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Green synthesis of zinc oxide nanoparticles using Azadirachta indica leaf extract

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Green synthesis of zinc oxide nanoparticles using Azadirachta indica leaf extract : Characterization and it's antimicrobial activity

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ABSTRACT
The synthesis of metal and semiconductor nanoparticles is an expanding research area due to the potential applications in the development of novel technologies. Especially, biologically synthesized nanomaterial has become an important branch of nanotechnology. The present work, described the synthesis of zinc oxide nanoparticles (ZnO NPs) using leaf aqueous extract of *Azadirachta indica*. and its antimicrobial activities. The nanoparticles were obtain characterized by UV–Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR) analysis. In this study we also investigated antimicrobial activity of green synthesized ZnO NPs.The aqueous leaf extract acts as a solvent with manifold roles as promoter, stabilizer and template for the synthesis of nanoparticles. The results of FTIR analysis revealed the presence of biomolecules such as Polyphenols, Carboxylic acid, polysaccharide, aminoacids and proteins. The results depicted increasing concentration of ZnO NPs due to the increase of H_2O_2 concentration from the surface of ZnO. Finally concluded the zinc oxide nanoparticles exhibited an interesting antimicrobial activity with both Gram positive *(Stayphylococcus Aureus*) and Gram negative bacterial (*E. coli*) at micromolar concentration.

Keywords: ZnO nanoparticle, UV-VIS spectrophotometer, Antibacterial.

INTRODUCTION

Nanotechnology is a science and engineering branch of recent well-established technology referring at the nanoscale, i.e. 1 to 100 nm. The field of nanotechnology is one of the most active research areas in modern material science. Over last decades, nanotechnology has established as the great innovation of science and technology. Metal nanoparticles are more therapeutic compared to others. The synthesis of nanoparticles by conventional physical and chemical methods has adverse effects like critical conditions of temperature and pressure, expensive and toxic chemicals, long reflux time of reaction, toxic byproducts [1].

Nanotechnology
It is the study of manipulating matter on a atomic and molecular scale. It deals with developing materials, devices and other structure possess at least one dimension size from 1 to 100 nanometers.

Methods for Synthesis of Zinc nanoparticles [2]
Most common methods for synthesizing nanoparticles:

- Physical methods
- Chemical methods
- Biological methods

Physical Methods
These methods involve use of physical forces to break down bulk material into very minute nanoparticles including **Ball Milling, Sputtering, Laser ablation and Evaporation.**
Ball Milling: Mechanical grinding of bulk materials into nanoparticles by using high energy balls.
Laser ablation: A laser beam is focused on bulk material causing ejection of nanoparticles
Sputtering: A high energy plasma is used to remove atom from target material that leads to formation of Nanoparticles [3].

Chemical Methods
Involes chemical reactions to create nanoparticles include Etching, Mechanical milling, Hydro Thermal Decomposition, Sol Gel, Chemical Vapor Deposition [4].
Etching: Process where chemical reactions remove specific atoms and molecules from the surface of a material to create nanoparticles with specific size distribution and shapes like rods, Nanowires or spheres.
Hydrothermal Decomposition: It uses high temperature and pressure in aqueous medium to firm nanoparticles were water act as solvent.

Green synthesis
Green synthesis of nanoparticles involves the use of plant or plant parts for the bioreduction of metal ions into their elemental form in the size range 1–100 nm without using chemicals. The green synthesis process exhibits enhanced efficiency, simplicity, and cost-effectiveness, enabling uniform capacity for larger-scale operations [5]. The physical process such as using a tube furnace is very time-consuming, requires a lot of space, and produces a lot of heat, which raises the temperature in the area around the source material and chemical process of producing nanoparticles is the usage of hazardous solvents and chemicals, which could significantly impact our already fragile ecosystem. Hence, there emerged a global demand for an alternative approach to nanoparticle synthesis, which subsequently gave rise to the concept of green nanotechnology [6].

