Green Synthesis of Zinc Oxide Nanoparticles using *Zephyranthes candida* Leaves

K.DHANUJA
21PCH008

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
May - 2023

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Signature of the Supervisor **Signature of the Head of the Department**

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K.DHANUJA

CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ZnO NPs	Zinc Oxide Nanoparticles
UV	Ultra Violet Spectroscopy
FT-IR	Fourier Transform Infrared Spectroscopy
TGA	ThermoGravimetric Analysis
XRD	X-Ray Diffraction
GPB	Gram Positive Bacteria
GNB	Gram Negative Bacteria

INTRODUCTION

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In recent years in the field of science and technology, one among the rapidly developing concepts is nanotechnology, which has brought tremendous development in nanomaterial which comprises distinctive physicochemical properties.Nanoparticles (NPs) are the building blocks of nanotechnology and can be synthesized chemically, physically, or biologically (Iravani *et al.*, 2014). Nanomaterials are particles that are nanoscale in size, and they are very small particles with improved thermal conductivity, catalytic reactivity, nonlinear optical performance, and chemical stability due to their large surface area-to-volume ratio.

There are many physical and chemical methods of preparing metal nanoparticles (NPs) and metal oxide nanostructures such as sputtering, lithography, and electrospinning but they are quite expensive, and involvement with toxic chemicals results in health risks.

Biological methods are referred to as green methods because they are more environmentally friendly(Bello *et al.*, 2017), non-toxic, and less harmful than chemical and physical methods.Therefore , the green synthesis method is approached which does not require any harmful chemicals, high-pressure reactors, or high temperatures and also it results in degradable waste with less risk of contamination at the end.Synthesis by microbes or plants is one example of a biological method.

Over the past few years, NPs has been synthesized using plants, fruits, flowers, algae, yeasts, bacteria, fungi but an extensive research has been carried out using plant extracts for the synthesis of NPs, and it was observed that compared to other means, plants are more suitable for the production of NPs, even at the small scale.The synthesis of nanoparticles is currently an important area of research, which seeks an eco-friendly approach and green materials for the current scenario(G. Rajakumar *et al.*,2017).

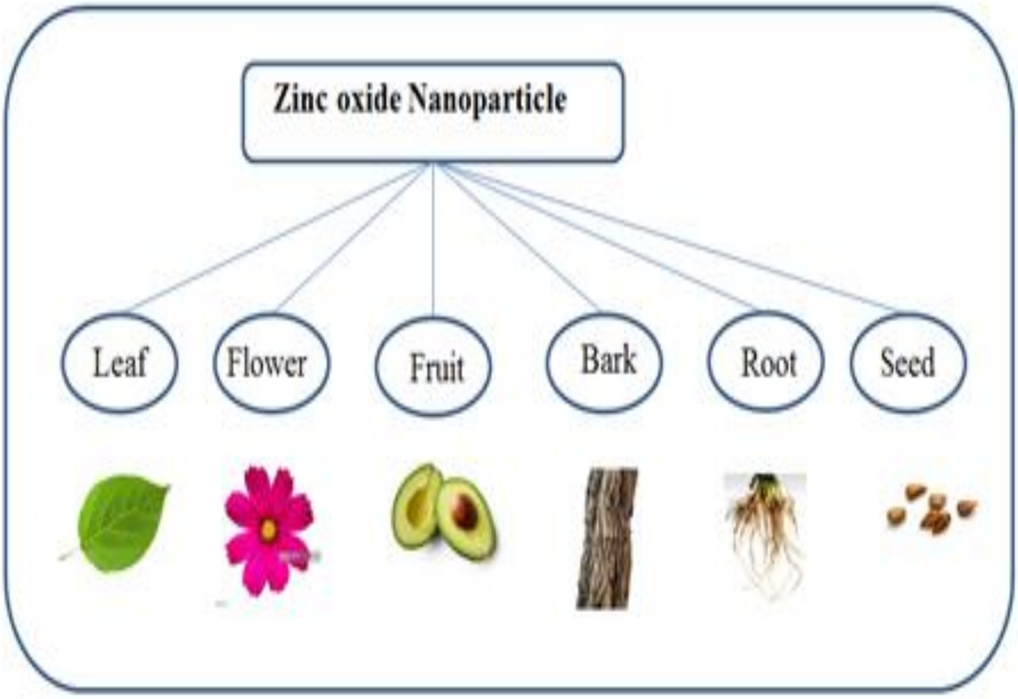

Fig 1.1 Various sources of plant parts required for nanoparticles synthesis(Umamaheshwari *et al*.,2018)

1.1 Advantages of biosynthesized nanoparticles using plant extracts

Natural product preparation from plant material is gaining popularity due to the use of fewer chemicals throughout the entire procedure because natural products present in plants have the ability to act as reducing and capping agents (Valli *et al.*, 2012).These sustainable techniques are eco-friendly, cost-effective, effectual, and scalable with desirable control features on the particle size and morphology of the NPs (Shi *et al*., 2017). Also, these biological methods are free from the generation of toxic chemical sludge and other by-products, which are the characteristics of physicochemical procedure (Shukla *et al.*, 2012).

The combination of nanotechnology and plant medicinal potential is causing a revolution in the pharmaceutical industry. Another significant reason to undertake nanotechnology is the enormous and effective use of nanoparticles for a variety of purposes. The size and structure of nanoparticles significantly affect their applications

(Kelly *et al.*, 2003). Currently, application of nano catalysts for biodiesel synthesis has drawn much attention, as a result of easy separation of products, less pollution, higher catalytic activity and reusability.

1.2 Zinc oxide nanoparticles

Zinc oxide is a multifunctional material with its unique physical and chemical properties, such as high chemical stability, high electrochemical coupling coefficient, high range of radiation absorption and high photostability.Zinc oxide (ZnO) is one such important metal oxide NPs which has gained scientific spotlight currently. The unique set of characteristics of ZnO NPs such as catalysis, photochemical capability, medicinal effects, fungicidal, antibacterial and UV filtration has made ZnO NPs as a multifunctional agent and more promising for wastewater remediation (Li *et al*., 2018).

Nanomaterials based on ZnO find an extensive range of applications such as energy storage, nanosensors, cosmetic products, nanooptical devices, nanoelectronic devices, and so on(fig 1.2). ZnO NPs have fascinated the research world through its significant applications in pigment electronics, spintronics and piezoelectricity fields.ZnO shows diverse group of growth morphologies such as nano rings, nano springs, nano combs, nanowires and nano cages..ZnO nanoparticles have drawn attention due to their antimicrobial activity; this finds application in food packaging.

Fig 1.2 Applications of biosynthesized ZnO nanoparticles (Sudipta *et al*.,2021)

Biocompatibility and biodegradability makes it a material of interest for biomedicine and in pro-ecological systems.Biodegradability and low toxicity are one among the most significant characteristics of ZnO nanomaterials compared to other metal NPs.Among all other types of metal oxides, ZnO NPs attract much attention because of its stimulating properties such as the high direct bandwidth of 3.3 eV at room temperature and high excitation energies of 60 meV , optical property, high catalytic activity, anti-inflammatory, wound healing,cost effective,high photosensitive and UV filtering properties.ZnO's superior photocatalytic abilities is because of its ability to absorb a wide range of the solar spectrum which affirms ZnO as an ideal semiconductor photocatalyst for the degradation of contaminants under solar irradiation hence utilized as a more environmentally friendly semiconductor photocatalyst as sunlight is a renewable and readily available energy source.

In biological applications, characterization of ZnO nanoparticles has also proved to be helpful in modifying and tuning their antibacterial and cytotoxic effects. It has been established that the antibacterial activity increases with decreasing the particle's size.In acidic and strong basic conditions, ZnO NPs could be dissolved slowly and this solubilized ZnO nanoparticles have shown that the release of Zn^{2+} ions can exert stress on cells and have adverse impacts on different organisms.Thus,ZnO nanomaterials have attained increased concern even in biomedical applications.

Although ZnO NPs could be prepared by a variety of methods such as chemical, precipitation, hydrothermal, solvothermal, microwave, sonication, etc., the biosynthetic routes are more examined nowadays, owing to their advantageous cut over the conventional method (Udayabhanu *et al.*, 2017). Green synthesis of ZnO NPs using plant-derived products is an essential leg of this biosynthesis category (Singh *et al.*, 2020; Al-Haddad *et al*., 2021).

1.3 General preparation of nanoparticles

The major steps involved in the preparation of nanoparticles that have to be evaluated from the point of green chemistry are (i) the solvent medium used for the synthesis, (ii) environmentally benign reducing agent, and (iii) the nontoxic material for the stabilization of the nanoparticles. The majority of the chemical and physical methods mentioned so far largely depend on organic solvents.This is principally due to the hydrophobicity of the capping agents used(P. Raveendran *et al*.,2003). Synthesis with bio-organisms is compatible with the principles of green chemistry: (i) ecofriendly approach, (ii) the reducing agent used, and (iii) the capping agent in the reaction. The synthesis of inorganic metal oxide nanoparticles using biological elements has received immense attention due to their unusual properties (optical, electronic, chemical, etc.)(P. Dhandapani *et al*.,2014)

The method primarily involves the production of the NPs using extracts derived from the plant parts, with or without the application of temperature and co-precipitation agents. Although the process chemistry of the NPs preparation is too complex, principally the different enzymes and phytochemicals present in the extract facilitate the reduction/oxidation of the precursor to ZnO NPs(Singh *et al.*, 2020; Bhuyan *et al.*, 2015).

A wide spectrum of metabolites available in these plant-derived extracts are rich antioxidants which readily reduce the precursor molecule to zinc ions and subsequently to ZnO NPs. Water-soluble phytochemicals such as quinones, organic acids and flavones are the critical components that aid the direct formation of ZnO NPs from their precursor molecule (Bhuyan *et al.*, 2015). The effective yield and characteristics of the resultant ZnO NPs from the plant-based biosynthetic methods are also impacted by process parameters such as temperature, pH and reaction time (Singh *et al.*, 2020).Fundamentally, the primary and secondary metabolites are present in plants, for example, saponins, tannins, starches, polypeptides, terpenoids, flavonoids, and phenolics, which act as reducing and capping agents. Mild solvents such as water, ethanol, and methanol are used for the extraction of the plant metabolites, which are allowed to react with the zinc salt solution in different conditions to achieve greatest yield.

