its cheaper availability, high stability etc., Here they discussed the syntheses from different sources from bacteria, fungi, and plant extract. They have various applications in the biological field. From this review we can know that ZnO nano particles are also reported to help to prevent dust formation for several years on oil paintings. The metal nano particles have been used in remediation, optics, catalysis and media recording .Some plant sources are mentioned in this paper, from which ZnO nano particles are already prepared also here explained the advantages of ZnO nano, they are nontoxic, easily accessible and low cost and have various biological functions. In this review they discussed about the synthesis from microorganisms, from biomolecules, also prepared from plasmid DNA and from some amino acids. Then explain about the characterization process, applications in various field .This review is very useful in focussing the applications of these nano particles in biological level
- **Bappi Paul *et al.*, (2017)** in this it is reported that the plant mediated green synthesis of zinc oxide nano particles using seeds extract from the tender pods of ***Parkia roxburghii***. It is a common plant found common in north east India. Different characterization studies are carried out in this study structural determination is done by powder XRD method, shows the formation of hexagonal close packing structure of ZnO nanoparticles. This study also explains about various applications of metal nano particles. The synthesised nano particles are highly efficient sono catalysts for degradation of MB and Rh B. And the nano particles are highly effective in the ultrasound assisted synthesis of 2- benzimidazole derivatives .catalytic properties of ZnO particles also explained here.
- **Deependra KumarBan et *al.*, (2014)** in this explained about the effect of zinc oxide nano particles on the production of β-Glucosidase in saccharomyces cerevisiae under various conditions. Here the nano particles are synthesised by chemical method .Results from the paper revealed that yeast culture administered with 5mm zinc oxide nano particles enhanced the intracellular BGL activity upto 28% compared to control with simultaneous growth of cells. This finding provide a new insight on the potential application of the zinc oxide nano particles as an external supplement to enhance the active production of BGL like important

industrial enzymes in ***Saccharomyces cerevisiae*** in both normal and alcohol stressed condition as well as to provide Barker's yiest in higher amount. The enhancement of BGL production in yeast using zinc oxide nano particles would not only be highly beneficial in biotechnology industry, but such strategy might encourage using various other metal and metal oxide nano particles for the production of varities industrially important enzymes in higher amounts.

- **Govindasamy rajakumar *et al*., (2017),** In this review zinc oxide nanoparticles are synthesised from ***Andrographis paniculata*** leaf extract .It is a common plant, after synthesising here study and evaluating antioxidant, anti -diabetic, anti-inflammatory activities. Green synthesised ZnO nanoparticles was carried out by using the reducing capacity and capping FT,XRD, FT-IR,SEM,TEM and SAED analysis . Nucleation and stability of ZnO nanoparticles studied using FTIR analysis. SEM, TEM analysis shows the hexagonal shapes of ZnO nanoparticles. The synthesised nanoparticles possess strong biological activities regarding antioxidant ,anti-diabetic and anti-inflammatory potentials ,These are utilized in various biological applications by the cosmetic ,food and biomedical industries .Green synthesis has more advantages than conventional physical and highly toxic chemical methods .The advantages of nano structured zinc oxide nanoparticles over other metal nanoparticles are due to their low cost ,characterization and anti-oxidant activity ,reducing power, anti-fungal activities are studied by different methods.
- **Pragathi jamdagni *et al*., (2016)** This study is mainly focus on the anti- fungal activity. It states a green approach for the synthesis of zinc oxide nanoparticles. The synthesis is done by using the aqueous flower extract of ***Nyctanthes arbortristis*** and zinc acetate. Synthesised conditions were optimised for narrow range synthesis of ZnO nanoparticles. Various analytical techniques were used for characterization such as UV-visible spectroscopy. FT-IR spectroscopy, X –ray diffraction, Dynamic light scattering and transmission electron spectroscopy. The nano powder was stored in dried form and was found to be stable after 4months. Size range is obtained from TEM, crystalline nature of nanoparticles were confirmed by XRD. Nanoparticle synthesis , now a day's become an important area of research, because of its exciting properties and applications like nano diagnostic ,nano medicine etc.,The study deals with chemical methods of preparation also. Anti-fungal properties showed good activity against 5 tested fungal phytopathogens.
- **Supraja *et al*., (2015)** In this study ZnO nanoparticles prepared using the stem bark extract of ***Boswellia avalifoliolata*** and studied their anti-microbial efficiency, the synthesis is done with zinc nitrate solution and bark extract ,confirmation of product is done by different

characterization studies like UV-Visible spectroscopic analysis , FT-IR. Morphology and crystalline phase of the nanoparticles were determining by TEM. Chemical and physical methods are so common in nanoparticle synthesis. But the use of toxic compounds limits their applications. So green synthesis is a new attempt and their applications are high. ZnO nanoparticles appear to be strongly resistant to bacteria and fungi. The anti -microbial study evaluated against pathogenic fungi and bacteria isolated from the biofilm formed in drinking water PVC pipelines by invitro using dark diffusion method .Plant extract was prepared from which the nanoparticles synthesised. Then the anti-microbial activity is studied according with some biological parameters. Spherical shaped ZnO nanoparticles were characterized by TEM. The result revealed that the synthesised nanoparticles showed good anti-bacterial activity compared to anti-fungal activity. The result help in development of new antimicrobial system based on the synthesised nanoparticles for medical application.

- **Anila p ashokan *et al*., (2016)** in this paper the ZnO nanoparticles with medical function, their effect on cancer cells also studied, most of the anti-cancer drugs are toxic, but from this procedure we get anti-cancer drugs .Morphological studies and characterization is done with different techniques. Nanoparticles have varities of applications in different fields diagnostic, anti-microbial activity, drug delivery etc., Among the inorganic NPs, ZnO nanoparticles are of particular interest because they can be prepared easily inexpensive and safe material for human beings and animals. Indeed, zinc oxide is non-toxic and chemically stable under high exposure to high temperature and is capable of high photo-catalytic oxidation. Plant-mediated biosynthesis of nanoparticles is advantageous over chemical and physical methods because it is cheap, rapid, and environment-friendly. Also, it does not require high pressure, energy, temperature, or the use of highly toxic chemicals. One of the green methods of synthesis of nanoparticles is the utilization of various plants and their parts.
- **Baskar G. *et al*., (2016)** in this study flower extract is used for the preparation of zinc oxide nanoparticles and the flower extract have anticancer activities so the nanoparticle also used for some medicinal purpose. ZnO nanoparticles are known to be one of the multifunctional inorganic nanoparticles. ZnO nanoparticles could be used effectively in agricultural and food safety applications and also can address future medical concerns. ZnO nanoparticles can be produced by different process such as chemical, metallurgical and hydrothermal process. Green synthesis is the simple, environmentally friendly, non-toxic and commonly used method.

- **Jose R. Peralta-Vide *et al*., (2016)** there view includes description of the traditional and green synthesis and applications of metal nanoparticles and highlights the factors limiting the use of plant based synthesis as a real alternative to the traditional synthesis of metal nanoparticles. In this study synthesis of various metal nanoparticles are done with different methods ,it gives different views about metal nanoparticles include gold, silver, copper ,titanium etc., Different characterization studies are done for these nanoparticles and nanoparticles and Nano plates of different forms and size has been reported. More than 50 plant species and several microbial species have been used. Extracts of leaves, flowers, and fruits, as well as severalplant-derived materials, have shown to reduce metals, forming nanostructures. The green-synthesized MNPs have shown similar properties as their chemically synthesized counterparts. However, almost all of the reports have shown a lack of regularity in size and forms of the synthesized MNPs and practically, none of them have quantified the production. Through this study green synthesis has been proposed as an alternative to reduce the use of hazardous compounds and harsh reaction conditions in the production of MNPs.
- **Dilaveez Rehana *et al.*,(2017)** this paper is about the Phyto fabricated green synthesis of zinc oxide (ZnO) nanoparticles using different plant extracts ,and also through chemical method and its characterization is done through different analysis FT-IR, XRD, SEM, TEM The present study also intends to screen α-amylase and α-glucosidase activity of ZnO nanoparticles synthesized using natural sources, which may minimize the toxicity and side effects of the inhibitors used to control diabetes also the anti-oxidant and anti- diabetic properties of zinc oxide nanoparticles are evaluated through this. Different phytochemical screening analysis is carried out for different phytochemical constituents. The results of antioxidant and ant diabetic activity show that the ZnO nanoparticles synthesized using the plant extracts of exhibited higher activity due to the presence of proteins and amino acids along with other phytochemicals constituents. The obtained results are significant, but detailed mechanistic investigations are needed to establish better models for application.
- **Nava *et al.*, (2017)** this work presents a study of the effects on the photo catalytic capabilities of zinc oxide nanoparticles prepared through green synthesis using different fruit peel extracts as reducing agents. Zinc nitrate was used as a source of the zinc ions different known fruit extracts are used for the synthesis. The Synthesized Samples were studied and characterized through Fourier Transform Infrared Spectroscopy (FTIR), X Ray Diffraction (XRD), and High Resolution Transmission Electron Microscopy (HRTEM). These analyses helps to confirm that the synthesised nanoparticles are zinc oxide and their

hexagonal crystal structure can be confirmed. According to the extract used surface morphology of the nanoparticles varies in its size and shape. The photo catalytic properties of the zinc oxide samples were tested through UV light and its degradation of methylene blue dye also studied. As in the peels of different fruit. From the review it is clear that the plant mediated synthesised.

- **Taranath *et al.*, 2015** This review is about the synthesis of Zinc nanoparticles from the aqueous leaf extract of ***Justicia adhatoda*** the characterization of nanoparticles was done by ultraviolet-visible (UV-Vis) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, Atomic force microscopy, These characterizations are help to confirm the nanoparticle and also to find out different properties The FTIR data revealed the possible biomolecules involved in bio reduction and capping of zinc nanoparticles for efficient stabilization. AFM and HR-TEM images have shown that spherical shape of nanoparticle. The biogenic zinc nanoparticle were evaluated for their toxic effect The investigation revealed that then mitotic index (MI) was decreased with increased concentration of zinc nanoparticles and exposure duration. The results revealed that zinc nanoparticles have induced abnormalities, and concluded that the cytotoxicity of zinc nanoparticles is directly proportional to the concentration and duration of exposure.
- **Narendhran *et al.*, 2015** in this study, Zinc oxide (ZnO) nanoparticles were synthesized using aqueous extract of **Lantana aculeate** Linn leaf and assessed their effects on antifungal activity against the plant fungal pathogens. Confirmational analysis was done by ultraviolet–visible spectroscopy, Fourier transform infrared spectrometer, energy-dispersive X-ray spectrometer, X-ray diffractometer, Field emission scanning electron microscopy, high-resolution transmission electron microscopy. The antifungal activity of ZnO nanoparticles were determined using the well diffusion method. Through characterization analyses it is clear that nanoparticles were highly stable and crystalline in nature. These results clearly indicated the benefits of using ZnO nanoparticles synthesized using biological methods and shown to have antifungal activities and also that it can be effectively used as antifungal agent in environmental aspect of agricultural development.
- **Ramaramesh Indra Priyadharshini *et al.*, 2014** in this paper metallic silver (Ag) and zinc oxide (ZnO) nanoparticles are synthesised using the extracts of macro-algae and also examined its anticancer activity against human prostate cancer cell lines the formation of silver nanoparticles and zinc oxide nanoparticles in the reaction mixture was determined and confirmed by ultraviolet-visible spectroscopy. X-ray diffraction, Fourier transform infra-red spectroscopy, energy dispersive X-ray, and field emission scanning electron microscopy

techniques are used for characterization. The silver and zinc oxide nanoparticles were spherical and rod shaped, respectively. The results strongly suggest that the synthesized ZnONPs showed effective anticancer activity againstprostate cancer cell lines than AgNPs.