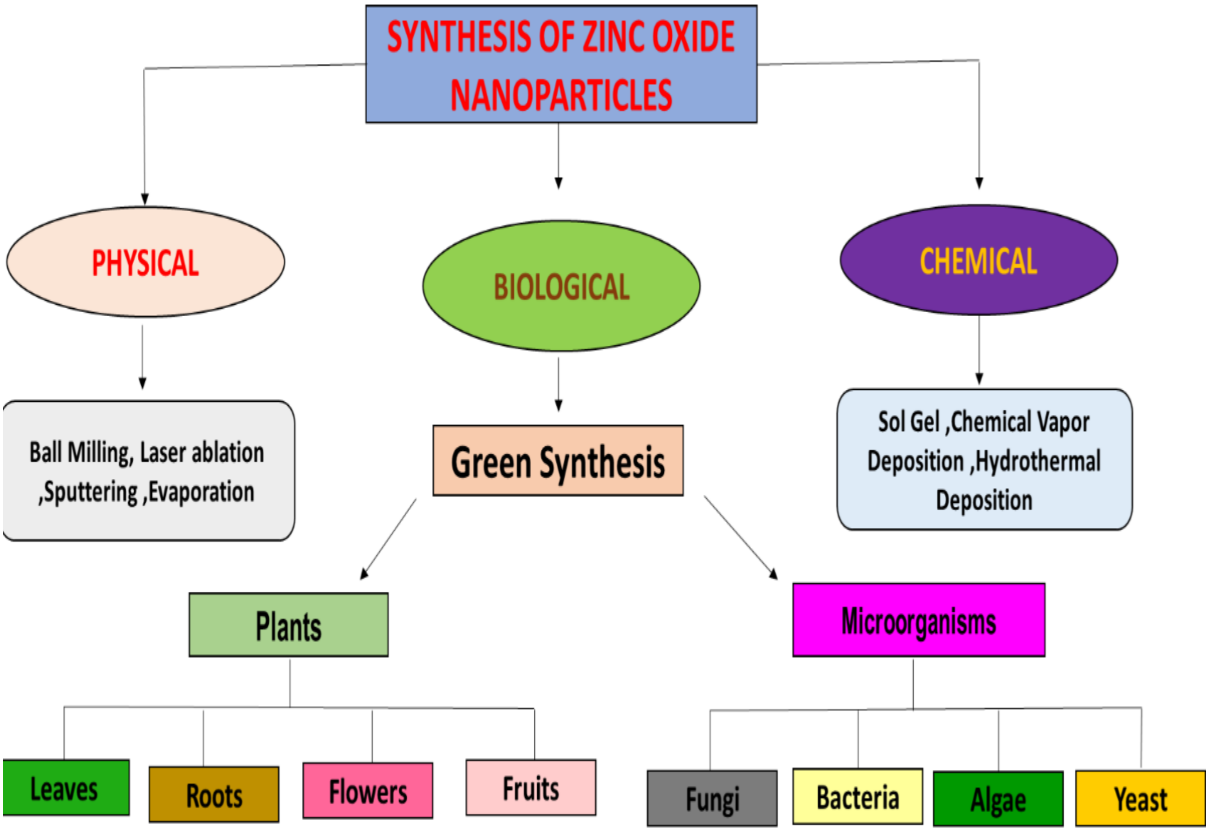


Fig 1. Different methods to synthesis of nano particles.

Medicinal properties of *Azadirachta indica*
Azadirachta indica (neem) is perhaps the most useful traditional medicinal plant in India. Each part of the neem tree has some medicinal property and is thus commercially exploitable. It is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products. It belongs to Meliaceae family, possess diverse medicinal properties [7].
Leaf extract of *A. indica* contains phytochemicals and enzymes which take partin the conversion of metal compounds into nanoparticles . The phytochemicals present in neem leaf extract actingas bioreductant are

flavones, organic acids, ketones, amides andaldehydes; out of which flavones and organic acids are water-solublephytochemicals that are responsible for the reduction of zinc ions into zinc nanoparticles [8].