A wide variety of plant-derived products such as seed of *Peganum harmala* (Fazlzadeh *et al.*, 2017); stem of *Boswellia ovalifoliolata* (Supraja *et al.*, 2016); leaves of *Punica granatum* (Singh *et al.*, 2019), *Mentha pulegium* (Rad *et al.*, 2019), *Costus woodsonii* (Khan *et al.*, 2019), *Couroupita guianensis* (Sathishkumar *et al.*, 2017); fruit of *Lycopersicon esculentum* (Sutradhar and Saha, 2016); peels of *Citrus sinensis* (Nava *et al.*, 2018), *Lycopersicon esculentum* (Nava *et al.*, 2017); hull of oak fruits (Sorbiun *et al.*, 2018) etc. have been reported for ZnO NPs synthesis by various research works. These biosynthesized ZnO NPs with notable features were employed for different study scopes of heavy metals elimination, dye degradation, semiconductors and photovoltaics, photocatalysis, antibacterial, and antimicrobial applications.Presently, valorization of agricultural biomass which are discarded as bio-wastes and eventually landfilled, have gained considerable weightage for NPs synthesis and especially for ZnO production (Lee et al., 2019).

1.4 Antibacterial property of ZnO nanoparticles

ZnO NPs created by green synthesis exhibit good antibacterial properties, but the principal antibacterial mechanism remains relatively unclear. For ZnO NPs to be widely used as an antibacterial material, it is first necessary to study the bactericidal mechanisms of ZnO NPs.(Mallikarjuna Swamy *et al.*,) found that ZnO NPs prepared from Aegle marmelos extract exhibited antibacterial properties, and concluded that the antibacterial mechanism was due to light causing the ZnO NPs to produce reactive oxygen species, causing cells to adopt a state of oxidative stress, leading to protein denaturation in the cells and affecting mitochondrial function and cell metabolic activity, and eventually causing death. (Sharmila *et al.*,) found that ZnO NPs prepared from Tecoma castanifolia leaf extract displayed antibacterial properties, and found that their effect was on the surface of bacterial cells, leading to the release of ion channels, resulting in an ionic imbalance within the cells that eventually resulted in their death.

1.5 Plant description

Fig 1.5.1 *Zephyranthes candida* plant

In this study, green synthesis of ZnO NPs using ***Zephyranthes candida*** plant extract has been carried out and studied.*Zephyranthes candida* (Lindl.) Herb. (Amaryllidaceae) is found throughout the world's tropical regions and commonly cultivated as an attractive flower and utilized as a medicinal plant in China**.** *Z.candida*'s leaves and rhizomes are quite bitter.*Zephyranthes candida* (ZC) has been mentioned in the Indian System of Traditional Medicine for the treatment of diabetes mellitus **(Pingili *et al.*,2017).**Preliminary phytochemical analysis of the crude methanolic extract of *Zephyranthes candida* showed the presence of, flavonoids, glycosides, terpenoids, saponins, alkaloids and tannins. Amino acids were absent in the extract.**(Shitara *et al.*,2014).**About 70 species of plants in the genus Zephyranthes have been reported to be rich sources of Amaryllidaceae alkaloids such as lycorine, haemanthamine, (+)-tazettine, trisphaeridine, and galanthamine, the latter of which is used in the treatment of Alzheimer's disease due to its selective and reversible inhibitory activity against acetylcholinesterase (AChE) **(Kojima *et al.*,1998 ; Bartolucci *et al*.,2001).**

Scientific classification of *Zephyranthes candida* :

Botanical name	*Zephyranthes candida*
Kingdom	Plantae
Order	Asparagales
Family	Amaryllidaceae
Genus	*Zephyranthes*
Species	*Z.candida*

Table 1.5.1 : Taxonomic classification of *Zephyranthes candida*

Uses :

- In Africa the leaves of *Z. candida* were used for diabetes mellitus.
- It was used for simple problems from headache, cough, cold, and boils to very complicated diseases such as breast cancer, tuberculosis, rheumatism, tumors

Fig 1.5.2 Aqueous extract of fresh leaves of *Zephyranthes candida*

OBJECTIVES

1. To bio-synthesize ZnO nanoparticles.

2. To use less chemicals and reduce waste generation.

3. To characterize the synthesized ZnO nanoparticles using UV-Visible spectroscopy, FT-IR, XRD, SEM, EDAX, TGA, 3-D Optical profilometer and antibacterial studies.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

In this study,we have mainly focused and investigated the antimicrobial activity of the synthesized ZnO nanoparticles using various plant extracts.

Depending on the composition of the cell wall, bacteria can be categorized as either Gram-positive or Gram-negative. It has been found that Gram-positive bacteria are more sensitive to ZnO NPs synthesized from plants than Gram-negative bacteria.

Inamdar *et al.*, found that under the same culture conditions, ZnO NPs prepared with *Mimosa pudica* leaf extract displayed stronger antibacterial effects on Gram-positive bacteria. The inhibitory concentration of ZnO NPs on S. aureus (Gram-positive bacteria) was 0.00025µg/mL, but 2.5µg/ mL and 0.0025µg/mL toward *E. coli* and *B. subtilis* (Gram-negative bacteria), respectively. This demonstrated that a lower concentration of ZnO NPs can inhibit Gram Positive bacteria compared with Gram-negative strains.

Velsankar *et al.*, found that *B. pumilus* (Gram-positive bacteria) were more sensitive to ZnO NPs prepared from *Echinochloa frumentacea* grain extract than *S. typhi* (Gram-negative bacteria). This wass likely due to differences in the composition of the cell wall between the two bacteria. The cell walls of Gram-positive bacteria contained peptidoglycan, teichoic acid, and abundant pores which allowed foreign molecules and nanoparticles to enter the cell, resulting in cell membrane damage and cell death. However, the cell walls of Gram-negative bacteria contained lipopolysaccharide, lipoprotein, and phospholipid, representing a barrier that only allows macromolecules to enter, hindering the entry of nanoparticles and weakening the action of ZnO NPs against Gram-negative bacteria.The antibacterial properties of ZnO NPs vary with the concentration of ZnO NPs.

Dobrucka and Długaszewska *et al.*, found that when cultured at concentrations of 125, 256, 516, and 128µg/mL, the diameters of zones of inhibition of ZnO NPs prepared from *Trifolium pratense* flower extract were 22, 26, 30, and 31mm for *S. aureus*, and 22, 26, 29, and 31mm for *E. coli*, respectively.

Zinc oxide NPs were synthesized from *Capsicum annuum* fruit extract using the solution combustion method. Later, ZnO nanoparticles were used to synthesize formamide esters and as a catalyst in the production of biodiesel. Some characterization techniques were used to investigate the shape, size, and morphology of the NPs. Using XRD and the Debye-formula, the size was determined to be 33.26 nm. According to the energy dispersive X-ray Diffraction study, the nanoparticle contained 55.33% zinc and 44.67% oxygen. The band and the characteristic peak of ZnO NPs in the FT-IR spectrum of ZnO NPs were discovered in the 680-400 cm^{-1} region. The formamide esters were tested for biological activity against a wide range of bacterial pathogens, and the results revealed that only a few molecules were effective.**(Lalithamba *et al*.,2018)**

Upadhyaya and his coworkers used a leaf extract of *Lawsonia inermis* to produce zinc oxide nanoparticles using a green synthesis method. As a precursor, zinc nitrate was used. Several techniques were used to characterize the synthesized nanoparticles. The leaf extract served as a capping agent. The nanoparticles formed were confirmed by SEM images, and their size was 75 nm. According to the XRD results, ZnO NPs have a hexagonal phase with a wurtzite structure. Green synthesized nanoparticles were found to have significant antibacterial activity against various bacterial strains when compared to other conventional method nanoparticles. This was due to hexagonal ZnO nanoplates' increased bioactive surface area. As a result, these nanopartic;es could be used in biomedical applications.**(Upadhyaya *et al*.,2018)**

The green synthesis method was used to create zinc oxide nanoparticles from leaf extracts of the *Costus pictus* D. Don plant. Several characterization tools were used to examine the synthesized nanoparticles. FTIR spectroscopy was used to analyze the functional groups, and the researchers believe that aglycone steroids played a significant role in stabilizing and capping zinc oxide nanoparticles. The nanoparticle's average crystallite size was calculated to be 29.11 nm. SEM and EDX techniques were used to investigate the surface morphology and elemental composition. The nanoparticles' size and structure were confirmed further using TEM and SAED. The average particle size was measured at 40nm. Furthermore, the synthesized nanoparticles demonstrated antibacterial, antifungal, and anticancer activity, and the researchers recommend that this method be used in biomedical applications.**(Suresh *et al*.,2018)**

Atalantia monophylla leaf extract was used in the green synthesis method to create zinc oxide nanoparticles. Several analytical methods were used to characterize the nanoparticles that were created. The absorbance peak in the UV-Vis spectrum was 352 nm, and the peak position in the fluorescence spectrum was 410 nm. The average particle size of the nanoparticles was 33.01 nm, which was comparable to what was observed using TEM. The functional groups were identified, and it was discovered that the amide groups played an important role in the stabilization of the nanoparticles as a capping agent. The spherical shape of the nanoparticles was confirmed by SEM imaging and TEM analysis. The antimicrobial activity of nanoparticles was tested using gram positive and gram negative bacterial strains, and the zone of inhibition was calculated. As a result, biosynthesized nanoparticles could be used in environmental and biomedical applications.**(Vijayakumar *et al*.,2018)**

Green synthesis method was used to make zinc oxide nanoparticles from *Prunus cerasifera* leaf extract. A few characterization tools were used to examine the synthesized nanoparticles. The UV-Vis spectrum revealed an absorbance peak at 380 nm. FT-IR spectra were used to identify various phytoconstituents and functional groups. The average crystallite size of 12 nm was calculated using XRD, and the wurtzite hexagonal structure was determined. The antimicrobial and antifungal activity was investigated, and the zone of inhibition was measured. The synthesized nanoparticles demonstrated effective photocatalytic degradation at a specific crystallite size of 12 nm. As a result, these biosynthesized nanoparticles could be used to clean organic pollutants and in environmental applications.**(Jaffri *et al*.,2018)**