- **Faezeh Farzaneh *et a*l., 2017** in this study, ZnO nanoparticles were synthesized using metal acetate, egg white as bio-template in water under microwave irradiation followed by calcination. The obtained ZnO nanoparticles are hexagonal structures were characterized by XRD, SEM, TEM, TGA/DSC, FT-IR techniques. The photocatalytic activity of ZnO nanoparticles was evaluated by photocatalytic degradation of organic dye pollutant under UV-light irradiation within 40 min. It was found that, the kinetic of photocatalytic behaviour of ZnO is firstorder. The white product characterized by different techniques as ZnO nanoparticles successfully catalyse the photodegradation. It was found that, the kinetic stability and reusability of the prepared catalyst was considerable. In conclusion, developed a simple, cost-effective and environmentally friendly synthesized method using water soluble egg white protein. .
- **Kavitha et al., 2017** Zinc oxide nanoparticles have been widely employed for various pharmacological applications. Several approaches were tried to synthesize ZnO nanoparticles. In this study, ZnO nanoparticles were biosynthesized using terpenoid fractions isolated from Andrographis paniculata leaves. This nanoparticle preparation has been confirmed by the colour change from green to cloudy-white and the peak at 300 nm by UV–Visible spectra. FTIR analysis showed the presence of functional group (i.e.) C=O which has further been confirmed by H1-NMR studies. From SEM and XRD analysis, it has been found that the hexagonal nanorod Therefore it is suggested that the synthesised Zn-TAP NPs are more suitable in drug delivery.
- **Alagesan Venkateasan 2017** ZnO NPs were efficiently synthesized from the leaves extract of ***Ipomoea pescaprae.*** The work focuses on phytochemical studies, phytomediated synthesis and characterization of ZnO NPs and its biological applications. Phytochemicals present in the extract may be responsible for reducing the metal salts into their respective nanosize particles. FTIR spectrum (ZnONPs) was recorded to identify the functional group of biomolecules involved in synthesis. ZnO NPs in various concentrations revealed potential antioxidant and antibacterial activities. ZnO NPs exhibited efficient dye degradation of methylene blue in the presence of sunlight. ZnO NPs exhibited concentration versatile properties and applications of ZnO NPs; these were widely studied by user-friendly approaches being green, simple and economy. The phytoconstituents such as flavonoids, phenolics, aminoacids, steroids, proteins, carbohydrate and terpenoid presentin the extract

may act as reducing agents for the preparation of ZnO NPs and the capping of ZnO NPs by the phytoconstituents provide stability to ZnO NPs as evident from FTIR studies. ZnO NPs have been prepared by using simple solution method via a green synthesis route. The synthesized ZnO NPs were found to have a crystalline structure with hexagonal wurtzite as evidenced by XRD method. The results proved that the ZnO NPs may serve as a potential antimicrobial agent. The cytotoxic effect ofZnO NPs showed high level of cytotoxic activity against the tested cancer cell lines. Hence, the results together suggest that the synthesized ZnO NPs would play a major role in target-based active molecules into specific cancer cells.

- **Akhilesh Kumar Singh *et al.*, 2017** The present study focused on the green and sustainable synthesis of zinc oxide quantum dots using zinc acetate and ***Eclipta alba*** leaf extract as a reducing agent. The synthesis of ZnO QDs was monitored by ultraviolet visible absorption spectroscopy. The transmission electron microscopy depicted homogeneous distribution of spherical ZnO QDs with mean particle size the selected area electron diffraction analysis revealed crystalline nature of ZnO QDs having a hexagonal wurtzite phase. They have many uses clinical products and fluorescence labeling including the antimicrobial agent. The present biogenic approach for the synthesis of ZnO QDs using biological agents is fast and cost-effective. No chemical reagent or surfactant template was used in this approach that consequently allows the bioprocess with the benefit of being environmental friendly.
- **Hemali Padalia *et al* ., 2017** In the present study, zinc oxide nanoparticles were synthesized using ***Salvadora oleoides*** leaf extract and effect of pH on formation of zincoxide nanoparticles was evaluated. The formation of zinc oxide nanoparticles was characterized by various spectral analysis like UV–Vis spectroscopy, zeta potential, TGA-DTA analysis, Fourier transform infrared spectroscopy, X-ray diffraction, and TEM analysis. The pH affected some characterization parameters distinctly while others were not affected. pH affected the size of nanoparticles synthesized. Zinc oxide nanoparticles exhibited good broad spectrum of antibacterial activity zinc oxide nanoparticles could be developed as antibacterialagents against a wide range of bacteria to control and prevent bacterial infections. The results of the present study suggested that synthesis of ZnO NPs by using ***S. oleoides*** leaf extract is a simple, lowcost, reproducible, and eco-friendly method. In the present work, though size, stability, and antibacterial activity were affected by pH, both particles synthesizedat different pH are functioning as good antibacterial agents.
- **Sangeetha Nagarajan *et al.*, 2013** in the present study by using seaweed, ZnO was rapidly biosynthesized at pH8. TEM analysis shows zinc oxide nanoparticles of different shapes like

spherical, triangle, radial, hexagonal, rod, and rectangle size. After changing temperature mono dispersed single zinc oxide nanoparticles size 36 nm was measured by AFM. Thus this method appears to be a potentially exciting tool for large-scale synthesis of nanoparticles. The findings also revealed that brown seaweeds have the natural potential for the synthesis of nanoparticles Chemical and physical methods of synthesis are costly and require extensive labour and time. Furthermore, large quantities of secondary waste are generated resulting from the addition of chemical agents for precipitation and reduction in the processes. The biosynthetic method employing plant extracts have drawn attention as a simple and viable alternative to chemical and physical methods. The green seaweed ***Calotropis procera*** has been used as zinc oxide nanoparticles. This biological approach appears to be a cost effective alternative to Conventional physical and chemical methods of synthesis.

- **Ramachandran Ishwarya et al., 2017** In this research, the synthesis of zinc nanoparticles was accomplished through new effortless green chemistry process, using the ***Ulva lactuca*** seaweed extract as a reducing and capping agent. It was characterized by powder X-ray diffraction, UV–visible, Fourier transform infrared spectroscopy, selected area electron diffraction analysis and transmission electron microscopy ZnO NPs were tested for their photodegradative action against organic dyes, as well as for antibiofilm and larvicidal activities. Overall, based on our results, we believe that the synthesis of multifunctional U. lactuca-fabricated ZnO NPs usin a widely occurring seaweed product can be promoted as a new potential eco-friendly alternative to chemical methods currently used for nanosynthesis of antimicrobials and mosquito larvicides
- **Ting-Ting Liu *et al*., 2015** zinc oxide nanopowders were synthesized by a sol–gel method with gum tragacanth and zinc nitrate as raw materials. Gum tragacanth was used as stabilizer to control the mobility of zinc cations and the growth of the nanopowders. Thermo-gravimetric analysis, x-ray diffraction, Fourier-transform infrared spectroscopy, transmission electron microscopy, energy dispersive x-ray spectroscopy, and scanning electron microscopy were used to characterize the as-prepared samples. This method can be used as an excellent alternative method for synthesis of ZnO nanoparticles with a plant extract as a raw material. Our experimental results show our method had the advantage of improving the electrical performance of ZnO varistors. ZnO nanoparticles with a narrow size range were synthesized by use of a gum tragacanth-mediated sol–gel method in aqueous solution. Gum tragacanth was used to terminate the growth of, and stabilize, the ZnO nanoparticles. Samples had a hexagonal ZnO lattice structure and the width of diffraction

peaks increased with increasing calcination temperature. The stable residues were ZnO nanoparticles.. Our results indicated gum tragacanth mediated synthesis led to a ZnO varistor with a high breakdown voltage which may be suitable for fabrication of voltage-switching devices in the near future.

- **Snehal Yedurkar et al., 2016** Green synthesis of metal oxide nanoparticles using plant extract is a promising alternative to traditional method of chemical synthesis. In this paper, we report the synthesis of nanostructured zinc oxide particles by biological method. Highly stable and spherical zinc oxide nanoparticles are produced by using zinc acetate and ***Ixora coccinea*** leaf extract. Formation of zinc oxide nanoparticles has been confirmed by UV-Vis absorption spectroscopy, X-ray diffraction, Fourier transform infrared spectroscopy, Dynamic light scattering analysis, zeta potential study and Scanning Electron Microscope with the Energy Dispersive X-ray studies. Dynamic light scattering analysis shows average particle size of nm whereas high zeta potential value confirms the stability of formed zinc oxide nanoparticles. The Scanning Electron Microscope reveals spherical morphology of nanoparticles and Energy Dispersive X-ray analysis confirms the formation of highly pure zinc oxide nanoparticle. It have applications in biomedical, cosmetic industries, biotechnology, sensors, medical, catalysis, optical device, coatings, drug delivery and water remediation, and also may be applied for electronic and magneto-electric devices. This new eco-friendly approach of synthesis is a novel, cheap, and convenient technique suitable for large scale commercial production. The rapid biological synthesis of zinc oxide nanoparticles using leaf extract of ***Ixora coccinea*** provides an environmental friendly, simple and efficient route for synthesis of nanoparticles. Zinc oxide nanoparticles have been successfully synthesized by using this method.
- **Sadhan Kumar Chaudhuri, *et al* ., 2017**, In this paper ,Green synthesis of zinc oxide nanoparticles was carried out using ***Calotropis*** leaf extract with zinc acetate salt in the presence of 2 M NaOH. Synthesized nanoparticles were characterized through UV–Vis spectroscopy, dynamic light scattering (DLS), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), EDX (energy dispersive X-ray), and AFM (atomic force microscopy). Effects of biogenic zinc oxide (ZnO) nanoparticles on growth and development of tree seedlings in nursery stage were studied in open-air trenches. The UV–Vis absorption maxima showed peak near 350 nm, which is characteristic of ZnO nanoparticles. Green-synthesized nanoparticles have a potential role in the form of nanofertilizers in the current scenario; this work may help in future development of nanonutrients for plant growth and development. This type of

nanofertilizer is a plant nutrient which is more than a conventionally used fertilizer because it not only supplies nutrients for the plant but also revives the soil to stay in organic state without any harmful factors of chemical fertilizers. One of the advantages of nanofertilizers is that they can be in very small amounts compared to chemical fertilizers. Nanopowders can be successfully used as fertilizers and pesticides as well for increased agricultural crop productivity. In the field of crop plant growth and yield, the role of various types of nanomaterials gradually increased the formulation of nanonutrients using nanoparticles and their foliar spraying in nursery seedlings may enhance the growth and development.

- **Sidra Sabir, *et al.*, 2014** in this paper discuss about zinc oxide nanoparticles are very much important due to their utilization in gas sensors, biosensors, cosmetics, drug-delivery systems, and so forth. As far as method of formation is concerned, ZnO NPs can be synthesized by several chemical methods such as precipitation method, vapor transport method, and hydrothermal process. The biogenic synthesis of ZnO NPs by using different plant extracts is also common nowadays. This green synthesis is quite safe and ecofriendly compared to chemical synthesis. This paper elaborates the synthesis, properties, and applications of zinc oxide nanoparticles. The green synthesis of ZnO NPs is much safer and environment friendly compared to chemical synthesis because it does not lead to formation of toxic byproduct chemicals. As far as synthesis of zinc oxide nanoparticles is concerned they can be synthesized by chemical methods but in recent times due to evolution of green chemistry, biogenic synthesis of ZnO NPs is also possible by using different plant extracts. The green synthesis of ZnO NPs is much safer and environment friendly compared to chemical synthesis because it does not lead to formation of toxic by product chemicals. As far as their usage is concerned nanoparticles play a significant role in agriculture, where colloidal solution of ZnO NPs is used in nanofertilizers. Application of these nanoparticles to crops increases their growth and yield.
- **Sorna Prema Rajendran *et al.*, 2017** the objectives of this present study are to synthesize iron oxide and zinc oxide nanoparticles from different concentrations of ***Sesbania grandiflora*** leaf extract using zinc nitrate and ferrous chloride as precursor materials and synthesized nanoparticles were characterized by different methods The SEM results reveal that zinc oxide nanoparticles were spherical in shape Application of synthesized nanoparticle on seafood effluent treatment was studied. Green synthesis of zinc oxide and iron oxide nanoparticles using ***Sesbania grandiflora*** leaf extract provides an effective route for eco-friendly method of synthesis of nanoparticles. SEM analysis showed that the aggregation and network formation of nanoparticles had been taking place. It was found that

increasing the leaf extract concentration increases the rate of reduction and the reduction of precursor into the nanoparticles.