It has strong antibacterial and antifungal properties against *E.coli.*, *Staphylococcus species and Pseudomonas aeroginosa,Candida* and *Aspergillus* and also exhibit antiviral properties by inhibit the replication of virus including herbs and Hepatitis .It act as Antioxidant which is rich in flavonoids and polyphenols.It treat in acne and promotes wound healing and boost immune system [9].

MATERIALS AND METHOD

Material Required

Distilled water, *Azadirachta indica* leaves, Magnetic stirrer, UV Spectrosphotometer, FT-IR spectrometer and antibiotics like Ampicillin and Streptomycin discs.

Preparation of plant extract

The *Azadirachta indica* plant leaves were collected from the campus of Deenbandhu Chhotu Ram University of Science and Technology, Murthal. Fresh 15 g green leaves of Azadirachta indica plant were gathered and washed multiple times in running tap water, which was further repeated with deionized water to dispose-off dirt particles. The fresh green leaves were chopped into fine fragments and soaked in an beaker containing of 250 ml of double-distilled water. The mixture was boiled at 60-80°C for 15-20 minutes on a hot magnetic stirrer plate. After boiling, a brown colored solution was obtained, which was then cooled down at room temperature and filtered using Whatman filter paper no 1 to remove the impurities. The resultant filtrate was preserved at 4°C for further studies.

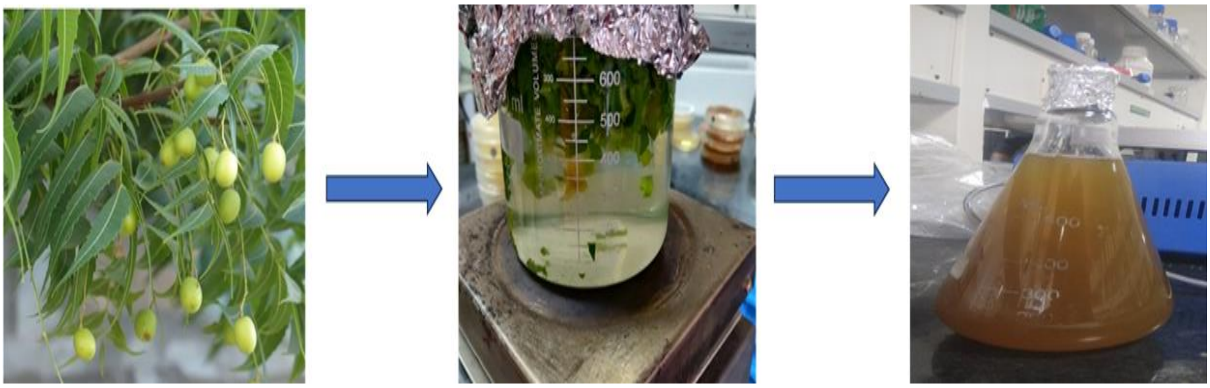
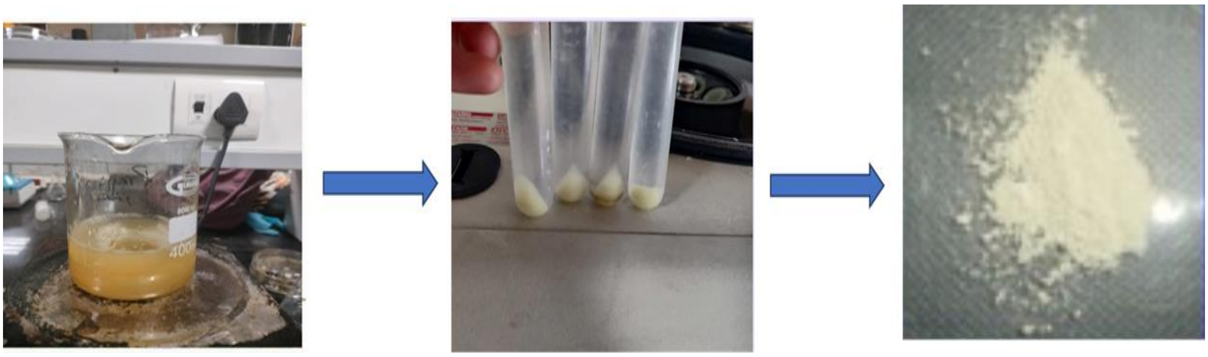


Fig 2. Extraction of Leaf extract.