At room temperature (25°C), 230 mL of 0.2 M Zinc acetate dihydrate and 100 mL of green tea leaves extract were used to make ZnO NPs. The microbicidal activity of synthesized NPs (100mg/mL) against pathogenic strains was determined to be 40.05 mm ±0.137 for S. aureus, 36.15 mm ± 0.304 for E. coli, and 40.10 mm ± 0.050 for A. niger. The crystalline structure of the prepared ZnO sample is hexagonal wurtzite, as calculated by the Bragg equation. The purity of ZnO NPs was confirmed by a UV-visible spectral peak at 350 nm, and FTIR results clearly documented the capping, reducing, and stabilizing phytochemicals found in green tea. The XRD diffractogram revealed a characteristic peak of ZnO NPs with sizes ranging from 30 to 40nm, which coalesced to form nanosheets as shown in the SEM images.**(Irshad *et al*.,2018)**

ZnO NPs were synthesized from a parsley extract at different temperatures (room temperature and 90°C) to determine the optimum time for ZnO NP preparation. X-ray diffraction (XRD), scanning electron microscopy (SEM), dynamic light scattering (DLS), and diffuse reflection spectroscopy were used to characterize the samples (DRS). The hexagonal wurtzite structure of the synthesized nanoparticles was revealed by the XRD pattern, which also confirmed their purity. DLS results revealed a single peak with a size of about 50 nm, which confirmed SEM findings. The antibacterial activities of the samples were determined using the agar diffusion method against Escherichia coli (E. coli). For antibacterial activity, a 0.02 molar concentration of sample was used, and the inhibition zone was measured at 4.8 mm at 90°C and 4.3 mm at room temperature.The smaller size of the nanoparticles resulted in higher antibacterial activity at higher temperatures.**(Haji Ashrafi *et al*.,2018)**

The ZnO nanoparticles were synthesized using *Moringa oleifera* (Drumstick) leaves as a natural precursor. According to XRD data, the average crystallite size of zinc oxide was around 52nm.UV-Vis peak obtained at 361 nm conformed to the formation of ZnO NPs. The degradation (%) of different dyes using ZnO NPs was compared in various dye solution pH ranges, and the results showed that synthesized nanoparticles exhibited excellent degradation at the shortest time range. Bacillus subtilis (gram positive) and E. coli (gram negative) bacteria were used to test the antibacterial activity of ZnO nanoparticles. The experimental results indicated that the nanoparticles had an effective growth inhibitory activity against both microorganisms and a strong activity alongside Bacillus subtilis and E. coli with 3.5 and 3.3cm inhibition respectively.
(Pal *et al*.,2018)

Zinc oxide nanoparticles were synthesized using *Tecoma castanifolia* leaf extract and characterized using UV-Vis spectroscopy, TEM, EDX, XRD, and FTIR . The phytochemical constituents of *T. castanifolia* leaf extract were investigated using GC-MS. ZnO NPs derived from *T. castanifolia* leaf extract demonstrated good antibacterial, antioxidant, and anticancer activities due to the synergistic effect of bioactive phytochemical constituents. Green synthesized ZnO NPs were tested for antibacterial activity against Gram positive *(Bacillus subtilis and Staphylococcus aureus)* and Gram negative *(Escherichia coli and Pseudomonas aeruginosa)* bacteria, and the zone of inhibition was determined. It demonstrated superior antibacterial activity against all bacterial strains tested. Furthermore, the results of the antioxidant activity revealed that increasing the concentration of ZnO nanoparticles increased the radical scavenging activity.It had an effective application in nano-drug delivery systems for delivering chemotherapeutic drugs in the body to the targeted site.**(Sharmila *et al*.,2019)**

Prunus dulcis was used to create ZnO nanoparticles (NPs) in a more environmentally friendly manner (Almond gum). The hexagonal phase with wurtzite structure was confirmed by X-ray diffraction analysis. The presence of the Zn band and other vibrational modes in the sample was confirmed by FTIR analysis. Using cyclic voltammetry (CV) behavioural studies, the supercapacitor properties of ZnO NPs were investigated, and the electrolyte was discovered using Electrochemical Impedance Spectroscopy (EIS). Zone inhibition of ZnO NPs on various gram - positive and gram negative, and fungal microorganisms demonstrated excellent antimicrobial effects in antimicrobial studies.**(Anand *et al*., 2019)**

Zinc oxide nanoparticles were produced using the green synthesis method and *Hydnocarpus alpina* alcoholic extract as a capping agent. Several techniques were used to characterize the synthesized zinc oxide nanoparticles. FE-SEM and XRD studies were used to investigate the size and surface morphology of zinc oxide nanoparticles, which revealed that the diameter of the nanoparticle was 38.84nm and in high purity. A GC-MS analysis revealed the presence of 19 phytochemicals, which may act as a capping or reducing agent in the formation of nanoparticles.Furthermore, the nanoparticles were tested against gram positive and gram negative

microorganisms, with the results revealing that the nanoparticles were especially active against *Proteus vulgaris* and *Salmonella entericatyphimurium*. The potential activity of nanoparticles in the scavenging of DPPH free radicals was discovered. Furthermore, a 96% effective photocatalytic activity against methylene blue degradation was observed in basic pH. As a result of these findings, it could be used in biomedical and industrial applications.
(Ganesh *et al.*,2019)

Zinc oxide nanoparticles were prepared using a novel and cost-effective green synthesis method using *Laurus nobilis* plant extract. A few analytical tools were used to characterize the prepared zinc oxide nanoparticles. The average crystallite size of the nanoparticles, which was between 20 and 35 nm, and hexagonal wurtzite structure were determined using XRD. The UV-Vis spectrum revealed a peak in absorbance at 375 nm. FTIR was used to analyze the functional groups present in both plant extract and ZnO nanoparticles, and the results revealed the presence of reducing groups such as polyphenols and flavonoids. The antibacterial activity of the ZnO NPs was also tested against E.coli, and the minimal inhibitory concentration was 1200μgml^{-1}. The photocatalytic degradation of ZnO NPs was also investigated and found to be significant. As a result, the biosynthesized nanoparticles could be used in the applications of pharmaceuticals and cosmetics industries.**(Chemingui *et al.*,2019)**

Strawberry plant leaves extract was used to create zinc oxide nanoparticles. The biosynthesis of ZnO NPs was confirmed using a UV-Vis Spectrophotometer and then characterized using Scanning Electron Microscopy (SEM). The nanoparticles produced antimicrobial activity against the pathogens Pseudomonas aeruginosa and Botrytis cinerea. Different concentrations of ZnO NPs were tested against P.aeruginosa, and the effective concentrations were found to be 26 and 42 mg/ml for non-calcined and calcined ZnO NPs, respectively. Similarly, it demonstrated antifungal activity by inhibiting the growth of B.cinerea, with the inhibition increasing with the concentration of NPs. As a result, it has the potential to be used as an antimicrobial agent against bacterial and fungal diseases.**(Bayat *et al.*,2019)**

Aqueous extract of *Medicago sativa L.* was used to make zinc oxide nanoparticles. Aqueous extract of *Medicago sativa* was investigated phytochemically. Physicochemical properties and antibacterial activity were investigated using a variety of analytical techniques. The average crystallite size of green synthesized ZnO NPs was found to be 13.942 ± 1.083 nm using XRD. The formation of nanoparticles was indicated by EDX, which also confirmed the chemical composition of the samples. According to the authors, the carboxyl group derived from alfalfa proteins and zinc-flavonoids/polyphenol complexes could be primarily involved in the formation of nanoparticles. The antimicrobial potential of the nanoparticles was tested using minimum inhibitory concentration (MIC) against bacteria *(Staphylococcus epidermidis, Lactococcus lactis, and Lactobacillus casei*) and yeast (*Candida albicans and Saccharomyces cerevisiae*) strains. The MIC for bio-ZnO nanoparticles was 0.58-9.31μg/mL.Bio-ZnO nanoparticles showed an inhibitory effect against all tested strains. **(Krol *et al*.,2019)**

Happy *et al*.,2019 investigated the antibacterial activity of the medicinal herb *Cassia alata* in the biosynthesis of ZnO NP. The presence of a strong peak at 320 nm in UV-Vis readings confirmed the synthesis of NPs, and the size, shape, and structure of the nanoparticles were identified using SEM and XRD. The average size range of nanoparticle crystallites was discovered to be 60-80 nm. EDAX was used to investigate elemental composition, and the FT-IR technique was used to identify functional groups. At a concentration of 50 μg/mL, ZnO NPs inhibited the growth of E.coli bacteria. As a result, ZnO NPs could be used in the pharmaceutical, cosmetic, and agricultural industries.

Ogunyemi *et al*.,2019 aimed to investigate the antibacterial activity of zinc oxide nanoparticles (ZnONPs) made from plant extracts of chamomile flower (*Matricaria chamomilla L*.), olive leaf (*Olea europaea*), and red tomato fruit (*Lycopersicon esculentum*). Other characterizations were also performed to investigate its morphological properties. According to SEM observations, *Olea europaea* had the smallest size range of 40.5 to 124.0 nm, whereas TEM and XRD revealed an average size of 48.2 nm. At 16.0mg/ml, zinc oxide nanoparticles synthesized by Olea europaea had the largest inhibition zone of 2.2 cm. The results revealed that the inhibitory effect of ZnONPs increased with concentration. Overall, the results showed that the size of the synthesis ZnONPs was critical for their efficient antibacterial activity, and they were discovered to be an effective antimicrobial agent.

Using *Silybum marianum* L. seed extract, a green microwave-assisted method was used to synthesize ZnO NPs. Crystalline phase, morphology, composition, surface area, optical, and thermal properties were all investigated. The efficiency of the ZnO NPs in treating alloxan-induced diabetic rats was compared to that of the plant extract and insulin treatments. Both ZnO samples were tested for antibacterial activity against E. coli to determine their potential antibacterial application. The MIC was determined to be 0.4 mg/mL. The findings confirmed that green production of ZnO NPs maintained its efficiency for bacterial growth inhibition and, to some extent, enhanced its antimicrobial activities.**(Arvanag *et al*.,2019)**

Nithya *et al*.,2019 used aqueous extract of *C.halicacabum* (0.2, 0.4, and 0.6 mL) at various concentrations to compare chemically synthesized ZnO NPs with biosynthesized ZnO NPs. To investigate its morphological properties, several characterization techniques were used. A study of UV-visible spectrometry revealed surface plasmon resonance at 387 nm. The average particle size determined by dynamic light scattering analysis was 48 nm, and the stability of the formed zinc oxide nanoparticles was confirmed by the zeta potential value. The mean crystallite size of Chem.-ZnO NPs was 65 nm, whereas the estimated sizes of biosynthesized using C.halicacabum leaf extract were 62, 55, and 48 nm at different concentrations (0.2, 0.4, and 0.6 mL). Antibacterial activity against various pathogens was investigated at various concentrations, including Gram-positive bacteria (*S. saprophyticus*, *B. subtilis*) and Gram-negative bacteria (*E. coli and P.aeruginosa*).The results clearly showed that biosynthesized nanoparticles, especially at higher concentrations, inhibited better than chemically synthesized nanoparticles.