- **Ayesha Naveed Ul Haq et al.,2017** among a variety of nanoparticles, zinc oxide nanoparticles have advantages because of the extraordinary physical and chemical properties. The current review illustrates a comparison between pollutants and hazards spawned from chemical, physical, and biological methods used for the synthesis of ZnO. Further, the emphasis is on devising eco-friendly techniques for the synthesis of ZnO especially biological methods which are comparatively less hazardous and need to be optimized by controlling the reaction conditions in order to get desired yield and characteristics. Referring to the varied and widespread use of ZnO in research and industry Chemical synthesis as evident from the name relies on intensive consumption of the chemicals, which are exhausted to the environment as waste material contributing to anthropogenic waste. Techniques employed for the chemical synthesis have their own limitations and drawbacks which can be dealt with green synthesis (plants, microbes, and waste) of ZnO, which offers a relatively pollution free mechanism but optimization of reaction conditions to get higher yield, desired characteristics, and instability put a limitation on its use. It is unpredictable to gauge stability of biosynthesized ZnO which may otherwise cause serious damage to biological systems. Thus, there is a need to devise and optimize reaction mechanism and techniques for both chemical and green synthesis.
- **Prasanta Sutradhar *et al*., 2015** With an increasing awareness of green and clean energy, zinc oxide-based solar cells were found to be suitable candidates for cost-effective and environmentally friendly energy conversion devices. In this paper, we have reported the green synthesis of zinc oxide nanoparticles by thermal method and under microwave irradiation using the aqueous extract of tomatoes as non-toxic and ecofriendly reducing material. The synthesised ZnONPs were characterised by UV-Visible spectroscopy, Infra-red spectroscopy, Particle size analyser, Scanning electron microscopy, Atomic force microscopy and X-ray diffraction study. A series of ZnO nanocomposites with titanium dioxide nanoparticles and graphene oxide were prepared for photovoltaic application. A facile approach has been reported using tomato extract, acting as reducing agent for the synthesis of ZnONPs of well-defined dimensions in bulk amount. This eliminated the need of toxic chemicals for the synthesis of nanoparticles. Besides, excellent reproducibility of these nanoparticles, without the use of any additional capping agent or stabiliser will have a great advantage in comparison with microbial synthesis, avoiding all the tedious and hygienic complications. To optimise the conditions, the synthesis has been done by thermal

method as well as under microwave irradiation using different power and the synthesised nanoparticles was successfully used to prepare nanocomposites for photovoltaic application.

- **Geetha M. Pinto *et al.*, 2016,** ZnO nanoparticles have multiple properties that are useful for biomedical applications. Reduction of materials to nanoscale offers advantage of developing new anticancer drugs. Notable application among them is its inherent anticancer cytotoxicity actions. ZnO particles can be prepared easily by different chemical, physical, and biological approaches. But the biological approach is the most emerging approach of preparation, because, this method is easier than the other methods, ecofriendly and less time consuming. The Green synthesis was done by using the aqueous solution of Ocimum tenuiflorum (Tulsi), Phyllanthus emblica (Gooseberry), Azadirachta indica (Neem) extract and zinc nitrate. The nanoparticles were characterized by different techniques, and their anti-microbial activity evaluated.
- **Smita Asthana *et al.*, 2016** The present study reports the synthesis of zinc nanoparticles, Zinc nanobeads. The nanoparticles synthesised were characterised by UV visible spectroscopy, colour change, pH change and particle size analysis. The nanoparticles synthesised were spherical and uniform in size with an average diameter of about 56. 8nm which was determined by dynamic light scattering method. The synthesised nanobeads were used efficiently for alizarin red degradation. In the present preliminarily investigatory study the nanobeads were applied for removal of alizarin red dye waste water.
- **Yin Zhang *et al.*, 2013** has been investigated for a wide variety of applications. One of the most important features of ZnO nanomaterials is low toxicity and biodegradability In this review article, we summarized the current status of the use of ZnO nanomaterials for biomedical applications, such as biomedical imaging (which includes fluorescence, magnetic resonance, positron emission tomography, as well as dual-modality imaging), drug delivery, gene delivery and biosensing of a wide array of molecules of interest. Research in biomedical applications of ZnO nanomaterials will continue to flourish over the next decade and much research effort will be needed to develop biocompatible/biodegradable With many attractive physicochemical properties and tremendous potential for various biomedical applications, ZnO nanomaterials are excellent candidates as biocompatible, biodegradable, "deliver and dissolve" nano platforms for cancer targeted imaging and therapy. Even though this research area is still nascent, various ZnO nanomaterials have already been evaluated for optical imaging and MRI in cells, as well as dual-modality MRI/optical imaging.
- **Saputra *et al.*, 2004** ZnO nanoparticles (ZnO NPs) were biosynthesized. The growth was observed by a sol-gel method. ZnO were successfully formed through the reaction of zinc

nitrate tetrahydrate Zn $(NO_3)_2.4H_2O$ precursor with aqueous leaf extract of ***Imperata cylindrica*** (ICL). The structural and optical properties of ZnO were investigated. The as-synthesized products were characterized by UV-Visible (UV-Vis), UV diffuse reflectance spectroscopy (UV-DRS), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDS). UV-Vis absorption data showed hydrolysis and characteristic of absorption peak at 300 nm of Zn $(OH)_2$. UV-DRS confirmed that ZnO NPs has the indirect band gap at 3.13eV. FTIR spectrum revealed the functional groups and indicated the presence of protein as the capping and stabilizing agent on the ZnO surface. Powder XRD studies indicated the formation of pure wurtzite hexagonal structure with particle size of 11.9 nm. The detailed morphological and structural characterizations revealed that the synthesized products were hexagonal nanochip. The ZnO NPs were successfully biosynthesized by sol-gel method using ***Imperata cylindrica*** *L*. leaf extract as base source and stabilizing agent. Hydrolysis process showed the characteristic absorption peak of Zn $(OH)_2$ at λmax 300 nm and UV-Vis-DRS data confirmed the indirect band gap at 3.13 eV. FTIR spectra revealed the functional groups of stretching bands of ZnO NPs around 600-400 cm-1. The XRD result showed the sample was well crystallized in a hexagonal wurtzite structure with crystallite size of 11.9 nm. SEM-EDS analysis showed the morphology of hexagonal nanochip with the compositions of Zn and O elements.

- **Sagar Raut *et a*l., 2013** In this research paper, we discussed on the synthesis and characterization of ZnO nanoparticles by green synthesis method. Here we utilized the leaves of ***Ocimum Tenuiflorum*** plant as reducing agent in the synthesis of ZnO nanoparticles. Green synthesis method avoids inert gases, high pressure, laser radiation, high temperature, toxic chemicals etc. as compared to conventional method like sol-gel technique method, laser ablation method, solvothermal method, inert gas condensation method, chemical reduction method etc. Prepared ZnO nanoparticles were characterized by X-Ray diffraction (XRD), scanning electron microscope (SEM) technique, Fourier Transform Infrared Spectroscopy (FTIR). The average particle is calculated as 13.86nm by using Scherrer's formula. In summary, ZnO nanoparticles are prepared with help of green synthesis method by using leaves extract of Ocimum Tenuiforum. Green synthesis methods are simple, non toxic, rapid method. The size and structure of nanoparticles is confirmed with the XRD technique. The synthesized ZnO average particle size is calculated as 13.86 nm by using Scherrer's Formula. The SEM image showed hexagonal shape nanoparticle formed with diameter range 11-25 nm. The characteristic peak of ZnO at 668.29 in IR absorption spectra is also noticed and carboxylic acid group, alkynes group, o-amino group are present in leaves extract of ocimum tenuiflorum. These ZnO nanoparticles can be used in various industrial applications like active medium for lasers, luminescent material for fluorescent tubes, paints, and so forth.

- **P. Ramesh *et al.,* 2014** Presently the progress of green chemistry in the synthesis of nanoparticles with the use of plants has engrossed a great attention. Zinc oxide nanoparticles have received potential interest due to their vast applications in the food industry. For such purpose, the development of novel and biological techniques is inconsiderable demand for raising these materials to an industrial level. This letter portrays a novel method for the biosynthesis of ZnO nanoparticles using a *Citrus aurantifolia* for the first time. The morphology structure and stability of the synthesized ZnO nanoparticles was studied using scanning electron microscope, UV-spectro photometer and Fourier Transform Infrared spectroscopy. The results depicted that the synthesized nanoparticles are moderately stable, roughly spherical shape .To conclude we have used unreported, inexpensive, nontoxic, ecofriendly and abundantly available ***Citrus aurantifolia*** for the rapid synthesis of ZnO NPs .This green synthesis approach shows that the environmentally benign and renewable *Citrus aurantifolia* extract canbe used as an effective stabilizing as well a sreducing agent for the synthesis of zinc oxide nanoparticles.

3. MATERIALS AND METHODS

Zinc oxide nanoparticles were synthesised through two methods ,the first one is through chemical method and the second one is green synthesis using plant extract .Zinc oxide ,starch and sodium hydroxide were used for chemical synthesis and zinc acetatedihydrate, Plant extract ,sodium hydroxide and ethanol were used for green synthesis .*Bauhinia variegata*leaves were collected from our university campus and confirmed the leaves with the help of botany department and they dried for one week .

3.1 Synthesis of ZnO nanoparticles

3.1.1. Chemical method

20ml of 0.1 M zinc oxide was taken in a conical flask .To the solution added 0.1% of starch solution (prepared by dissolving 0.01g of starch in 10ml of water).The above solution was stirred for 1hr on a magnetic stirrer, then added 2ml of 0.2M NaOH and kept in incubation for 24 hrs.

3.1.2 Green synthesis

Green synthesis of zinc oxide nanoparticles were done with *Bauhinia variegata* leaves extract.

3.1.2.1 Preparation of leaf extract

Fresh leaves of *Bauhinia variegata* were thoroughly cleaned with running tap water to remove debris and other contaminations, followed by distilled water and air dried at room temperature .Leaves were finely chopped into small pieces .The aqueous extract of sample was prepared by boiling the freshly collected cut leaves (10g), with 100 ml of distilled water, at 60◦c for about 20 minutes, until the colour of the aqueous solution changes from watery to light brown.

Then the extract was cooled to room temperature and filtered using what Mann filter paper. The extract was stored in a refrigerator in order to be used for further experiments

3.1.2.2 Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per standard methods **(Prashant Tiwari, *et al*, Jan-March 2011).**

1. Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

b) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c) Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

b) Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

c) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides:

Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

4. Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

5. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

b) Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

6. Detection of phytosterols

a) Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of 2golden yellow colour indicates the presence of triterpenes.

b) Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

7. Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

8. Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

9. Detection of flavonoids

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

10. Detection of proteins and aminoacids

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

b) Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid

3.1.2.3 ZnO Synthesis from plant extract

For the synthesis of ZnO nano particles,50ml of 0.5M zinc acetate dehydrate solution was prepared using distilled water.1ml of aqueous leaf extract was introduced into the above solution after 10 minutes stirring . In order to maintain the pH 12 NaOH solution was added drop by drop, which resulted in a pale white aqueous solution .This was then placed in a magnetic stirrer for 2 hrs. The pale white precipitate was then taken out and washed over and over with distilled water and then with ethanol to remove impurities.

Then a pale white powder of ZnO nanoparticles was obtained after drying in oven. **(Snehal yedurkar *et al.*, 2016.)**

3.2 Characterization of Nano particles

Characterization of nanoparticles was done by different methods. UV-visible spectral analysis was used to analyse the absorbance .Fourier transform infra spectroscopy (FT-IR) gives specific signals for NPs .shape of the nanoparticle were characterized by scanning electron microscopy, thermo gravimetric analysis, LASER analysis is used to topography and roughness of nanoparticles.

- **UV-visible analysis**

UV-visible spectroscopy is usually conducted to confirm the synthesis of ZnO NPs Peaks. Which studied in the range of 200-400 nm. Different peaks are obtained in this range. Absorbance also obtained through this analysis. Conducting electrons start oscillating at a certain wave length range due to surface Plasmon resonance. **(Santhosh Kumar j. *et al*., 2017)**

- **FT-IR analysis**

Specific signals obtained by IR spectroscopy according to the vibrations of the molecule. FT-IR spectra and functional group involved in ZnO NPs synthesis illustrated peak in the range of 500-4000cm^{-1}.The sample pellet was placed into the sample holder and FT-IR spectra were recorded in FT-IR spectroscopy.