Extraction of Zn nanoparticles with help of salt Zinc Oxide

1.9g of Zinc oxide was prepared in 90 ml of distilled water is placed in magnetic stirrer and it stirrered for 2-3 hours ,after completely dissolving ZnO salt in solution , 10 ml of plant extract of *Azadirachta indica* added drop wise which transforms transparent colour into a pale yellow and add further dropwise addition of 1M NaOH for maintaining thee pH at 8. The mixture was then placed at constant stirring for 90 minutes on a magnetic stirrer, which led to the formation of a pale yellow colored precipitate, observed to be settled down at the bottom of the mixture. The resultant residue was obtained by centrifugation [8000 rpm, 8 minute] and, washed with distilled water twice. A final washing with ethanol was carried out at 8000 rpm for 8 minutes to remove any impurities, or excess of plant extract. The precipitate was spread into another cleaned petridishes and placed for drying at 55°C for 8-10 hr in a hot air oven, with help of spatula yellow precipitate were scratched on petridishes and trans formed into yellow powder. The resultant yellow coloured powder was collected and stored in tight vials for further examination.



(a). (b). (c)

Fig 3. (a) Plant extract is dropwise added in ZnO solution after zinc ions dissolved then NaOH used or added to maintaining the temperature. (b) The four tubes are centrifuged and dry the pellet in hot air oven by spreading it into another clean Petridishs and place at 55°C in hot air oven. (c) After dry scratch it and it convert into pale yellow powder.

CHARACTERIZATION

Characterization to confirm the synthesis of ZnO NPs and to determine their shape, size, and morphology, various analytical techniques, viz., UV-Vis spectroscopy, Fourier Transform Infra-Red (FTIR) spectroscopy, were performed.

Ultraviolet-Visible spectroscopy

The Principle of UV-Visible Spectroscopy is based on the absorption of ultraviolet light or visible light by chemical compounds, which results in the production of distinct spectra [10]. When matter absorbs ultraviolet radiation, the electrons present in it undergo excitation. This causes them to jump from a ground state (an energy state with a relatively small amount of energy associated with it) to an excited state (an energy state with a relatively large amount of energy associated with it). It is important to note that the difference in the energies of the ground state and the excited state of the electron is always equal to the amount of ultraviolet radiation or visible radiation absorbed by it. The wavelengths of light for UV-visible absorption are from about 200 nm to 800nm (UV - 200-400, Vis – 400-800).

Procedure

1. 1 mg ZnO nanoparticles were taken and disperse in 10ml of distilled water so that it becomes liquid solution.
2. Because it doesn't get completely soluble in solvent then it taken for sonication for approx 10 mins
3. Black solution was prepared,which was solvent without ZnO nanoparticles.
4. Bio Genix spectrophotometer was turned on and allowed it for stabilize about 10 min.
5. UV visible mode selected in software .
6. Medium scanning method choosen for better resolution.
7. Two clean Quartz cuvette one was for black solution and second was for ZnO nanoparticles sample solution .
8. First Blank solvent was placed in Quartz cuvette and inserted into reference or sample loader.
9. For setting baseline Blank scan was run.
10. Dispersed ZnO solution was poured into clean Quartz cuvette and placed into reference or sample loader.
11. Scan was started from 200 to 800nm.
12. Spectrum displayed absorbance vs wavelength data.

Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared spectroscopy (FT-IR) is a technique that has been used over the years in chemical analysis for the identification of substances and is one that may be applied to the characterization of microorganisms [11]. FT-IR used to identify the probable functional groups in biomolecules contained with the presence of a natural extract that are due to the reduction of the zinc ion into ZnO NPs, a Fourier transform infrared spectral analysis (FTIR) was performed [12]. FTIR spectra for the synthesized dried nanopowder and *Azadirichta Indica* leaf extract were analyzed using FTIR (Perkin elmer) in the range of $4000\text{-}400^{-2}$ and a resolution of 4 cm^{-1}. For Synthesis of zinc oxide nanoparticles the most preferable method was ATR

(Attenuated Total Reflectance) by directly placing dry ZnO powder in ATR crystal.FTIR spectrum of dried and powdered ZnO NPs was obtained, DCRUST, Murthal [13].

Procedure

1. About 1 mg of dry ZnO nanoparticles powder was placed on ATR crystal and gently press it to ensure proper contact.
2. Then Perkin elmer FT -IR instrument was Turned On and was allowed to warmed about 15-30 min.
3. Perkin elmer software was opened on computer device.
4. Resolution was set about $4cm^{-1}$ and peakes were scanned.
5. Background scan was performed by using ATR crystal.
6. Sample was inserted and scan was run and FT -IR spectrum displayed transmittance vs wavenumber.

Evaluation of the antimicrobial response of the synthesized NPs

The bio-synthesized nanoparticles were exploited to evaluate their antimicrobial ability against four clinical multi-drug-resistant uropathogens using antibacterial assay. Antimicrobial ability of the bio-synthesized ZnO NPs was determined by employing the agar disc diffusion method against two highly drug-resistant [14]. *Escherichia coli* and *Staphylococcus aureus*, Each strain's fresh 24h bacterial culture was spread into NA plates. The Streptomycin and Ampicillin discs with varying concentrations (30µg/ml and 30 µg/ml) and the synthesized 10 ul ZnO NPs liquid sample were impregnated on the surface of the plates and then placed at 37◦ C for 20-24h in an incubator.

Ampicillin and. Streptomycin are two standard antibiotic discs (30 µg), was also placed into NA plates along with ZnO NPs and used as a positive control and Deionized water used as negative control .After completion of incubation, the level of zones produced around each disc were examined.

RESULTS AND DISCUSSION

UV SPECTROSCOPY

When light is absorbed by matter, the energy content of the atoms or molecules increases. When ultraviolet radiation is absorbed, electrons are excited from their ground state and moved to a higher energy state. The powder was resuspended in deionized water to note the UV-Visible spectra showing an absorption peak at aprox. 375 nm.

The following peak shows Blue shift or Hypsochromic shift means tha UV visible t absorption of nanoparticles shift towards lower wavelength which have higher energy .

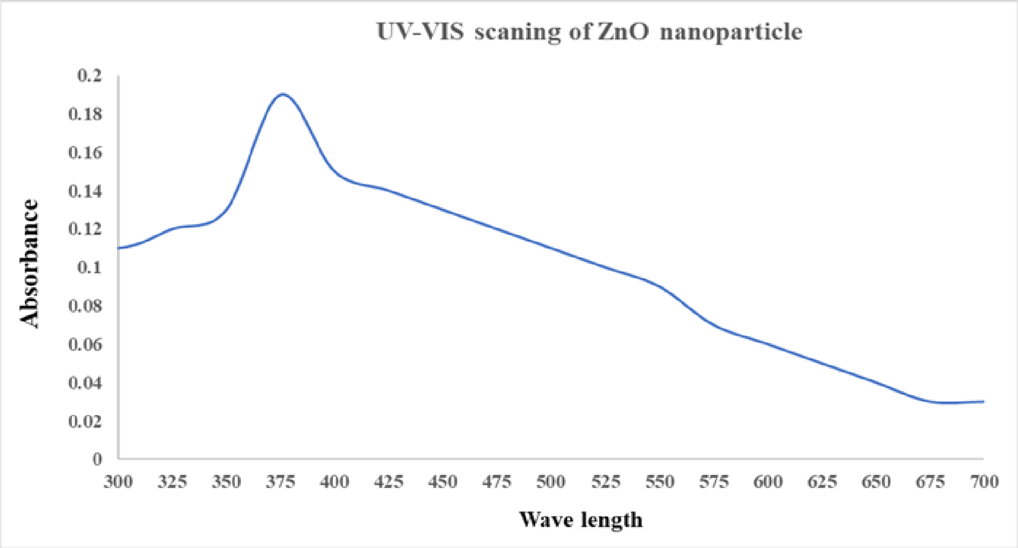


Fig. 4. UV Spectroscopy graph which shows the highest absorption peak at 340nm under standard conditions.