Biosynthesis of zinc oxide nanoparticles (ZnO NPs) was synthesized using *Berberis aristata* leaf extract. The antioxidant activity of *B. aristata* leaves extract synthesized ZnO nanoparticles showed 32.06% inhibition at 1 g/ml and 61.63% inhibition at 5 g/ml. The antibacterial activity of the synthesis ZnO NPs was tested using the Agar well diffusion method against eight pathogens: *Klebsiella pneumoniae, B. subtilis, B. cereus, E. coli, Serratia marcescens, Staphylococcus aureus, Salmonella Typhi, and Proteus spp.* The antibacterial activity of nanoparticles was found to be good against six pathogenic bacteria, but not against two pathogenic bacteria, *Proteus spp.* and *S. typhi*. The minimum inhibitory concentration of B. aristata ZnO NPs against *B. subtilis* and *S. marcescens* was reported to be 64 g/ml.Thus,it could be used in many fields including drug, pharmaceuticals and biomedical industries.**(Chandra *et al*.,2019)**

Mentha pulegium L. leaf extract was used to create zinc oxide nanoparticles (ZnO NPs). Its morphology and formation were studied using a variety of characterization techniques. The UV-Vis spectra revealed an absorbance peak at 370 nm, and its purity was confirmed by EDAX, which revealed a high zinc content of 56.26% and oxygen content of 43.74%. The nanoparticles were semi-spherical in shape, with a diameter of 38-49 nm, as shown by the SEM image. Using XRD, the average crystallite size was determined to be 44.94 nm. The antibacterial activity of biosynthesized ZnO-NPs against Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria(*Escherichia coli*) was determined using the disc diffusion method . The results revealed that both Gram positive and Gram negative bacteria demonstrated excellent activity, with the maximum zone of inhibition observed at the concentration of 200 μg/mL.**(Rad *et al*.,2019)**

ZnO NPs were made using a green synthesis method that included orange-peel extract as a reducing agent. The microstructure, morphology, and bactericidal activity of ZnO NPs against *E. coli* and *S. aureus* were found to be significantly affected by annealing temperature and synthesis pH. Without UV light, the bactericidal rate against E. coli was greater than 99.9% for all samples, while the rate against *S. aureus* ranged between 89 and 98%. The bactericidal activity against *S. aureus* varied greatly depending on the synthesis parameters. It provides a straightforward and environmentally friendly method for the green synthesis of ZnO NPs from fruit peel, with the potential to reduce the use of toxic chemicals as well as the cost of nanoparticle production.**(Thi *et al*.,2020)**

Response Surface Methodology was used to optimize the green synthesis of zinc oxide nanoparticles (ZnO NPs) using loquat seed aqueous extract (*Eriobutria japonica*) (RSM). The optimized ZnO NPs were characterized using XRD (X-ray diffraction), EDX (Energy-dispersive X-ray spectroscopy), and FTIR (Fourier-transform infrared spectroscopy). Different doses of the green synthesis ZnO NPs (100, 150, and 200 µg/disc) were loaded on a blank disc and incubated for 24 hours before the bactericidal activity of the ZnO NPs was measured using a Caliper based on the diameter of the zone of inhibition. These nanoparticles' antibacterial activity against some food pathogens revealed a dose-dependent increase in bactericidal activity against Gram-positive bacteria.**(Shabaani *et al*.,2020)**

Using Indian bael (*Aegle marmelos)* juice as fuel, microwave irradiation was used to create zinc oxide nanoparticles (ZnO NPs). The excitation wavelength was 370 nm, and the emission peaks were 388 and 468 nm, corresponding to Zn vacancies and O vacancies, respectively. Under UV irradiation, the ability of the synthesized zinc oxide nanoparticles to degrade methylene blue dye was tested. The dye removal efficiency of nanoparticles after 35 minutes of UV (λ=617 nm) irradiation was 96%. ZnO nanoparticles were tested for antimicrobial activity against various strains. Their findings revealed that all of the nanoparticles exhibited a wide range of antibacterial activity by inhibiting the growth of food-borne pathogens (Gram positive and Gram negative), providing a foundation for future research.**(Mallikarjuna Swamy *et al*.,2020)**

Cinnamomum verum plant extract was used in the green synthesis of zinc oxide nanoparticles. Green synthesis ZnO NPs inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* with MICs of 125 g mL^{-1} and 62.5 g mL^{-1}, respectively. ZnO-NPs inhibited test pathogens more effectively than plant extract. SEM images revealed a specific particle agglomeration, which was confirmed by TEM studies. As a result, the prepared ZnO NPs may be useful as an antimicrobial agent against harmful pathogens.**(Ansari *et al*.,2020)**

Zinc oxide nanoparticles were produced using four different plant extracts (ZnO-NPs). According to TEM images, the ZnO NPs synthesized from *Beta vulgaris* (BE1), *Cinnamomum tamala* (VA1), *Cinnamomum verum* (PA1), and *Brassica oleracea* var. *italica* (BR1) were spherical, whereas those obtained from *Cinnamomum tamala* were ZnO nanotubes. They demonstrated biological activity when tested for antimicrobial activity against *E. coli, S. aureus, Candida albicans,* and *A. niger*. BE1, VA1, PA1, and BR1 were found to be active against *E.coli*, and VA1, PA1, and BR1 were found to be active against *S.aureus*, but BE1 was inactive. Further research in the fields of water remediation and photocatalytic properties should be conducted.**(Pillai *et al*.,2020)**

Zinc oxide nanoparticles were created using an aqueous extract of *Ailanthus altissima* fruit.The nanoparticles prepared ranged in size from 5 to 18 nm on average.
The FT-IR analysis revealed that the obtained ZnO-NPs were stabilized by interactions with flavonoids and phenolic compounds in the fruits extract and demonstrated antibacterial activity against two bacterial strains, *E. coli* and *Staphylococcus aureus,* and were considered a potential additive to substitute toxic chemical and physical antibacterial materials.**(Awwad *et al*.,2020)**

Synthesis of ZnO nanoparticles using *Euphorbia hirta* leaves extract was done in a green way. The leaf extract acts as a biological reducing agent in the synthesis of zinc nitrate-derived ZnO nanoparticles. Various analytical methods were used to characterize the nanoparticles.UV-Visible spectrum revealed a strong absorption peak at 370 nm, and the functional group was investigated using FT-IR spectroscopy. XRD technique reflected the hexagonal phase of ZnO.The size of the nanoparticle was determined using SEM to be between 20 and 25 nm. Using the disc diffusion method, antibacterial activity against various bacterial strains such as *Streptococcus mutans, Streptococcus aureus, Clostridium absonum, Escherichia coli,* and *Proteus mirabilis* was studied, and the zone of inhibition was measured.The zone of inhibition increased with the increase in the concentration of ZnO nanoparticles.Similarly antifungal activity was also performed against few fungal strains.Hence the authors concluded that this cost effective method could be used for biomedical applications.**(Ahamad *et al*.,2020)**

Zinc oxide nanoparticles were created using *Veronica multifida* leaf extract and zinc acetate dihydrate (ZnO NPs). Different spectroscopic techniques were used to evaluate the properties of the synthesized nanoparticles, including size, shape, and functional groups. UV-Vis spectral scanning was performed at wavelengths ranging from 200 to 800 nm, and functional groups were identified using the FT-IR technique. XRD, SEM, and TEM images confirmed the crystal structure and average size of ZnO NPs. ZnO NPs had an average crystallite size of 29.5 nm. The antimicrobial activities of ZnO NPs were evaluated using the disc diffusion method. Various concentrations of ZnO NPs (5, 2.5, 1, 0.5, and 0.25 mg mL^{-1}) were applied to the inoculated agar plate surface and inoculated at 35^0C for 16-20 h.Minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) assays revealed that ZnO NPs had a strong bacteriostatic effect but are not bactericidal on both gram-positive and gram-negative bacteria at the concentrations used. Bactericidal activity required higher concentrations.**Dogan *et al*.,(2020)**

The authors synthesized NPs-PL and NPs-PF, which were synthesized using aqueous extracts of *Punica granatum* (pomegranate) leaves and flowers respectively. Various spectroscopic techniques were used to characterize the synthesized nanoparticles. Pomegranate leaf and flower extract absorption bands were 284 and 357 nm, respectively, according to UV-Vis analysis. Pomegranate leaf-mediated zinc oxide nanoparticles and pomegranate flower-mediated zinc oxide nanoparticles had crystalline particle sizes of 57.75 and 52.50 nm, respectively. The antibacterial activity of biosynthesized zinc oxide nanoparticles against 13 pathogenic strains was investigated using the agar well diffusion method. Depending on the bacterial strain, the minimum concentration of zinc oxide required to effectively inhibit the growth of microorganisms used in the study ranged from 0.6 μg/mL to 2500 μg/mL.**Ifeanyichukwu *et al*.,(2020)**

The antibacterial and photocatalytic properties of ZnO nanoparticles derived from *Prosopis juliflora* leaf extract were studied using a hydrothermal method at 170°C. Several techniques were used for characterization, and FESEM and HRTEM revealed that the ZnO particle size was 65nm and its morphology was spherical and hexagonal in shape. Under UV light, the ZnO nanoparticles effectively degraded the methylene blue dye. Antibacterial activities against *E. coli, R. Rhodochrous, B. Subtilis,* and *V. Cholera* were tested. This bio-synthesized nanoparticles had better MIC values against various pathogens and could be used as an antibacterial agent. The nanoparticle concentration was varied between 50 and 100 μg/ml, with the highest activity detected at 100 μg/ml. A larger zone of inhibition of 25 mm was established.In comparison to other gram-positive bacterial strains, the gram-negative bacteria *V. Cholera* had a larger zone of inhibition of 25 mm.**Mydeen *et al*.,(2020)**