- **SEM Analysis**

SEM analysis is used to visualize shape and size of nanoparticles. Scanning electron microscope was adjusted in different magnification and used to determine the shape of ZnO NPs.SEM images in different magnification ranges can be evaluated. **(SanthoshKumar *et al*., 2017).** SEM image was usually used to study the morphology of synthesised nanoparticles **(Kalpana handore. et al., 2014)**

- **3D Optical Profilometry**

LASER and AFM analyses are given us insight about the topography, roughness of nanoparticles. LASER imaging was conducted in different magnification range image clearly demonstrate smooth nanoparticle with capping of phyto- chemicals over the surface of nanoparticle **(Santhosh Kumar j. *et al*., 2017)**

It is commonly used for obtaining colloidal solution of nanoparticles. This analysis also helps in determine the morphology and surface studies.

- **Thermo gravimetric analysis**

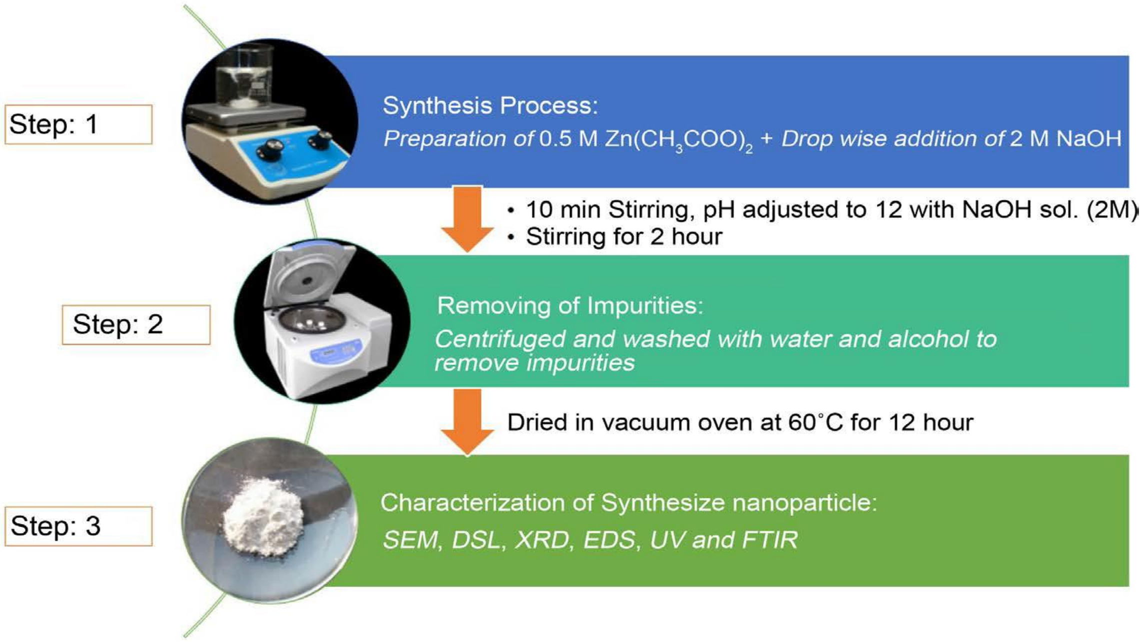
TGA is a method of thermal analysis in which changes in physical and chemical properties of materials are measured as a function of increasing temperature or as a function of time with constant temperature. It is a temperature based study.

- **Anti-microbial activity studies**

Agar disc diffusion method is used for antimicrobial stusies and by this technique we can say that ZnO nanoparticles are how much effective to the microbes like *E-coli, K-pneumoniae, s-aureus.*

These NPs exhibit antifungal, anticorrosive, antibacterial properties due to increased specific surface area, reduced particle size, enhanced particle surface reactivity and abrasive surface texture or surface defect ZnO NPs have recently shown to provide effective pathogen growth control as they have lower toxicity as well as positive impact on soil fertility. The chemically synthesized ZnO NPs have been found to be convincing antibacterial agents because they have the ability to absorb UV radiation and the inhibition efficiency of microbes studied.

The cultures were stocked at 4˚C after sub culturing incubation of 24hrs at 37˚C in incubator. Mueller hinton agar were prepared and sterilized at 121˚C for 15 minutes the anti-bacterial assays were carried out the agar well-diffusion method **(Jesteena *et al*., 2017)** Mueller Hinton agar plate were prepared and after solidification 60µl of culture were poured and spreaded with sterile cotton swab and kept for drying 2-3 minutes .Wells were made with cork borer in the diameter of 5mm and added the sample separately around 50µl in each well. All the plates were incubated at 37˚Cfor 24hrs. The diameters of inhibition zone produced by the extract were measured in mm after the incubation period.



3.1. Work plan of the synthesis (Snehal yedurkar, et *al*., 2016)

4. RESULT AND DISCUSSION

Many metal oxide nanoparticles are synthesised through different method using different green medium, ZnO NPs have attracted great attention because of their superior optical properties and its applications in different fields like medicine agricultural etc., and Visual colour change is the preliminary test for nanoparticle synthesis.

The present investigation dealt with **"*Bauhinia variegata* leaves mediated green synthesis of Zinc Oxide Nanoparticles and its antimicrobial activity"**. In this study, Zinc oxide nanoparticles were synthesised from *Bauhinia variegata* leaves extract. Characterization studies were carried out with the synthesised nanoparticles. Anti-microbial activity of the zinc oxide nanoparticles was also studied using three different microbes. The synthesis of zinc oxide nanoparticles was carried out using 2 different synthesis methods.

- ❖ Chemical method
- ❖ Plant mediated synthesis

4.1 Synthesis of ZnO NP Using Chemical method (ZnONP-C)

20ml of 0.1 M zinc oxide was taken in a conical flask. To the solution added 0.1% of starch solution (Prepared by dissolving 0.01g of starch in 10ml of water). The above solution was stirred for 1hr on a magnetic stirrer, then added 2ml of 0.2M NaOH and kept in incubation for 24 hrs.

4.1.1Characterization

The characterization of ZnO NP-C was carried out using the methods

- ➢ Optical Characterization
  - ❖ UV –Visible analysis
- ➢ Thermal Characterization
  - ❖ TGA analysis
- ➢ Surface characterization
  - ❖ FT-IR analysis
  - ❖ 3D Optical Profilometry
  - ❖ SEM

4.1.2 Visual observation

The chemically synthesised ZnO NPs was white in colour and the yield of product is 200mg.

Fig: 4.1 Chemically synthesised ZnO NPs

4.1.3 Optical characterization

UV –visible analysis

UV-visible spectroscopy is usually conducted to confirm the synthesis of ZnO NPs. Conducting electrons started oscillating at a certain wavelength range due to **Surface Plasmon resonance** (SPR) effect which represented the UV-visible spectra of freshly prepared ZnO NPs.

Surface Plasmon resonance is the resonant oscillation of conduction electrons at the interface between negative and positive permittivity material stimulated by incident light .SPR is the basis of many standard tools for measuring adsorption of material onto planar metal surfaces or onto the surface of metal nanoparticles.

UV–VIS absorption spectrum of ZnO NP synthesized by chemical reduction method using 0.1% starch as stabilizing agent. The absorption spectrum was recorded for the sample in the range of 200-400 nm. The peaks were found in 340nm and 380 nm.

The spectrum showed the absorption peak at 340nm corresponding to the characteristic band for zinc oxide nanoparticles. **(Deependra Kumar Ban *et al.*, Snehalyedukar *et al.*, 2016).** Peak obtained at 380 nm clearly demonstrated the presence of ZnO NPs in the reactionmixture. **(Santhoshkumar *et al.*, 2017)**

The particle diameter obtained from the spectrum = 11.99nm.

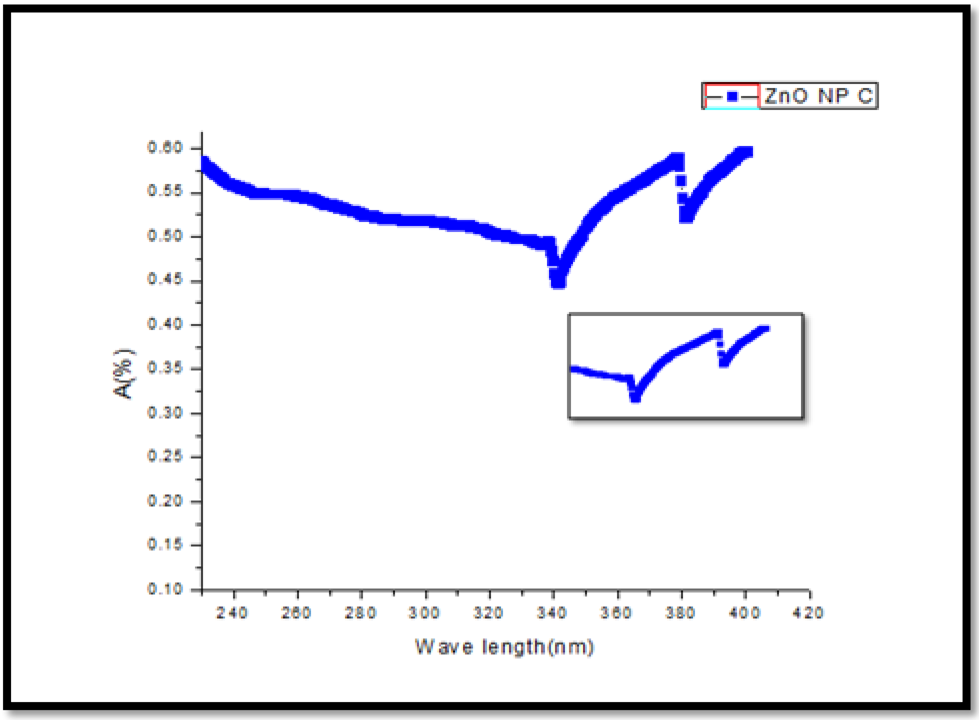


Fig: 4.2 UV Spectrum of chemically synthesised ZnO NPs;

Inset Surface Plasmon Resonance

4.1.4 Thermal characterization

Thermo gravimetric analysis

Thermal gravimetric and differential thermal analyses indicate the thermal stability of nanoparticles within the temperature range 0 to 600 °C. **(HemaliPadalia et al., 2017)**
The amount and rate of weight changes in a material are measured by using TGA either as a function of temperature with increasing temperature or isothermally as a function of time. It is used to characterize any material which exhibits a weight change and detects phase changes due to oxidation, decomposition or dehydration. It is connected to a computer which acts as a controller to control the functions like to run data analysis 46 programs, store experimental data and to setup and control experiments.