Blue shift occurs because of solvent polarity effects, in polar solvent π-π transitions occurs which displays Blue shift UV absorption spectrum due to increased stabilization of ground state over excited state.ZnO nanoparticles which absorb 375 nm UV visible spectrum range falls in near ultraviolet (UV-A) region [15].

FT-IR Spectrometry

Fourier transform infrared spectroscopy (FT-IR) is used to get specific signals Obtained by substance specific vibrations here it is used to examine Aqueous leaf extract of *Azadirachta indica* in reducing and stabilizing the nanoparticles. The technique of FTIR is used to extract particular signals from substance specific vibration of molecules. FTIR images in the 4000-500cm- identify functional molecules. The highest peak is between 1976-1884The peaks obtained in FTIR spectrum are 1976, 1650, 1148 were attributed to C=O stretching, alkene (C=C stretching), O=C stretching. The peak at. 2750 shows the =C-H stretch and the peak at 780 shows =C-H bending vibrations. These functional groups are responsible on the reduction and stabilization of Zinc nanoparticles on the surfaces of the Azadirachta indica leaf.

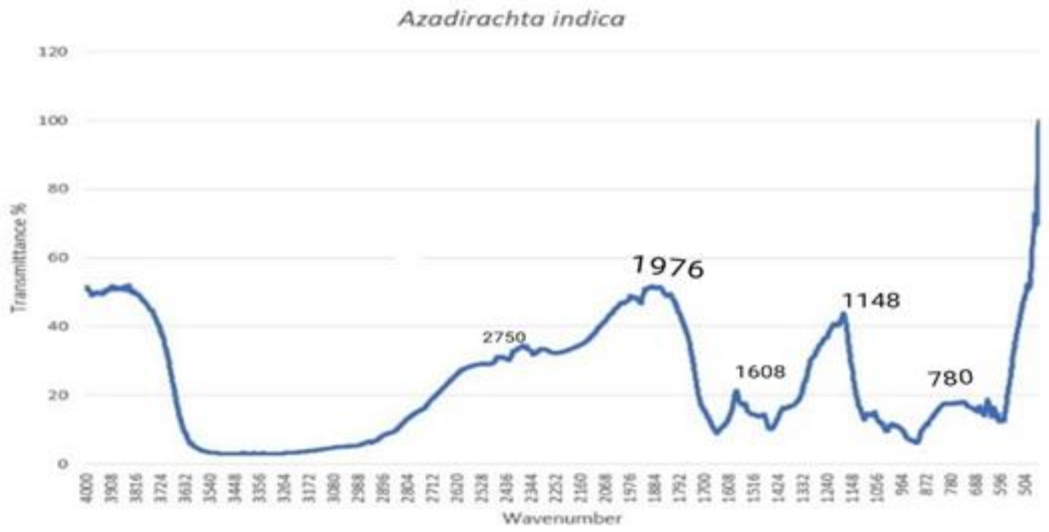


Fig 5. Graph of FT-IR to show vibrations of bending and streching of functional group of molecule.