Agarwal *et al.*, synthesized zinc oxide nanoparticles (ZnO NPs) and investigated the antibacterial potential of *Mucuna pruriens* seed extract functionalized ZnO NPs (ZnO NPs). The formation of nanoparticles was confirmed using UV-Vis spectroscopy, with a peak at 344 nm. SEM images revealed that the average size of the nanoparticle was 60 nm. The FT-IR technique was used to examine the functional groups. The broth dilution technique was used to conduct an antibacterial test against *Bacillus subtilis*. The results showed that the MIC concentration was 20 μg/mL and the IC50 concentration was 70 μg/mL. *Bacillus subtilis* growth was inhibited by ZnO NPs in a concentration-dependent manner. As a result, ZnO NPs have the potential to be used in medical applications against multi-drug resistant bacteria.**Agarwal *et al*.,(2020)**

Capparis zeylanica leaf extract was used to create zinc oxide nanoparticles (ZnO NPs). Various characterization techniques were applied to these bio-synthesized nanoparticles. The nanoparticles produced were pure, spherical, and had an average size of 32-40 nm. Methylene blue dye was degraded. Using the agar well diffusion method, ZnO nanoparticles were tested for anti-microbial activity against Gram-positive bacteria (*Staphylococcus epidermidis, Enterococcus faecalis*), Gram-negative bacteria (*Salmonella paratyphi, Shigella dysenteriae*), and fungi (*Candida albicans, Aspergillus niger*). The maximum inhibition zone for the ZnO samples was recorded by *S. epidermidis* (423.78 mm) at concentrations of 100 g/ml. As a result, biosynthesized ZnO nanoparticles could be used in drug delivery and photocatalytic applications.**Nilavukkarasi *et al*.,(2020)**

The green synthesis method was used for the synthesis of zinc oxide nanoparticles by using algae extract of *Tetraselmis indica*.The prepared nanoparticles were characterized by various analytical tools.The absorption peak showed by the UV spectrum was at around 370 nm.Using XRD,the mean crystallite size of the nanoparticles were calculated and it was found to be 27 nm.By FT-IR,the absorption peak obtained at 470cm^{-1} confirmed the presence of ZnO nanoparticles.Using EDAX, the elemental composition of 74.05% zinc and 25.95% oxygen was noted.The size and agglomeration of the nanoparticles were analyzed by using SEM and TEM.Antibacterial activity against various gram positive and gram negative bacterial strains were performed and the zone of inhibition was measured.The nanoparticles were resistant to *S.aureus* and *E.coli* in a selective manner. Similarly, the nanoparticles demonstrated hemolytic and free radical scavenging activity. The findings indicated that these nanoparticles could be used in the textile, biomedical, cosmetics, and food packaging industries.**(Thirumoorthy *et al*.,2021)**

The green synthesis method was used to create zinc oxide nanoparticles from *Cassia auriculata* leaf extract. As a precursor, zinc acetate was used. The antibacterial activity was investigated. Several other characterizations of the prepared nanoparticles were also performed. The UV-Vis spectrum revealed a band gap value of 3.3 eV, indicating a red shift with an absorption peak at 376 nm. Photoluminescence analysis was used to investigate the emission properties of the nanoparticles. The average crystallite size of the nanoparticles was calculated using the Debye Scherrer equation and XRD data to be 24-30 nm. The capping agent was identified by analyzing it with FTIR spectroscopy The elemental composition of the ZnO NPs was determined using EDAX, and the results revealed that 65% of Zn and 25% of O were present. Significant antibacterial activity was observed against the bacterial strains. As a result, the green nanoparticles created may have a wide range of applications in the food processing industries.**(Ramesh *et al*.,2021)**

The authors synthesized zinc oxide nanoparticles (ZnO NPs) using a leaf extract of *Parthenium hysterophorus*. SEM and TEM images revealed the surface morphologies of spherical ZnO nanoparticles with a size range of 10 nm. The hexagonal wurtzite structure of ZnO NPs was revealed by X-ray diffraction structural analysis. Characterizations in UV-Vis and FT-IR were also performed. Both bacterial and fungal growth were effectively inhibited by these nanoparticles. Umavathi et al.

concluded that antimicrobial activity increased with increasing ZnO nanoparticle concentration, and the zone of inhibition was dependent on species specificity and particle size of ZnO nanoparticles (10 nm). Because of the high surface and volume-relationship, this had a significant impact on antimicrobial activity.**Umavathi *et al*.,(2021)**

Thymbra spicata (TS) leaf extract was used to create nanostructured ZnO using the Successive ionic layer adsorption and reaction (SILAR) method. The crystallite sizes of the green synthesized samples were determined by XRD to be in the range of 15.58 nm to 18.09 nm. SEM images of ZnO samples revealed hexagonal wurtzite structure. Seed-borne plant bacterial disease agents were tested for antibacterial activity. The mean zone of inhibition in *C. michiganensis subsp. Michiganensis* was 28.25–37.25 mm, 13.50–22.13 mm in *P. cichorii*, 15.0–21.92 mm in *P.syringae pv. Phaseolicola*, and 11.37–18.12 mm in *P. caratovorum subsp. Carotovorum*. Green synthesized nanostructured ZnO had significantly higher inhibition zones than pure ZnO nanoparticles against all tested bacterial strains based on inhibition zone diameter values.As the bacterial agent is known to be transmitted through seeds, the authors concluded that seed treatment is one of the most appropriate ways of applying green synthesized nanostructured ZnO for the management of seed-borne bacterial diseases. **(Sahin *et al*.,2021)**

Alvarez-Chimal *et al.,* created zinc oxide nanoparticles (ZnO-NPs) and investigated their structural and antibacterial properties against *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli,* and *Pseudomonas aeruginosa*, as well as bacteria commonly found in human mouths like Aggregatibacter actinomycetemcomitans. Most of the bacterial strains were sensitive to both synthesis and commercial ZnO-NPs, producing zones of inhibition with the same diameter, but *Prevotella intermedia* was the most sensitive, with larger zones of inhibition. Several characterization techniques were used to determine its shape and size, and the results revealed that ZnO-NPs with a quasi-spherical shape in the size range of 5-30 nm were synthesised.**(Alvarez-Chimal *et al*.,2021)**

ZnO NPs were synthesized using *Ruellia tuberosa* for antimicrobial and bioremediation purposes. The green synthesis ZnO NPs were tested against pathogenic *E. coli* and *S. aureus* using the agar well diffusion method, and it was discovered that when compared to larger sized particles, smaller sized ZnO NPs directly penetrated the cell, damaged the membrane, causing oxidative stress, and ultimately leading to cell death via apoptosis. Smaller NPs with a higher surface area generated more hydroxyl radicals, resulting in increased photocatalytic activity. Antibacterial activity and characterization of various NP coated cotton fabrics revealed that they were effective against both gram negative and gram - positive pathogens.**(Vasantharaj *et al*.,2021)**

Zinc oxide nanoparticles (ZnO-NPs) were synthesised from aqueous fruit extracts of *Myristica fragrans*. The synthesised NPs had significant larvicidal activity (77.3±1.8) against *Aedes aegypti*, the mosquito that transmits dengue fever. At 400 g/mL, the maximum inhibition was calculated to be approximately 73.23±0.42 for -amylase and 65.21±0.49 for -glucosidase. Because ZnO-NPs are biocompatible, they could be used in therapeutic applications. The anti-bacterial activity of ZnO-NP-coated ciprofloxacin, imipinem, vancomycin, and amoxicillin-clavulanic acid antibiotics increased to 32.2, 27.3, 15.8, and 22.2% against *E. coli*; 41.4, 31.8, 15.8, and 23.9% against *K. pneumoniae*; 35.3, 24.1, 10.5, and 11.2% against *P. aeruginosa*; and 55.8, 30, 55.7, and 12.8% against *S. aureus*, respectively.Using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, different concentrations of biogenic ZnO-NPs from 25 to 400 g/mL were tested for antileishmanial activity against amastigotes and promastigotes, and it was discovered that at the highest concentration of 400 g/mL, the NPs possessed the potent mortality rate of 71.50±0.70 for promastigotes and 61.41±0.71 for amastigotes.**(Faisal *et al*.,2021)**

The metabolites found in the cell filtrate of *Arthrospira platensis,* a newly isolated and identified microalga, were used to successfully synthesize zinc oxide nanoparticles (ZnO-NPs). EDX analysis confirmed the presence of Zn and O in the nanostructure. Data analysis revealed that the activities of biosynthesized ZnO-NPs were dose-dependent. When used as an antimicrobial agent, they formed clear zones with diameters of 24.1±0.3, 21.1±0.06, 19.1±0.3, 19.9±0.1, and 21.6±0.6 mm against

Bacillus subtilis, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans*, respectively. These activities decreased as the concentration of NPs decreased. ZnO-NPs were more effective in vitro against cancerous (Caco-2) cells (IC50 = 9.95 ppm) than normal (WI38) cells (IC50 = 53.34 ppm).**(El-Belely *et al*.,2021)**

Alcohol-free *Artemisia pallens* plant extract as a reducing agent,was used to create zinc oxide nanoparticles (ZnO NPs). FT-IR was used to identify the Zn-O vibrational band at 478.34 cm^{-1}. XRD, SEM, and TEM analysis confirmed the nano size and crystalline nature of ZnO nanoparticles, and energy dispersive X-ray spectroscopy confirmed the composition, and the crystallite size calculated using the Debye-Scherer equation was found to be 23-27 nm. The antibacterial activity of the synthesis ZnO NPs was tested using the zone inhibition method against gramme positive *Bacillus subtilis, Staphylococcus aureus,* and gram negative *Eschercia coli*. The zone of inhibition for *B. subtilis, S. aureus,* and *E. coli* was measured to be 12 mm, 7 mm, and 6 mm, respectively. The resulting ZnO nanoparticles could be used as nano-carriers in anti-cancer drug delivery system.**Gomathi *et al*.,(2021)**