The thermo gravimetric analysis of ZnO was performed on a Perkin–Elmer Thermo gravimetric analyser and showed a loss of weight due to the loss of water and a continuous loss of weight for ZnO nanoparticles. **(Elizabeth Varghese *et al*., 2015)**

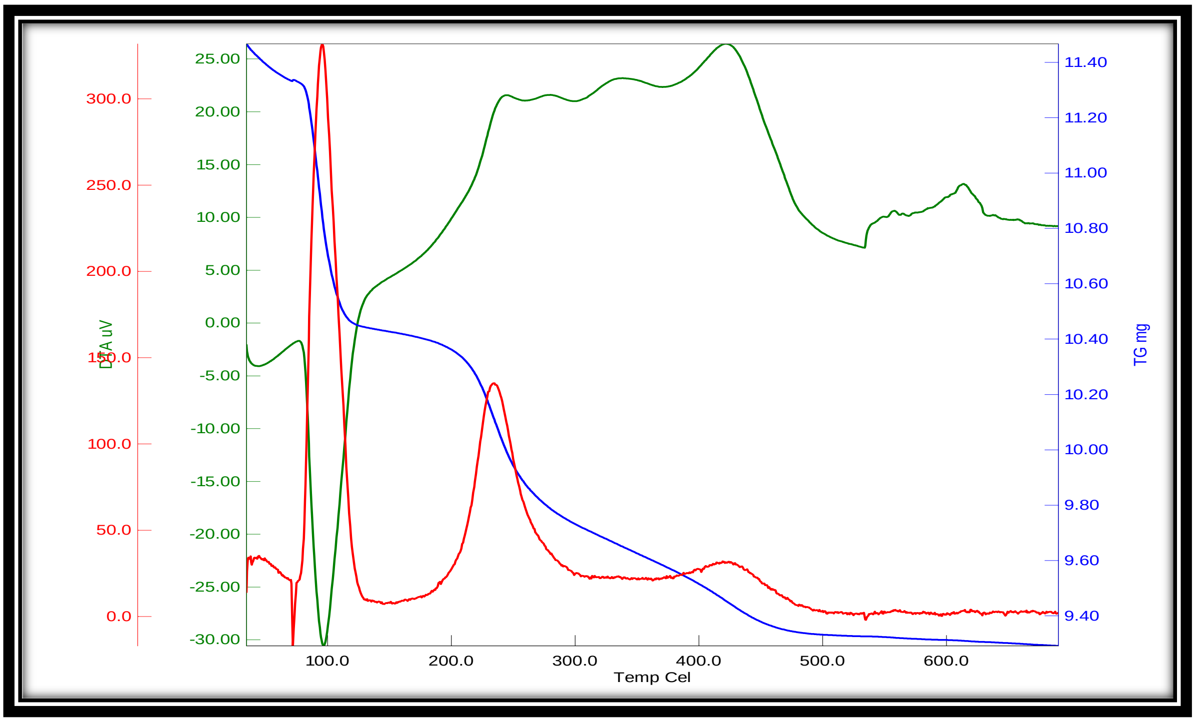


Fig: 4.3 TGA image of chemically synthesised ZnO NPs

Surface characterization

4.1.5 FTIR analysis

FT-IR Spectral studies gave informations regarding chemical bonding between Zn and O. FT -IR is an effective method to reveal the composition of the product.

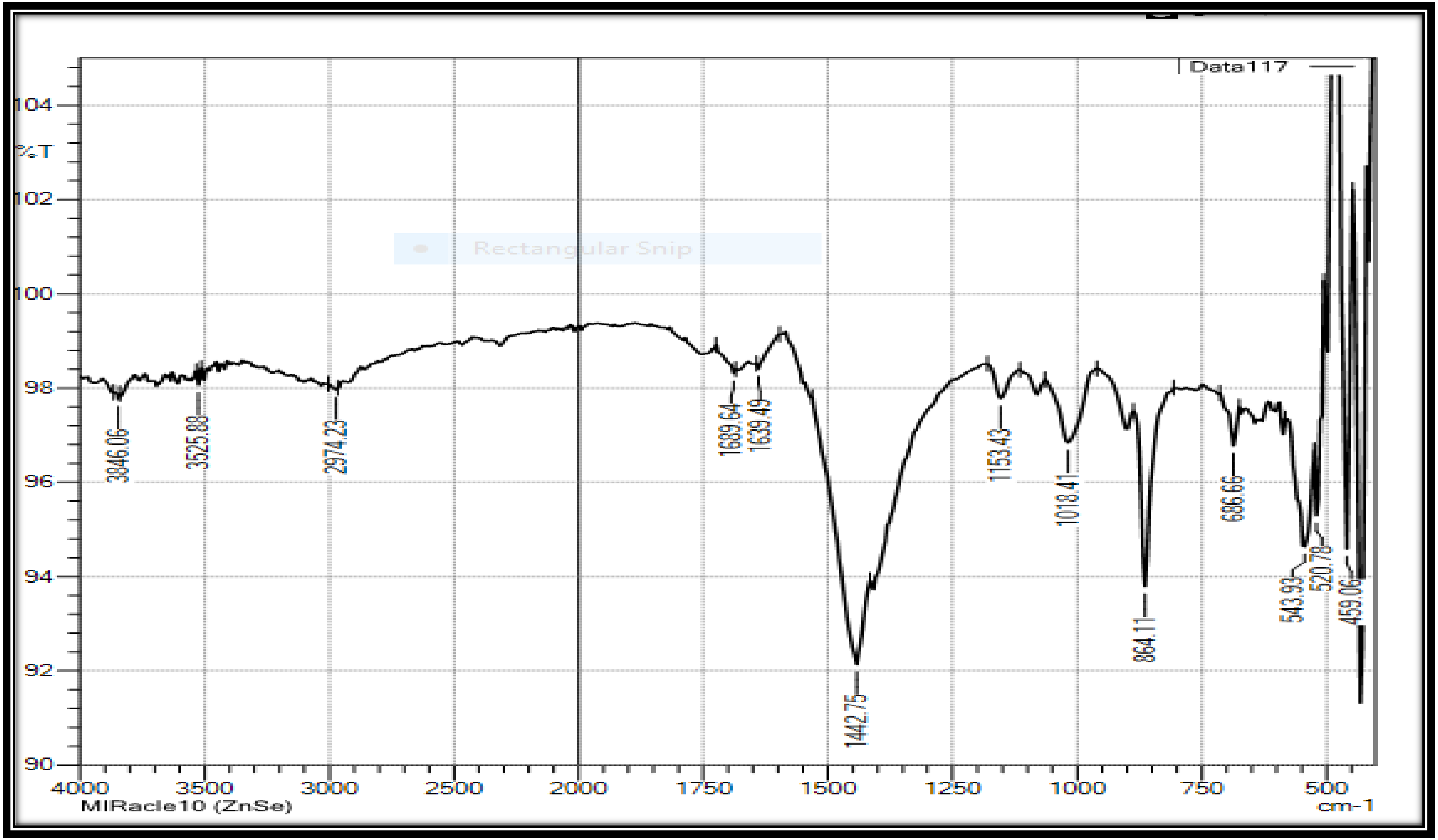


Fig: 4.4 FTIR Image of chemically synthesised ZnO NPs

Vibration frequency range	**Functional group**
3000-4000 cm^{-1}	OH stretching vibration
1400-1600 cm^{-1}	C=C stretching
1018,864 cm^{-1}	C-O stretching
650 and 543 cm^{-1}	Zn-O Bond

Table 4.1 FT-IR Spectral frequency range and assigned functional group

The peak obtained at 3525- 3846 cm^{-1} demonstrated the OH stretching vibration and are narrow band at 1442 cm^{-1} range demonstrated the C=C stretching in aromatic ring and a band is obtained in the range of 864 cm^{-1} indicated the C-O stretching. A very small peak obtained at 650 cm^{-1} and 543cm^{-1} demonstrated the probale presence of C-Alkyl chloride and Hexagonal phase

4.1.6. 3D Optical Profilometer

Optical profilometer studies give us insight about the topography, roughness of nanoparticles. Images were clearly demonstrated the smoothness of nanoparticles.

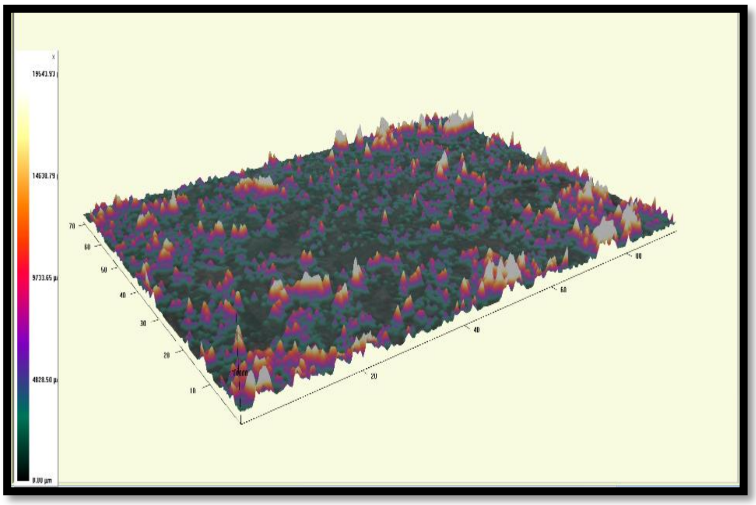


Fig: 4.5 3D Optical profilometer image of ZnONP-C

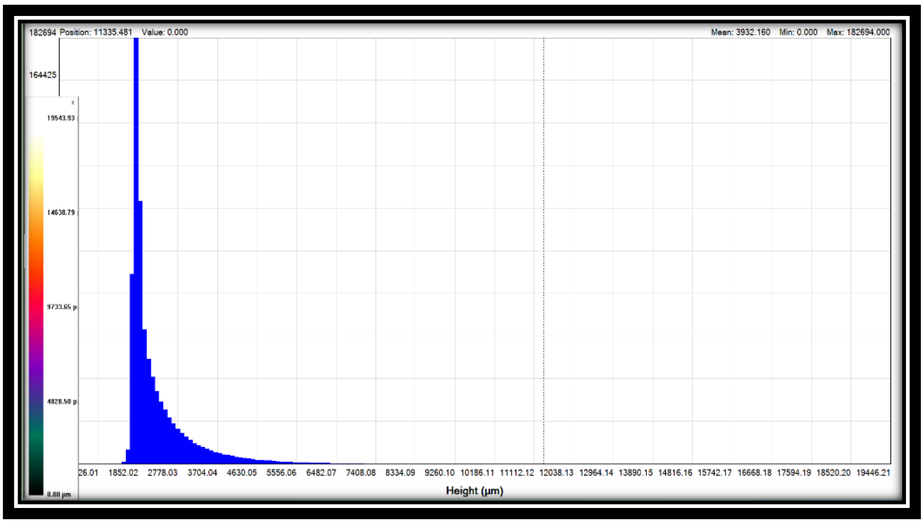


Fig: 4.6 Histogram image of ZnONP-C

Sample	Ra	Rq
ZnONP-C	378.92	517

Table 4.2 3D Optical Profilometer-ZnONP-C

4.2. Plant mediated synthesised ZnO NPs (ZnO NP-BVL)

The green synthesis of nanoparticles greatly reduced the use of physical and chemical methods. Various chemical methods have been proposed for the synthesis of zinc oxide nanoparticles (ZnO NPs), such as reaction of zinc with alcohol, vapour transport, hydrothermal synthesis, precipitation method etc. The use of green synthesis method by the researchers is rapidly increasing due to usage of less toxic chemicals, eco-friendly nature and one step synthesis of nanoparticles. The biological system involved in the green synthesis of nanoparticles is plants and their derivatives, microorganisms like bacteria, fungi, algae, yeast **(Santhoshkumar.j*et al*., 2017).** Here the synthesis was done with the leaf extract of *Bauhinia variegata*, a locally available flowering plant in the species of Fabaceae. Its common name is kachnar.

Phyto chemical screening of crude BVL extract

Phyto constituents	Alkaloids	Vit C	Flavonoids	Saponins	Amino acids	Fats &oils	Phytosterol	Tannins	Carbohydrates	Terpenoids
Results	+	-	+	-	+	-	-	+	+	-

+ present; - absent

Table 4.3 Phyto chemical screening of crude BVL extract

The results indicated the presence of phytochemical constituents like flavonoid, tannins, Alkaloids, Carbohydrate, terpenoids. In the BVL extract .Presence of above constituents and based on literature *Bauhinia variegata* shows pharmacological actions like chemoprotective antitumor activity, anti-inflammatory, anti-diabetic, antioxidant and etc., The FT-IR spectrum of *Bauhinia variegata* leaf extract is as shown. UV spectrum and FT-IR spectrum of *Bauhinia variegata* leaf extract is as shown.

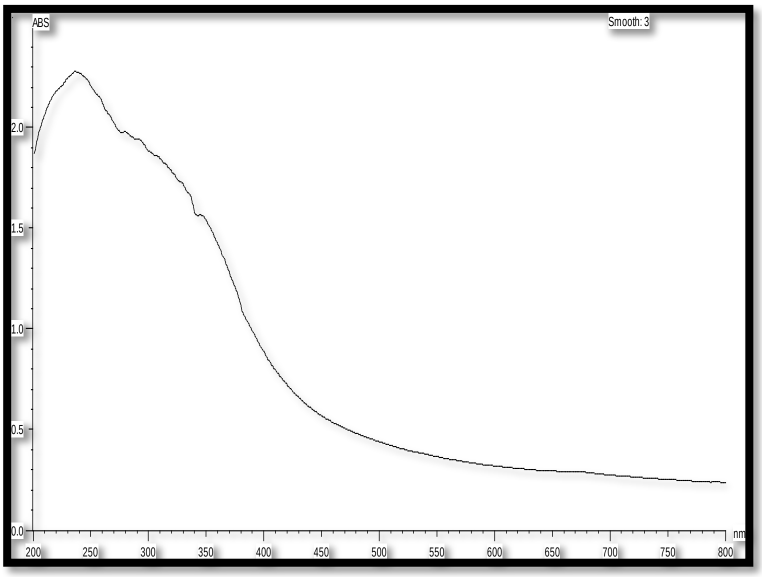


Fig: 4.7 UV spectrum of BVL Extract

The UV –Visible spectrum of BVL extract was recorded in the range 200-800, the peak obtained at 250nm

FT-IR Spectrum showed the peaks in the following ranges.