Antimicrobial response against pathogens

The agar disc diffusion method was used to analyse the efficiency of the antibacterial ability of the bio-formulated ZnO nanoparticles against clinical MDR Gram-negative and Gram positive uropathogens [16]. Zinc oxide nanoparticles (ZnO NPs) are one of the most widely used nanoparticulate materials due to their antimicrobial properties, but their main mechanism of action (MOA) has not been fully elucidated [17] .The levels of zonations were obtained within the range of mm, and the maximum antimicrobial efficacy was found to be against *E coli* was 13 mm around Streptomycin disc and 9.5 mm around ZnO nanoparticle liquid sample ,followed by *Staphylococcus Aureus* was 9 mm around ZnO nanoparticles liquid sample. Thus, the synthesized ZnO NPs were found to be entrusted with strong antimicrobial ability against all the tested uropathogens. In the present study, Ampicillin and Streptomycin are type of aminoglycosides, one of the most potent antibiotics commonly used for the treatment of UTIs, was used as the control for comparative evaluation of the antimicrobial potential of biosynthesized nanoparticles [18]. Ampicillin and streptomycin was observed to be 100% resistant against the all tested uropathogens. Hence, from the present study, it can be concluded that bio-synthesized ZnO NPs have better antimicrobial potential than the commonly used empiric antibiotic therapy against the MDR uropathogens.

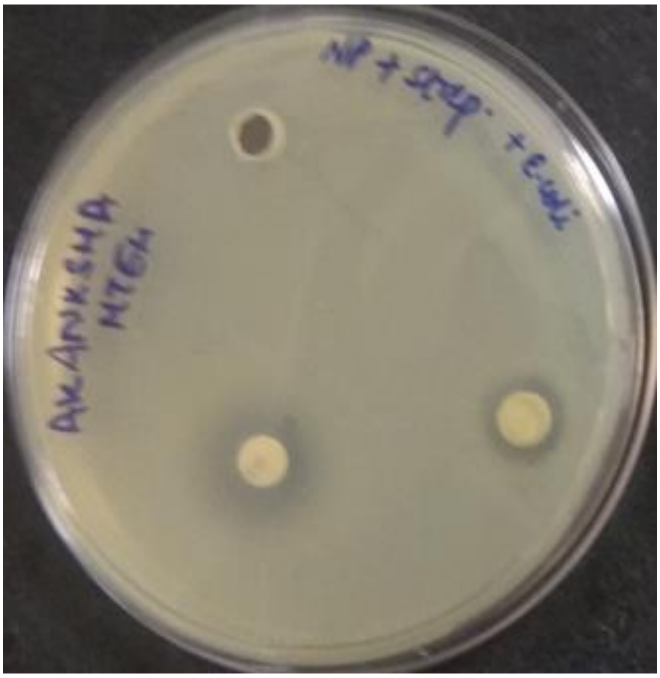


Fig 6. Well diffusion test against *E. coli* bacterial.

Fig 7. Well diffusion test against *Staphylococcus aureus*.

Table.1. Inhibition zones of ZnO nanoparticles with bacterial strains.

Samples	***E. coli*** **(Zone of Inhibition in mm)**	***S. aureus*** **(Zone of Inhibition in mm)**
ZnO nanoparticles (10ul)	9.5	9
Streptomycin (30ug)	13	-
Ampicillin (30ug)	-	No Zone
Blank	No Zone	No Zone