ZnO nanoparticles were synthesized from date pulp waste (DPW) using a green synthesis method that produces no byproducts and is environmentally friendly. The primary application of these DPW synthesized NPs was in wastewater treatment, which also aided in the solution of the dry pulp waste disposal problem. According to the photoluminescence study, the violet-blue emission properties of DP-ZnO-NPs favour their use in fluorescence labelling applications. The IR spectrum of the Zn-O bond was distinguished by a distinct peak at 445 cm^{-1}. The average crystallite size for the DP-ZnO-NPs was 29.3 nm using Debye Scherrer's equation and XRD data. The hexagonal wurtzite phase of ZnO was indicated by the Raman high-intensity peak at 440.2 cm^{-1}. The ZnO NPs were found to be pure, with no impurities, according to X-ray photoelectron spectroscopy.According to SEM analysis, the particles were uniform and there was no agglomeration. The results of the TEM also revealed that the particles were spherical. The EDX spectrum elemental analysis revealed that the samples contained 63.3% Zn and 36.7% O, indicating that they were made up of single-phase ZnO constituents. Because of the presence of oxygen reactive species (superoxides, hydroxyl radicals, and hydrogen peroxide) on their surface, DP-ZnO-NPs containing DPW extract demonstrated excellent photocatalytic activity for dye degradation as well as antibacterial activity. Other applications, such as energy storage, optical, and biosensing, necessitated additional research..**(Rambabu *et al*.,2021)**

Utilizing an extract from *Eucalyptus lanceolatus* (leaf litter), zinc oxide nanoparticles (ZnO NP) were created and then variously characterized. A hexagonal structure with an average size of 100 nm was visible in the TEM image. Agar well diffusion was used to test the antimicrobial activity of ZnO NPs against pathogens (*Bacillus subtilis* and *Pseudomonas aeruginosa)* that caused diseases in maize plants. Fungicidal activity against *Fusarium oxysporum, Pencillium chrysogenum,* and *Bipolaris bicolor* grown on PDA medium was tested and found to be effective. The results showed that the antibacterial activity of ZnO NPs increased significantly with increasing ZnO NP concentrations. Thus, the antimicrobial activity of ZnO NPs in the current study demonstrated their potential for effective management of biotic stresses caused by microbial infection in maize plants.**Sharma *et al*.,(2022)**

Zinc oxide nanoparticles were biosynthesised using the plant extract *Foeniculum vulgare*. The colour of aqueous zinc ions changed from yellow to white when they were reduced into zinc oxide nanoparticles by a medicinal plant extract of *Foeniculum vulgare*. The formation of ZnO NPs was indicated by this colour shift. The nanoparticles were characterized using FTIR, SEM, EXD, SEM, and XRD. FTIR spectra revealed carbonyl and hydroxyl functional groups at 1010 cm^{-1} and 3550 cm^{-1}, respectively. The size and colour of the NPs, which were white and 22.94 nm in size, were examined using SEM. The diameter of NPs was calculated using the Debye-Scherrer equation using the XRD technique.. The EXD analysis revealed the presence of 24.31% oxygen and 75.69% zinc. Both crude plant extract and ZnO NPs were subjected to a phytochemical screening test, with the NPs exhibiting higher levels of flavonoids, alcohol, and phenolic content. Antioxidant and antibacterial studies were also conducted, with the NPs serving as a potential antioxidant agent but showing less antibacterial potential when compared to the crude plant extract.**(Khoso *et al*.,2022)**

Zinc oxide nanoparticles were synthesized from leaf extracts of *Aloe vera (AV), Azadirachta indica (AI),* and *Amaranthus dubius (AD)* using a simple green synthesis process. XRD revealed hexagonal phases in AV-ZnO, AI-ZnO, and AD-ZnO with crystallite sizes of 35.8 nm, 30.83 nm, and 33.1 nm, respectively. UV-Vis characteristic absorption ranged from 200 to 450 nm. The ZnO NPs were rod-shaped with a rough spherical appearance, as revealed by FESEM and TEM images. Antimicrobial activity of synthesized AV-ZnO, AI-ZnO, and AD-ZnO NPs against gram-positive (S. aureus and B. subtilis) and gram-negative (P. aeruginosa and E. coli) bacterial strains was tested. For all of the tested pathogens, the diameters of the inhibitory zones of the AD-ZnO NPs increased when compared to the AI-ZnO and AV-ZnO NPs.**Almarhoon *et al*.,(2022)**

Green synthesis was used to produce zinc oxide nanoparticles from *Thymbra Spicata* L. plant extract. A few analytical methods were used to characterize the nanoparticles that were produced. SEM images were used to examine the size and surface morphology of zinc oxide nanoparticles. Apart from zinc, elemental analysis by EDX revealed the presence of metals. XRD analysis confirmed the nanoparticles' hexagonal wurtzite structure. The FTIR technique was also used to identify the functional groups that were present. The zinc oxide nanoparticles' UV-Vis spectrum revealed an absorbance peak at 360 nm. The DPPH test was used to analyze the antioxidant activity of the nanoparticles, and the DPPH Radical scavenging activity of Ts-ZnO NPs at 250 μg/mL was found to be 79.67%. The antimicrobial activity of the nanoparticles synthesized against various pathogens was determined.As a result, the synthesis ZnO nanoparticles could be used as an antimicrobial, antioxidant, and environmental and biomedical agent.**(Gur *et al*.,2022)**

Aqueous extract of *Phoenix roebelenii* palm leaves was used in a green chemistry approach to synthesize zinc oxide nanoparticles (ZnO NPs). X-ray powder diffraction (XRD), diffuse reflectance spectroscopy (DRS), high-resolution transmission electron microscopy (HRTEM), selected area electron diffraction (SAED), simultaneous thermogravimetric analysis (TGA) and differential thermal analysis (DTA), photoluminescence (PL) spectroscopy, and Fourier transform infrared (FTIR) spectroscopy were used to investigate the physical properties of the nanoparticles. The antibacterial activity of ZnO NPs was significant, with maximum inhibition zones of 21.4 mm for *S. typhi*, 15.8 mm for *E. coli*, 15.1 mm for *S. pneumoniae*, and 15 mm for *S. aureus*. The antibacterial activity of the as-prepared ZnO NPs was concentration dependent.When compared to gram-positive bacteria, this biosynthesized sample performed better against gram-negative bacteria. As a result, they have the potential to be used as an effective antibacterial agent in industrial and pharmaceutical applications such as food preservation packaging.**(Aldeen *et al*.,2022)**

Acalypha indica leaf extract was used to create zinc oxide (ZnO) nanoparticles, and their photocatalytic degradation and antibacterial properties were measured and investigated. The crystallinity and phase of synthesized ZnO nanoparticles were determined by assigning the XRD pattern to planes (100), (022), (101), (102), (110), (103), and (200) that correspond to hexagonal Wurtzite structure. Synthesized ZnO nanoparticles exhibited antibacterial properties against four bacterial strains, including *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Escherichia coli*, with the highest zone of inhibition observed against *Escherichia coli* (25.2 mm). This biosynthesized ZnO nanoparticles exhibited greater antibacterial activity due to their small size and stability, and the antibacterial activities of smaller nanoparticles were greater than that of bulk nanoparticles due to their higher surface area..**(Kamarajan *et al*.,2022)**

Zinc oxide (ZnO) NPs were synthesized using an aqueous leaf extract of *Pelargonium odoratissimum* (L.) as a reducing agent. With an IC50 value of 28.11±0.01 mL-1, the DPPH experiment demonstrated ZnO nanoparticles' ability to scavenge free radicals. ZnO NPs were discovered to have superior antibacterial properties against both GNB and GPB in pharmaceutical and biomedical research, as well as being more effective as an anti-inflammatory. The hexagonal wurtzite crystalline structure of ZnO NPs was supported by the peaks in the XRD pattern and the diffraction rings on the SAED image.**(Abdelbaky *et al*.,2022)**

Three different leaf extracts of *Aloe vera* (AV), *Azadirachta indica* (AI), and *Amaranthus dubius* (AD) were used to synthesize zinc oxide nanoparticles using a cost-effective and simple green synthesis method . Various techniques were used to investigate the structure, size, shape, and bandwidth of the synthesized nanoparticles. XRD studies revealed that the sizes of the three samples, AV-ZnO, AI-ZnO, and AD-ZnO, were 35.8 nm, 30.83 nm, and 33.1 nm, respectively. UV studies also yielded band gap energies of 3.10 eV, 3.12 eV, and 3.07 eV, respectively. The surface morphology of the nanoparticles was examined using TEM and FESEM, and the results revealed that the nanoparticles were rod-shaped and spherical in appearance.EDAX's elemental analysis confirmed the presence of Zn and O. Antibacterial activity was tested against gram positive and gram negative bacteria, and the nanoparticles were especially effective against a few gram negative and gram positive bacterial strains, with AD-ZnO showing the highest inhibition zone among these three extracts. Hence,it could be used in biomedical applications.**(Almarhoon *et al*.,2022)**

Table 2.1 -Plant-mediated synthesis of ZnO NPs from various leaves extracts

S.No	Plant	Common name	Part taken for extraction	Size (nm)	Shape	Reference
1	*Citrus sinensis*	Orange	Dried fruit peel	12(XRD)	Hexagonal wurtzite	**Lalithama *et al.*,(2018)**
2	*Tecoma castanifolia*	Yellow bells	Fresh leaves	70-75(TEM)	Hexagonal wurtzite	**Upadhyaya *et al.*,(2018)**
3	*Eriobutria japonica*	Loquat	Dried seeds	18-27(FE-SEM) 14.81 (XRD)	Hexagonal wurtzite	**Suresh *et al.*,(2018)**
4	*Prunus dulcis*	Almond gum	Gum	30(XRD) 25(HR-SEM)	Hexagonal wurtzite	**Vijaykumar *et al.*,(2018)**
5	*Aegle marmelos*	Indian bael	Fruit pulp	17(XRD) 20(TEM)	Hexagonal wurtzite	**Jaffri *et al.*,(2018)**
6	*Cinnamomum verum*	Cinnamon	Bark	30-40(XRD)	Hexagonal wurtzite	**Hajiashrafi *et al.*,(2018)**
7	*Syzygium Cumini*	Java plum	Fresh leaves	10-12.55 (XRD)	Hexagonal wurtzite	**Sadiq *et al.*,(2021)**
8	*Hibiscus sabdariffa*	Hibiscus	Dried flower 1%,4%, 8%	38.63, 9.05, 8.71(XRD) 5-40 (TEM)	Hexagonal wurtzite	**Soto Robles *et al.*,(2019)**
9	*Tilia Tomentosa*	Ihlamur	Dried leaves	22(XRD) 80(SEM)	Hexagonal	**Shashanka *et al.*,(2020)**
10	*Averrhoe Carrambola*	Star fruit	Dried fruit	19.6(XRD)	Hexagonal wurtzite	**Begum *et al.*,(2018)**

MATERIALS AND METHODS

MATERIALS AND METHODS

The methodology pertaining to the title "Green Synthesis of Zinc Oxide Nanoparticles using ***Zephyranthes candida*** leaves" is presented in this chapter.Zinc oxide nanoparticles were synthesized through green synthesis using plant extract. Zinc acetate dihydrate, plant extract,sodium hydroxide and ethanol were used for green synthesis. ***Zephyranthes candida*** leaves were collected from our university campus and confirmed with the help of the botany department and they dried for one week.