Vibration frequency range	**Functional group**
3000-4000 cm^{-1}	OH stretching vibration
1400-1600 cm^{-1}	C=C stretching
1018,864 cm^{-1}	C-O stretching

Table 4.4 FT-IR Spectral frequency assigned functional groups for BVL Extract

The peaks obtained at 1512 $cm^{-1}$was due to C=C stretching,Weaks peaks are obtained at 590,1029,1643,3838cm^{-1}

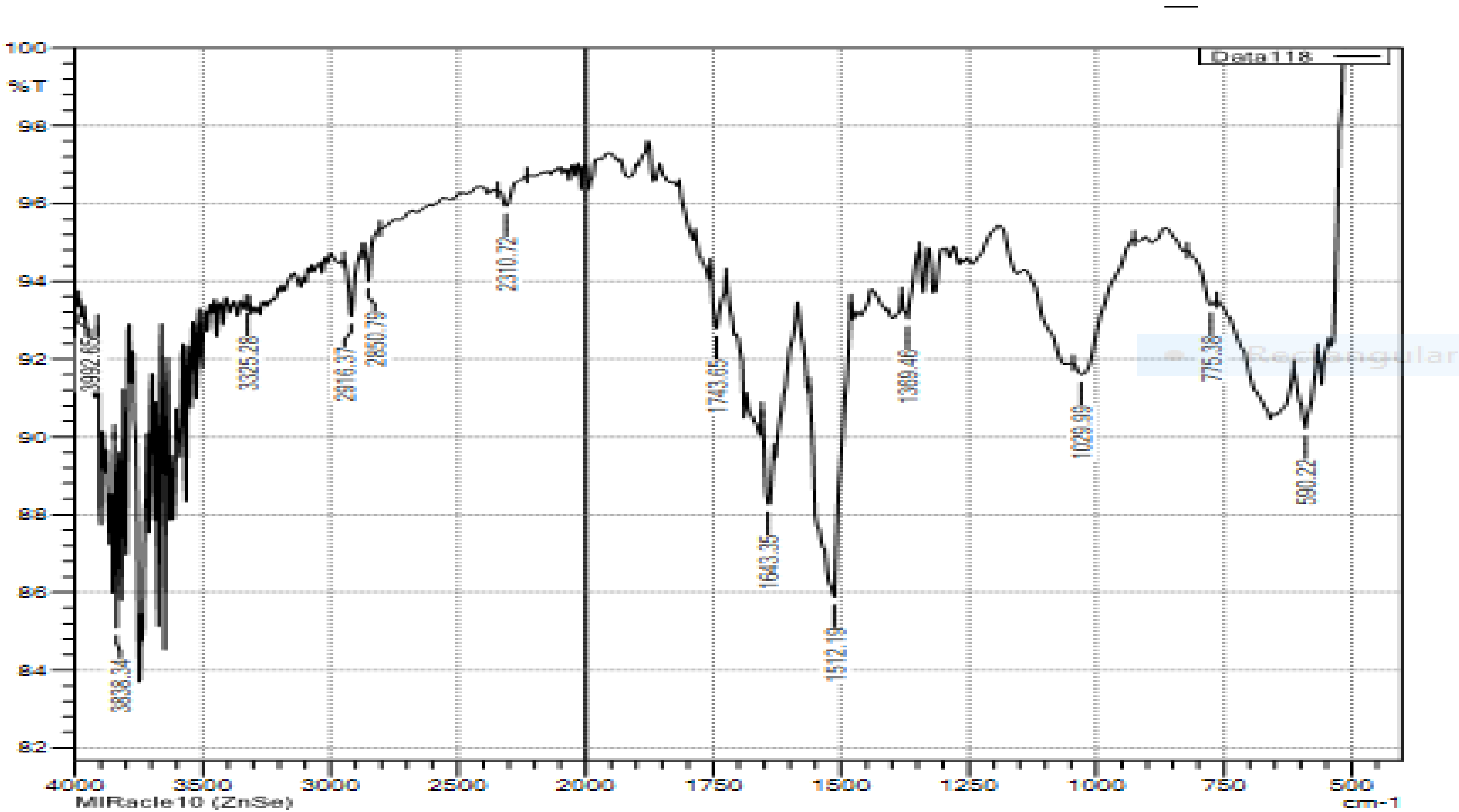


Fig: 4.8 FTIR Spectrum of BVL extract

4.2.1 Visual observation

The ZnO NPs synthesized using *Bauhinia variegata* leaves extract shows a colour changes from half white to pale yellow represents the synthesis of ZnO NPs after drying for long time the colour again changes to pale white. **(santhosh kumar *et al*., 2017)**

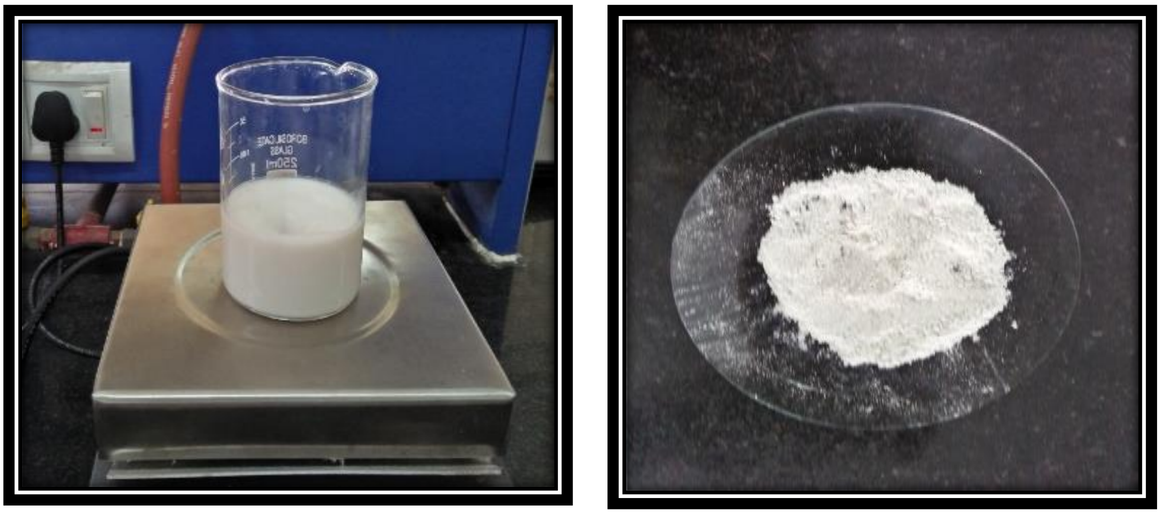


Fig: 4.9 *Bauhinia variegata* mediated synthesised ZnO nanoparticles

4.2.2 Optical Characterization

UV –visible analysis

The optical absorption spectra of zinc oxide nanoparticles were recorded using UV/VIS 3000+ Double Beam UV Visible Ratio-Recording Scanning Spectrophotometer UV-Vis absorption spectrum of zinc oxide nanoparticles. The absorption spectrum was recorded for the sample in therange of 200 - 400 nm. The spectrum showed the absorbance peak at 340 nm corresponding to the characteristic band of zinc oxide nanoparticles **(SnehalYedurkar *et al*., (2016)**

UV-visible spectroscopy is usually conducted to confirm the synthesis of ZnO NPs. Conducting electrons start oscillating at a certain wavelength range due to surface plasmon resonance (SPR) effect. (Fig: 4.7) represents the UV-visible spectra of freshly prepared ZnO NPs. Peak obtained at 380 nm clearly demonstrates the presence of ZnO NPs in the reaction mixture. **(Santhoshkumar *et al*., 2017).** This characteristic band may be attributed to the intrinsic band – gap absorption of ZnO nanoparticles owing to the e^- transition from the valence band to the conduction band ($O_{2p} \longrightarrow Zn_{3d}$).

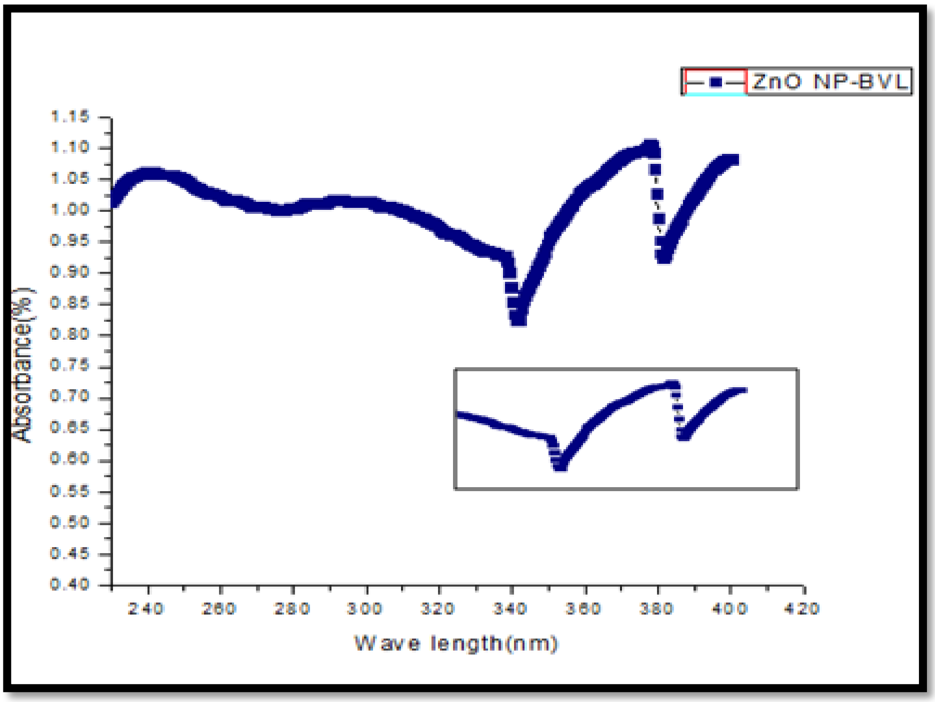


Fig: 4.10 UV spectrum of *Bauhinia variegata* mediated green synthesised ZnO NPs

From the graph it was clear that two peaks were obtained at 340 and 380 nm. By analysing different studies it is confirmed that absorbance peak at 340 nm corresponding to the characteristic band of zinc oxide nanoparticle and band at 380 nm also demonstrated the presence of ZnO nanoparticle in the reaction mixture.

Particle diameter obtained from the spectrum=21.23nm

4.2.3 Thermal Characterization

Thermo gravimetric analysis

Thermal gravimetric and differential thermal analyses indicate the thermal stability of nanoparticles within the temperature range 0 to 600 °C. **(HemaliPadalia et al., 2017)**

The thermo gravimetric analysis of ZnO was performed on a Thermo gravimetric analyser and showed loss of weight due to the loss of water and a continuous loss of weight for ZnO nanoparticles. **(Elizabeth Varghese *et al.*, 2015).** The amount and rate of weight changes in a material are measured by using TGA either as a function of temperature with increasing temperature or isothermally as a function of time. It is used to characterize any material which exhibits a weight change and detects phase changes due to oxidation, decomposition or dehydration. It is connected to a computer which acts as a controller to control the functions like to run data analysis 46 programs, store experimental data and to setup and control experiments.