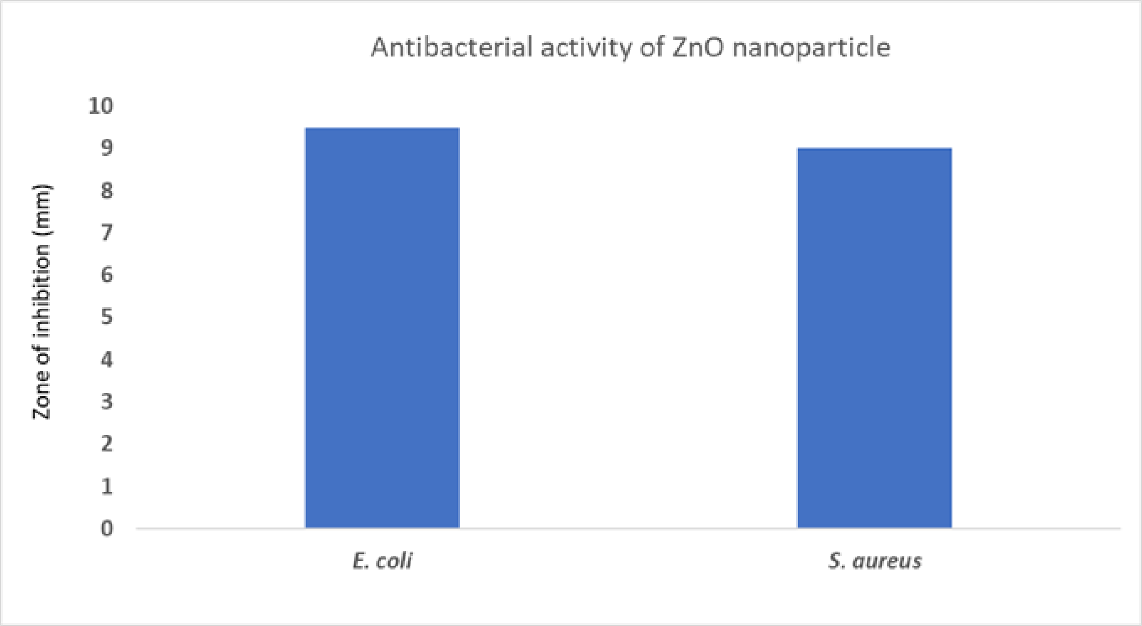


Fig 8. Antibacterial activity of ZnO nanoparticle against *E. coli* and *S. aureus*.

CONCLUSION AND SUMMARY

Zinc Oxide nanoparticles have potential characteristics in terms of drug delivery,oncology,and nano medicine,has biomedical applications like they are used in Antifungal ,Anti bacterial and Antiviral agents.Zinc oxide nanoparticle are also used to make biosensors which detect biomolecules,glucose level in blood as glucometer,It also exhibit selective toxicity against cancerous cell.

This thesis is relsted to green synthesis of Zinc Oxide nanoparticles by using *Azadiricta* Indica which provides an eco friendly, cost effective and sustainable approach ,This bioactive components in Neem act as natural reducing and stabilizing agent which also help in eliminating the use of toxic chemicals. The UV Spectrosocopy analysis shows as absorption about 375nm peak, this blue shift in absorption suggest small partical size due to quantum confinement effect . Fourier Transform Infrared Spectrometer (FT -IR) ued to confirm of presence of functional groups and ZnO bonding nanoparticles synthesized by using Neem extract.

ZnO nanoparticles exihibit Antimicrobial properties due to Zn^{2+} which penetrates by cell membrane and discrupt the cell wall of pathogenic bacteria which kill the pathogenic bacterial cells. ZnO nanoparticles shows strong antimicrobial activity against gram positive bacteria Stayphylococcus Aureus and gram negative bacteria E.coli. Such MDR pathogens hamper the conventional empiric antibiotic therapy and ring an alarm for researcher communities to look for an alternate therapy to cure several infections. In the past few decades, the scientific community has been fascinated by exploring and evolving green chemistry to produce nanoparticles as its environment-friendly. The presence of phytochemicals in the plants acts as a conjugating operative that helps reduce, stabilize, and further facilitate the shape and size-controlled nanoparticle synthesis.

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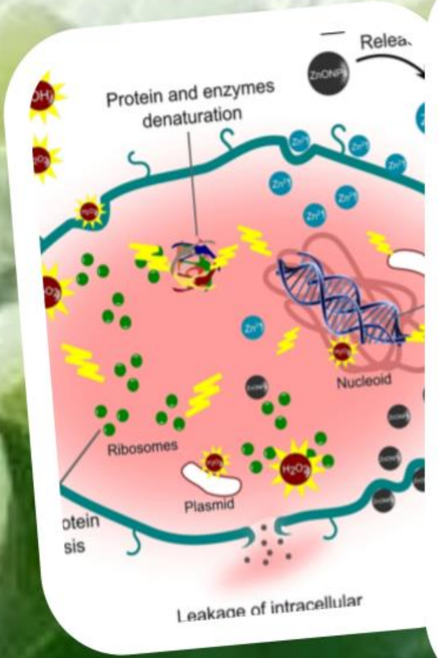

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