Green synthesis

Green synthesis of zinc oxide nanoparticles were done with ***Zephyranthes candida*** leaves extract.

Preparation of leaf extract

Fresh leaves of ***Zephyranthes candida*** were thoroughly cleaned with running tap water to remove debris and other contaminants, 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 whatMan filter paper. The extract was stored in a refrigerator in order to be used for further experiments.

3.1 Phytochemical screening test

Phytochemical examinations were carried out for the extract as per standard methods (Tiwari et al., 2011)

1. Detection of alkaloids: Extracts were dissolved individually in distilled water 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 a-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 hydrolyzed 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 hydrolyzed 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 ammoniacal layer indicates the presence of anthranol glycosides.

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

4. 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.

5. 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 2 golden 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 the brown ring at the junction indicates the presence of phytosterols.

6. 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.

7. 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.

8. 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.

9. Detection of proteins and amino acids

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 a few minutes. Formation of blue colour indicates the presence of amino acid.

ZnO Synthesis from plant extract

For the synthesis of ZnO nanoparticles, 50 ml of 0.5M zinc acetate dihydrate solution was prepared using distilled water. 1 ml 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 the oven. **(Snehal yedurkar *et al.*, 2016)**

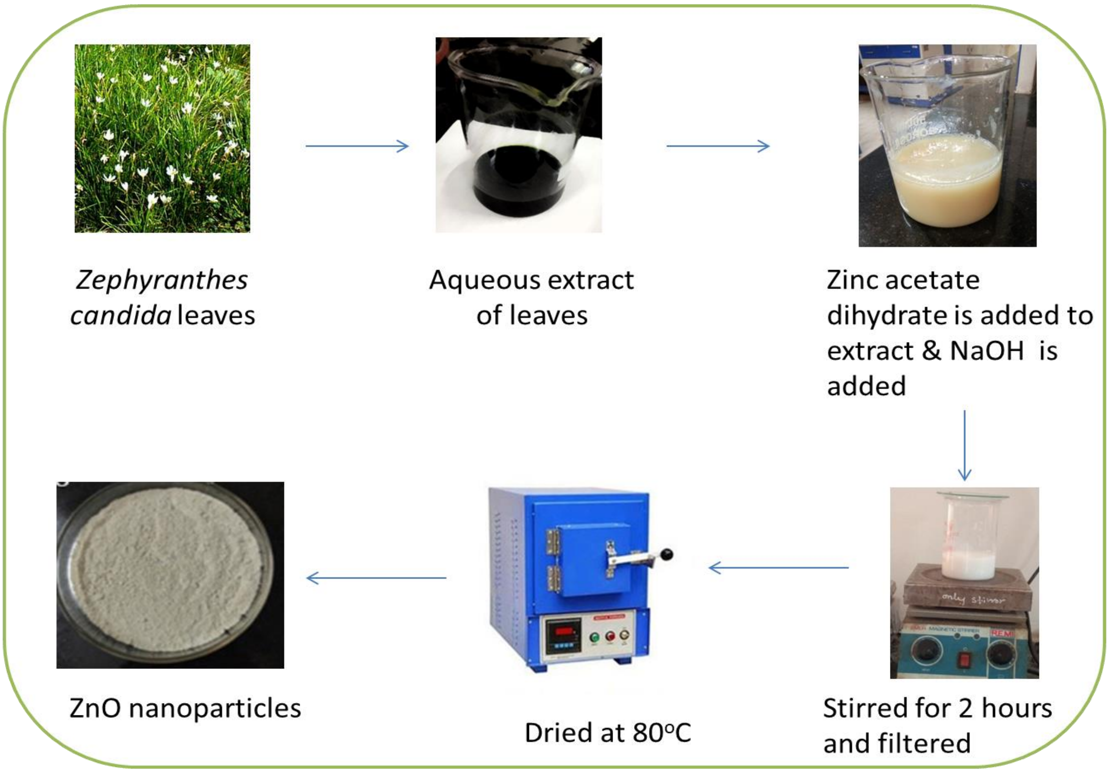

Fig 3.3.1 Biosynthesis of ZnO nanoparticles using *Zephyranthes candida* leaves extract

3.2 Characterization of synthesized ZnO nanoparticles

Biogenically synthesized ***Zephyranthes candida*** ZnO nanoparticles were characterized by using the UV(UV-visible),XRD (X-ray Diffraction), FT-IR(Fourier Transform Infrared),Cyclic Voltammetry(CV),3-D Optical Profilometer and Thermogravimetric Analysis(TGA).Antibacterial tests are also carried out to determine the potential activity of the synthesized nanoparticles against different bacterial strains.

UV-visible analysis

UV–visible spectroscopy was used to examine the bio-reduction of aqueous zinc ions to metallic ZnO NPs and the optical characteristics of the ZnO NPs. The UV absorption spectra were obtained using distilled water as a blank with wavelengths ranging from 200 to 800 nm

FT-IR analysis

Several functional groups present in the ZnO NPs were identified using Fourier transform infrared spectroscopy (FTIR). The FTIR spectrum was obtained by placing powdered ZnO NPs on a KBr pellet and examining it in the wavelength range of 4000–400 cm^{-1}.

3D Optical Profilometry

LASER and AFM analyses have given us insight about the topography, roughness of nanoparticles. LASER imaging was conducted in different magnification range images clearly demonstrating smooth nanoparticles with capping of phyto- chemicals over the surface of nanoparticles **(Santhosh Kumar j.*et al.*, 2017)**. It is commonly used for obtaining colloidal solutions of nanoparticles. This analysis also helps in determining morphology and surface studies.

XRD

X-ray diffractometer (XRD) was used to evaluate the crystalline nature of the ZnONPs produced using a biological method. The XRD (XRD-6000 Shimadzu, Japan) was equipped with a nickel filter and a Cu-kα (λ = 1.5412 A°) radiation source. The diffraction angle varied at 40 kV and 30 mA. The crystallite size (D) of selected samples was estimated using Scherer's equation $D = K\lambda / \beta\cos\theta$,where D is the mean grain size, K is the constant (0.98), λ is the wavelength of the X-ray, β is the full width at half maximum (FWHM) width of the diffraction peak and θ is the diffraction angle.

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.

Antibacterial study

Procedure: Growth Method (Kirby-Bauer method)

The growth method is performed as follows,

At least three to five well-isolated colonies of the same morphological type are selected from an agar plate culture. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as Nutrient broth.The broth culture is incubated at 35^0C until it achieves or exceeds the turbidity (usually 2 to 6 hours).The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain turbidity. This results in a suspension containing approximately 1 to 2 x 10^8 CFU/ml for Staphylococcus aureus, Bacillus subtilis, Escherichia coli and pseudomonas aeruginosa.

<u>Inoculation of Test Plates</u>

Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level.This will remove excess inoculum from the swab.The dried surface of a Nutrient agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times,rotating the plate approximately 60 each time to ensure an even distribution of inoculum. As a final step, the rim of the agar is swabbed.The lid may be left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.The media was punctured by making a well of 6 mm in diameter and filled with 50µl of a sample. Further the petri plates were placed inversely for complete diffusion and inhibition zones were examined by measuring the diameter (mm) formed around the well after 24 hrs incubation at 37°C. The zones were measured by using standard (Hi-Media) scale.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

The present investigation entitled **"Green synthesis of Zinc Oxide Nanoparticles using *Zephyranthes candida* leaves"** deals with the synthesis of Zinc oxide nanoparticles from *Zephyranthes candida* leaves extract.Characterization studies were carried out with the synthesized nanoparticles.Antibacterial activity of the zinc oxide nanoparticles was also studied using four different bacterias.

4.1 Qualitative Phytochemical Analysis

Plant extract is commonly employed as a potential substitute for stabilising and reducing agent due to the presence of various essential bio-components such as terpenoids, alkaloids, phenolics, tannins, proteins, amino acids, polysaccharides, enzymes, vitamins, and saponins. The phytochemical analysis of the plant extract of ***Zephyranthes candida*** leaves is shown in table 4.1.1.

Phytochemical screening of the *Zephyranthes candida* leaf extract shows the presence of alkaloids, flavonoids, glycosides, saponins and tannins (Table 4.1.1). **Shitara *et al.*,2014** has also reported that the phytochemical analysis of the crude methanolic extract of *Zephyranthes candida* indicated the presence of Flavonoids, Glycosides, Saponins, Alkaloids and Tannins.

S.No.	**Chemical constituent**	**Phytochemicals test**	**Result**
1	Carbohydrates	Fehling's test	-
		Molisch's test	
2	Alkaloids	Dragendorff's test	+
3	Glycoside	Legal's test	+
4	Phenolic test	Ferric chloride test	-
5	Tannins	Gelatin test	+
6	Saponins	Foam test	+
7	Flavonoids	Alkaline reagent test	+
		Lead acetate test	+
8	Phytosterols	Salkowski's tets	-
9	Proteins	Xanthoproteic test	-
10	Amino acid	Ninhydrin test	-

+ presence - absence

Table 4.1.1- Phytochemical constituents of *Zephyranthes candida* leaf extract

Presence of OH groups in flavonoids can reduce the zinc compounds into ZnO NPs and also act as capping or stabilizing agent of the NPs. Therefore, separate capping or stabilizing agents are not necessary in this synthesis NPs approach **(Umamaheshwari *et al*.,2021).** In the reduction process, it is assumed that zinc acetate acts as the precursor whereas the leaf extracts acts as reducing agent. Reactions involved in the formation of ZnO NPs from zinc acetate are shown by **(Happy *et al*.,2019)** as

$Zn(CH_3COO)_2.2H_2O + 2NaOH \rightarrow Zn(OH)_2 + 2CH_3COONa + 2H_2O$

$Zn(OH)_2 + 2H_2O \rightarrow Zn^{2+} + 2OH + 2H_2O \rightarrow [Zn(OH)_4]_2$

$[Zn(OH)_4]_2 \rightarrow ZnO_2^{-2} + 2H_2O$

$ZnO_2^{-2} + 2H_2O \rightarrow ZnO + 2OH$

Saponins may be responsible to produce the stable nanoparticles, because saponins might be the surface-active molecules stabilizing the nanoparticles.Also,other organic molecules such as alkaloids and tannins are responsible for the reduction of the zinc ions and the formation of stable nanoparticles.Based on the results of phytochemicals screening, we concluded that the formation of ZnO nanoparticles using the plant extracts may be primarily due to the presence of flavonoids,glycosides and alkaloids.