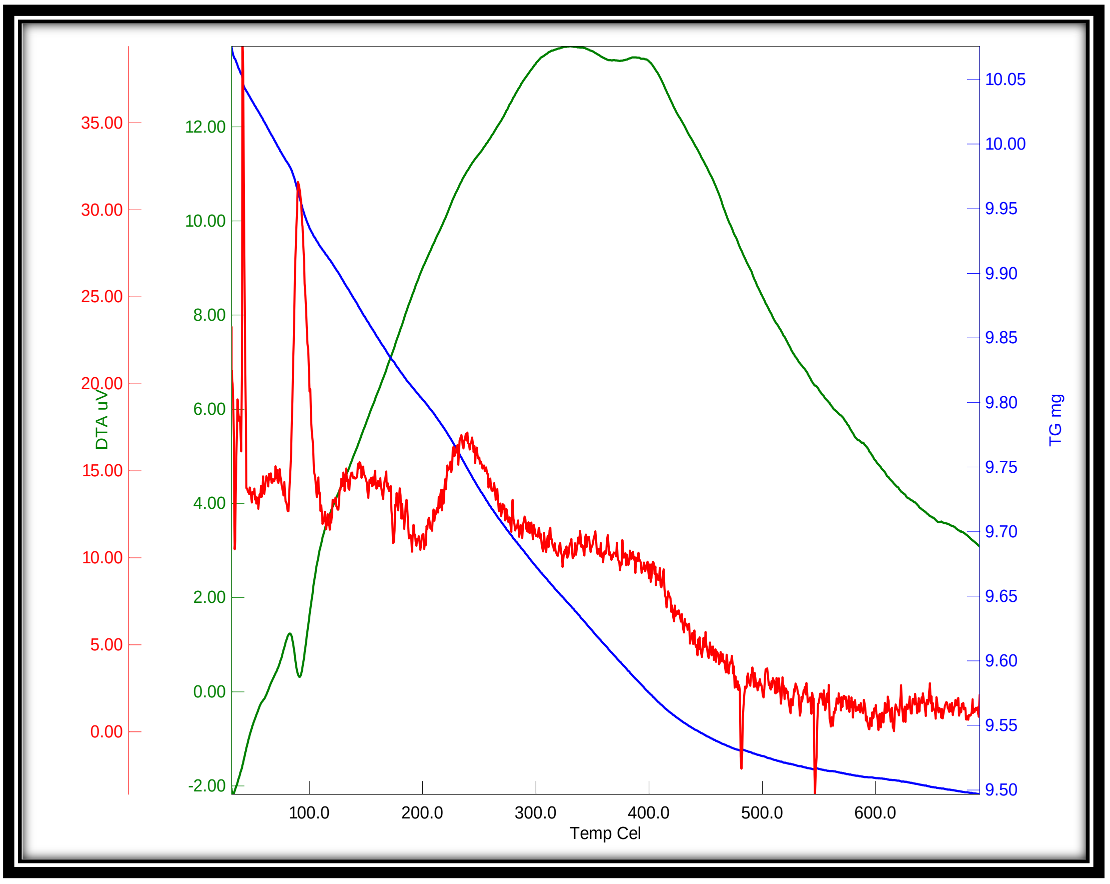


Fig: 4.11 TGA image of plant mediated synthesised ZnO NPs.

4.2.4 Surface characterization

4.2.4.1 FT-IR Analysis

FT-IR is an effective method to reveal the composition of products. Typical FTIR spectrum of zinc oxide nanoparticlesindicates absorption spectrum peak at 533 cm^{-1} is the characteristic absorption of Zn-O bond and a peak at 3456 cm^{-1} can be attributed to the characteristic absorption of hydroxyl. These data are similar to the results observed by others. *Bauhinia variegata* mediated synthesised ZnO NPs exhibited peak at 543cm^{-1} which corresponds to hexagonal phase Zn-O Stretching vibrations **(Pave *et al.*, 2017)**.

Vibration frequency range	**Functional group**
3029-4456 cm^{-1}	OH stretching vibration
1400-1600 cm^{-1}	C=C stretching
1018,864 cm^{-1}	C-O stretching
650 and 543 cm^{-1}	Zn-O Bond

Table: 4.5 FT-IR Spectral frequency and assigned functional group for ZnO NP

The FT-IR spectral peaks of BVL extract are due to nitro compounds 1512 $cm^{-1}$of nitro compounds, 1643 cm^{-1} of C = O stretching of COOH groups, 1743 cm^{-1} C=O stretch, 1025 cm^{-1} C=N stretching of aliphatic amines have disappeared in the FT-IR spectral peak of ZnO –BVL indicating that plant metabolitessuch as alkaloids , carboxylic acids and flavanoids are bound to the surface of ZnO NP and these compounds are responsible for the reduction of Zn ions to ZnO NP. Further the stability of the ZnO NP is expected to be presence of free amino acids and carboxylic groups that have interacted with the Zn surface.

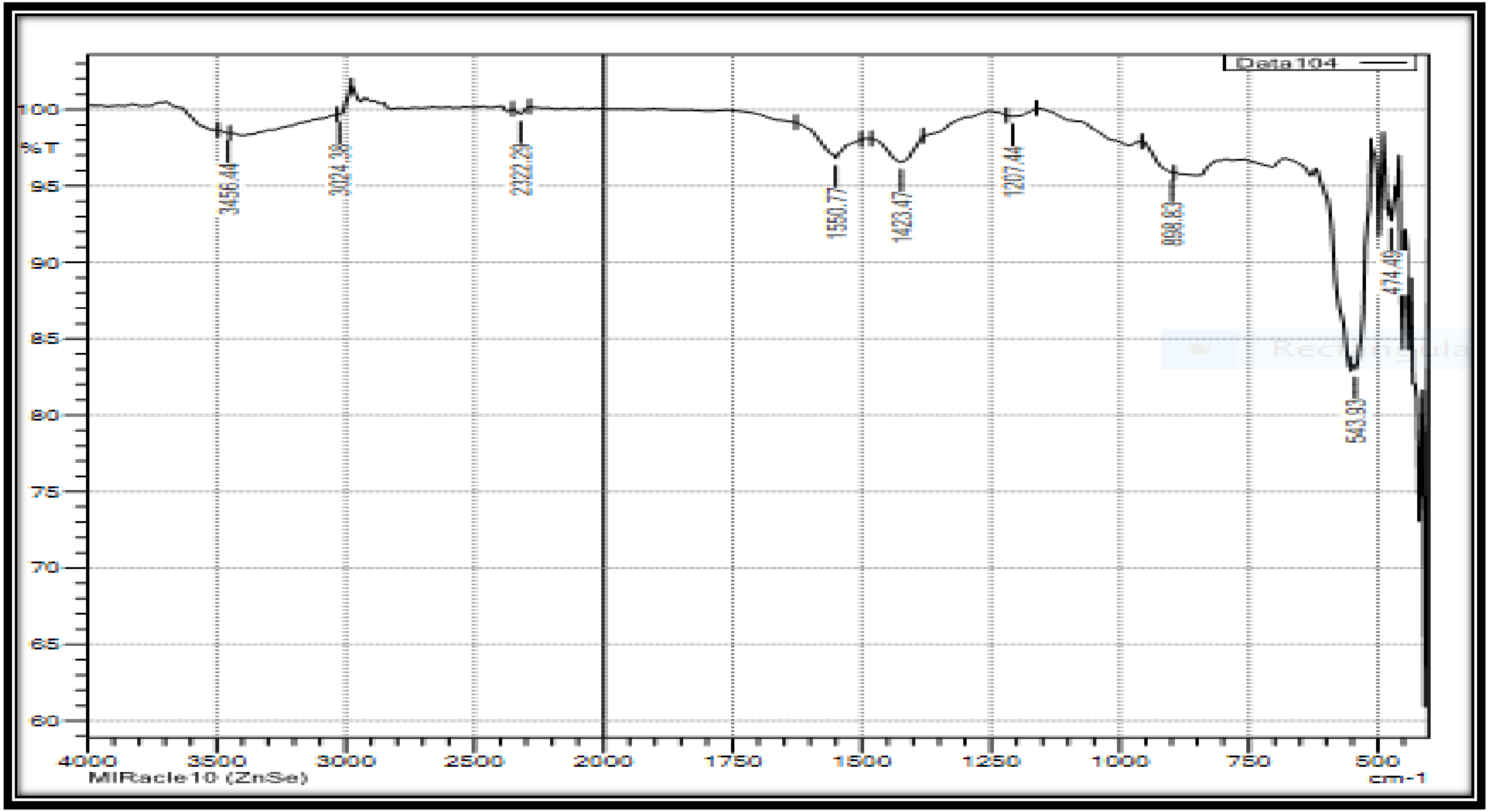


Fig: 4.12 FTIR image of plant mediated synthesised ZnO NPs.

4.2.4.2 Scanning electron microscopy

The SEM images of the plant mediated ZnO- NP- BVL exhibited varieties of typical shapes as spherical and hexagonal nanoparticles.

Also the SEM images show distinguished spherical and hexagonal morphology ans are generally in random and not uniform.this was in accordance with those desceibed in previous literature **(Gomathi *et al.*, 2017)**. The nanoparticles were found to be aggromerated (Fig 4.15 c)

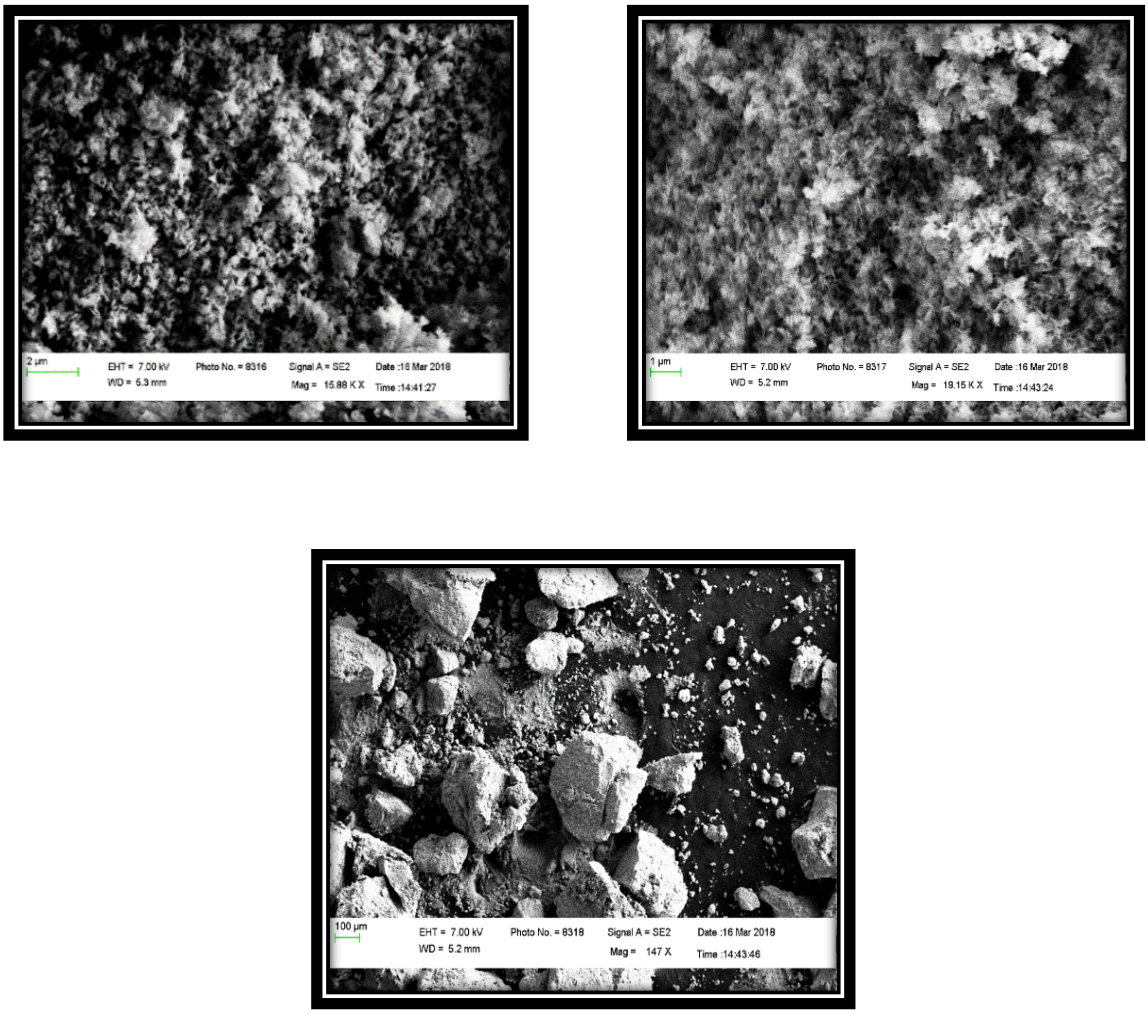


Fig: 4.13 SEM Image of ZnO NPs

4.2.4.3 3D Optical profiler

Optical profilometer studies give us insight about the topography, rough- ness of nanoparticles. Images were clearly demonstrated the smoothness of nanoparticle with capping of phytochemicals over the surface of nanoparticle **(santhoshkumar.j*et al*., 2017.)**

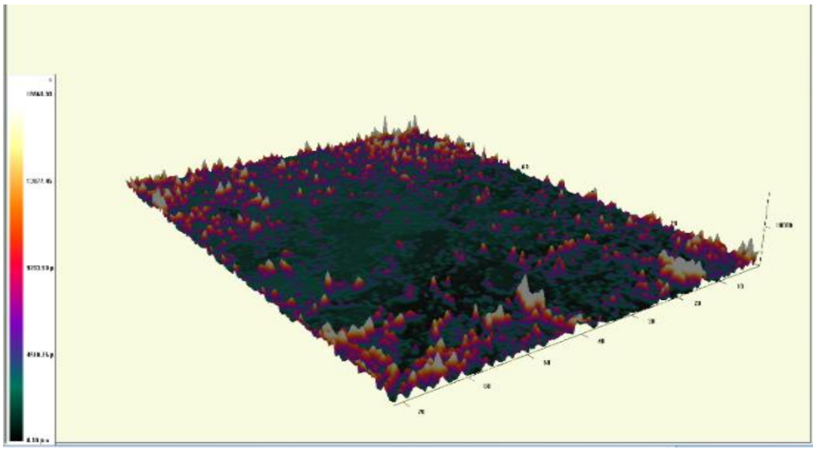


Fig: 4.14 3D Optical Profilometer image of ZnONP-P

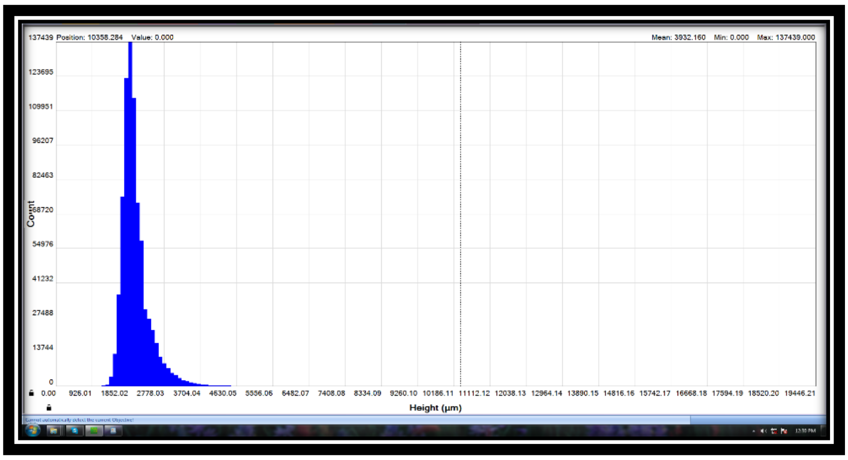


Fig: 4.15 Histogram image of ZnONP-P

Sample	Ra	Rq
ZnO NP-P	397.9	542.94

Table: 4.6 Ra and Rq values for ZnONP-P

Estimation of band gap energy

Owing to the electron transition,the characteristic absorption band at 340 nm and 380 nm was attributed to the intrinsic band gap absorption of ZnONP-C and ZnONP-P. From the variation of the kubelka-Munk function with photon energy, the band gap energy of ZnO nanoparticles was assessed. The band gap for as-synthesized ZnO through chemical method and green method were found to be 3.6 eV and 3.2 eV respectively.

Sample	**Band gap in eV**
ZnONP-C	3.6
ZnONP-P	3.2

Table: 4.7 Band gap energy values of ZnONP-C and ZnONP-P

From this data it was revealed that ZnO nanoparticles synthesized from both chemical and green method was found to have approximately same band energy gap.

Particle size determination

The synthesized ZnO NP-C and ZnONP-P were calculated with the use of wavelength at the edge of the UV-Visible spectra (λ_e) through a method described by Henglein and coworkers using the below mentioned equation.(**DilaveezRehana,*et al* 2017).**

$$2R_{ZnO}= 0.1/(0.1338-(0.00002345*\lambda_e))$$

The results were shown in table.

Sample	**$\lambda_e$excitation (nm)**	**Calculated ZnONPs diameter (nm)**
ZnONP-C	340	12
ZnONP-P	380	21

Table: 4.8 Particle size of synthesized ZnO NPs

Anti-microbial activity of synthesised ZnO

ZnO NPs have recently shown to provide effective pathogen growth control as they have lower toxicityas well as positive impact on soil fertility. Thechemically synthesized ZnO NPs have been found tobe convincing antibacterial agents because they have the ability to absorb UV radiation. There are numerous reports stating that Gram-positive bacteria are probably extra sensitive to ZnO NPs than Gram negative bacteria (**Ajey Singh *et al*., 2017)**

Cultures of staphylococcus aureus, Escherichia coli, and klebsiella pneumonia were used for anti-bacterial studies.

Agar disc diffusion technique was adopted to perform the assay.

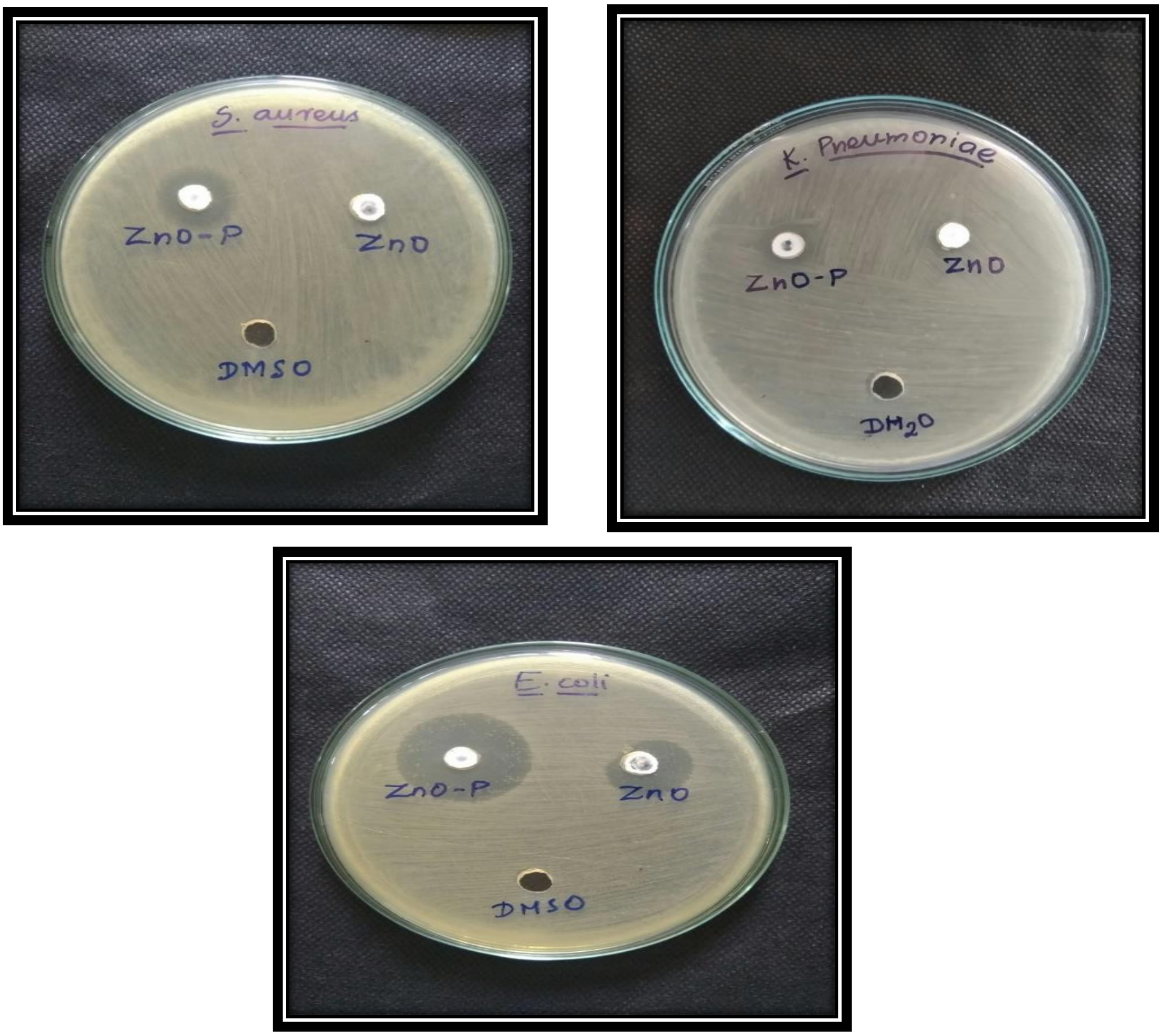


Fig: 4.16 Zone inhibition of E.coli, K.pneumonia and S.aureus on ZnO NPs

Organisms	ZnO-P	ZnO	DMSO
E.Coli	11	4	0
K. pneumonia	4	1	0
S.aureus	**5**	0	0

Table: 4.9 Zone of inhibition of microbes

Maximum inhibition was showed by E.coli and inhibition is minimum for K. pneumoniae. This proves that the plant mediated synthesised ZnO NPs may be used for microbial activity study.**(AlagesanVenkateasan, *et al*., 2017).**

The enhanced antimicrobial activity of nano particles can be attributed to their increased surface area avai9lable for interactions which enhanced bacterial effect.

The mechanism by which the nano particles where able to penetrate the bacteria is not completely understood, but study suggest that when bacteria where treated with ZnO particles changes to place in its member in morphology that produced a significant increased in its permeability affecting proper transport through the plasma membrane (Auffan *et al*, 2009).

The synthesized NPs exhibit antifungal, anticorrosive, antibacterial properties due to increased specific surface area, reduced particle size, enhanced particle surface reactivity and abrasive surface texture or surface defect. Zone of inhibition obtained using nanoparticle was much higher than the standard disc used which depicts the need of further engineering of nanoparticle to obtain desirable effects. Thus we can say that the zinc oxide nanoparticles are inhibit the microbial growth in *in-vitro* antimicrobial activities. **(santhoshkumar *et al*., 2017)**

SUMMARY AND CONCLUSION

This study conclusively reported eco friendly approached for synthesize of ZnO nano particles. A novel approach for one part biosynthesis of ZnO nano particles has been demonstrated.

A ZnO nano particle has been achieved using physical and chemical method extract from *Bauhinia variegata*. As synthesized ZnO nano particles where quit effective comparable with that of chemically synthesized ZnO nano particles. Thus this new protocol may serve as viable alternative to numerous existing procedures.

The presence of phytochemicals in the leaf extract itself helps in the synthesis of metal oxide nanoparticle ZnO NPs was synthesized by the green synthesis method using *Bauhinia variegata* extract is simple and cost effective. Prepared ZnO NPs were characterized using several techniques such as SEM, FTIR, Solid UV –Vis and TGA and 3D optical profiler .The UV-Vis absorption peak at 340 nm confirmed the presence of ZnO in the nano scale. The FT-IR studies showed an absorption peak at 543^{cm-1} (Zn-O linkage) which indicated the formation of zinc oxide nanoparticles. The SEM analysis of ZnO NP showed the morphology of the samples.

The antimicrobial activity of the synthesised ZnO NPs also studied using 3 different microbes, E.Coli, K.Pneumonial and S.aureus. The inhibition efficiency of ZnO NPs is higher for gram negative microb E.coli.

Biosynthesized ZnO nano particle prepared from *Bauhinia variegata* leaves are expected to have notable applications in pharmaceutical bio medical fields such as drug delivery and in cosmetics industries.

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