Fig.4.1.1 Phytochemical examinations of *Zephyranthes candida* plant extract

4.2 UV-Visible Spectroscopy

The absorption spectra of zinc oxide nanoparticles were recorded using a double beam double beam spectrophotometer.The green synthesis of zinc oxide nanoparticle synthesized using *Zephyranthes candida* leaf extract was analyzed by measuring the UV-Visible spectrum which clearly indicated intense characteristic absorption peak at **251 nm** which is the characteristic band of ZnO **(Balogun *et al*.,2020).**

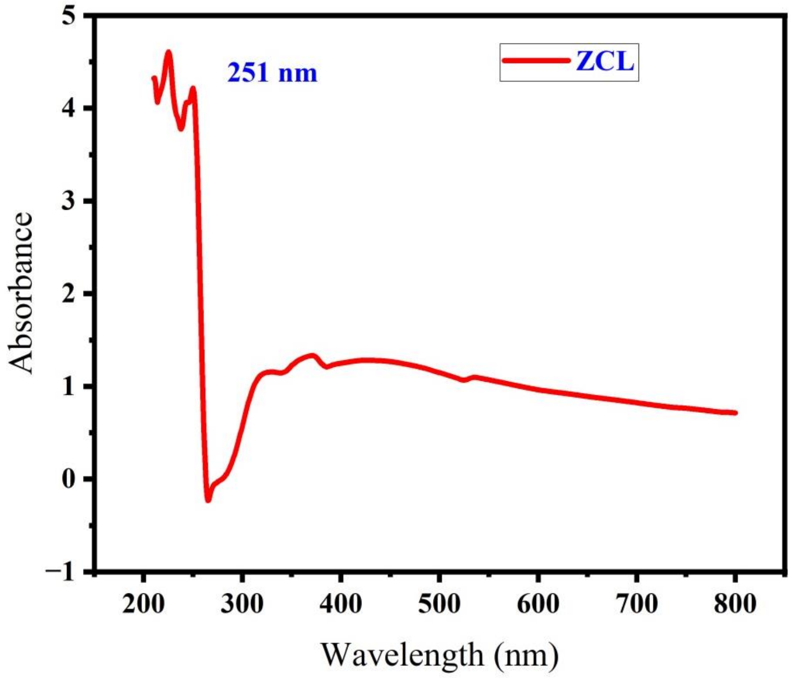

Fig 4.2.1 UV-Vis spectrum of green synthesized ZnO NPs

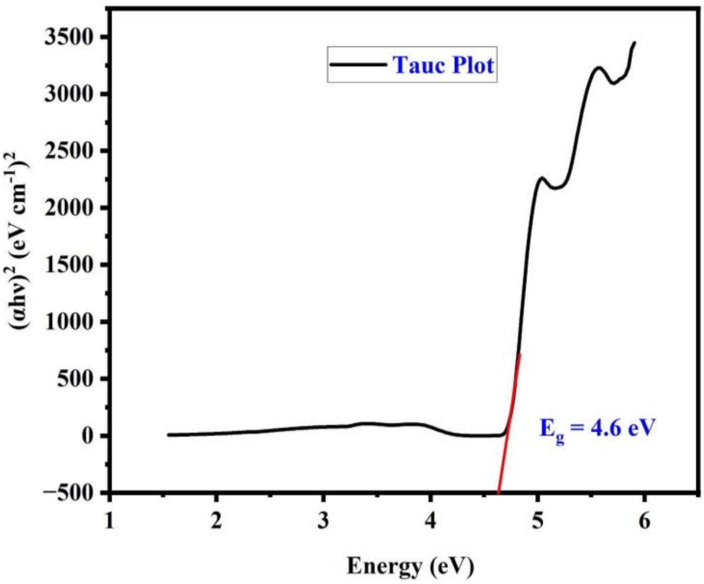

Fig.4.2.2 Band gap energy of green synthesized ZnO NPs

The band gap energy (E_g) of ZnO was obtained from the wavelength value corresponding to the intersection point of the vertical and horizontal part of the spectrum, using the equation given below,

$\mathbf{E_g = hc/\lambda\ eV}$,where hc = 1240 eV where, Eg is the band gap energy (eV), h is the Planck's constant ($6.626x10^{-34}$ J s), c is the light velocity ($3x10^8$ m/s) and λ is the wavelength (nm). From Fig.4.2.1 the absorption edge are positioned at 251 nm, corresponding the band gap value of 4.6 eV.

4.3 FT-IR Spectroscopy

Infrared studies were carried out to determine the quality and composition of the nanoparticles, as well as the presence of phytochemicals in the extract. The phytochemicals, such as alcohols, phenols, amines, carboxylic acids, and others, can interact with the zinc surface and stabilize ZnO NPs.FT-IR spectral studies give information regarding chemical bonding between Zn and O and it is used to identify the chemical bonds present in the synthesized nanoparticles.The analysis was done at a frequency range of 400-4000 cm^{-1} at room temperature.

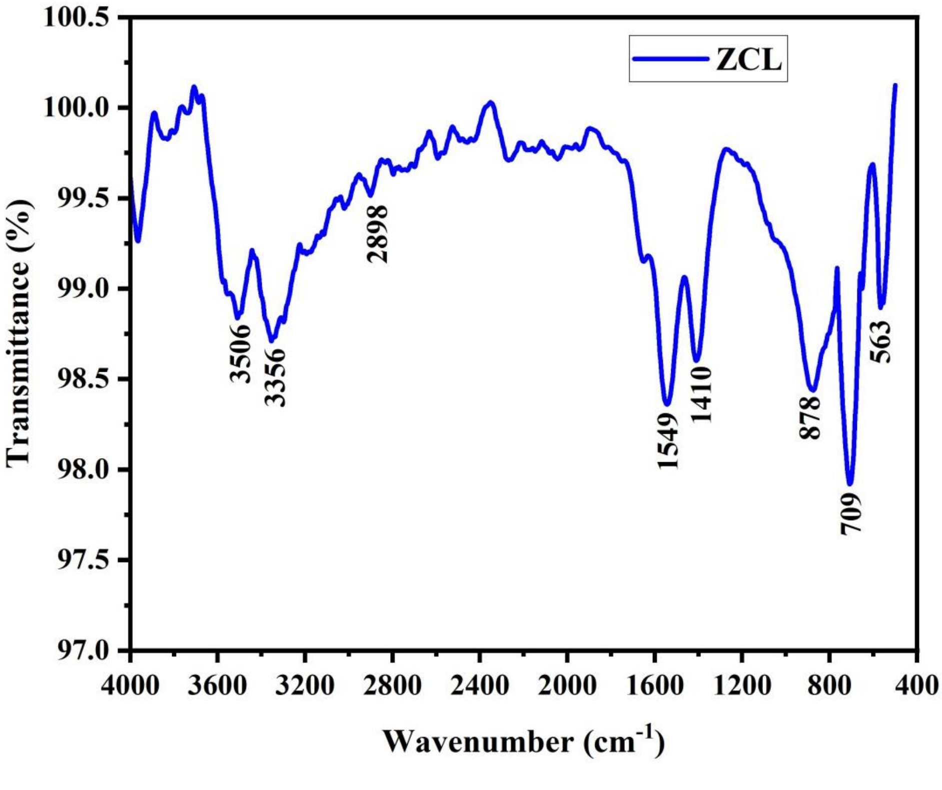

Fig 4.3.1 FT-IR spectrum of green synthesized ZnO NPs

Vibrational frequency (cm^{-1})	Functional group	Reference
3506	O-H stretching	**Vijayakumar *et al*.,2016**
3356	O-H stretching	**Vijayakumar *et al*.,2016**
2898	Aromatic C-H stretching	**Bala *et al*.,2015**
1549	Aromatic C-C stretching	**Ramesh *et al*.,2021**
1410	C=O stretching	**Bala *et al*.,2015**
878	C-H bend in alkane group	**Vijayakumar *et al*.,2018**
709	C-H bending	**Pillai *et al*.,2020**
563	ZnO stretching	**Umamaheshwari *et al*.,2021**

Table 4.3.1 Spectral range and functional group of green synthesized ZnO NPs.

Fig.4.3.1 shows the FTIR spectrum of synthesized ZnO NPs. A broad stretch at 3306 and 3356 cm^{-1} shows the presence of O–H stretch and hydrogen bonded groups in alcohol or phenol or water molecules in the extract. A peak at 2898 cm^{-1} is due to the presence of aromatic C-H stretching. The strong and intense absorption peaks at 1549 and 1410 cm^{-1} indicates the aromatic stretching vibration of C-C and C=O respectively. The peak around 878 and 709 cm^{-1} indicates the C-H out of plane bending vibrations.The peak at 563 cm^{-1} is allotted to Zn–O stretching.The bands at 3306, 1549 and 1410 cm^{-1} may indicate the participation of flavonoids and glycosides that have functional groups of alcohols and ketones in bioreduction reactions. Therefore, flavonoids and glycosides are suggested to be responsible for the bioreduction.

4.4 X-Ray Diffraction

X-ray diffraction is taken in order to confirm the ZnO phase of the nanoparticles. The XRD patterns of the obtained ZnO nanoparticles are shown in Fig.4.4.1.XRD pattern shows the orientation and crystalline nature of zinc oxide nanoparticles.Green synthesized Zinc oxide nanoparticles using *Zephyranthes candida* leaf extract shows good crystallinity,and the peak positions with 2 theta values are **31.6, 34.3, 36.1, 47.4, 56.5, 62.7 and 68.**

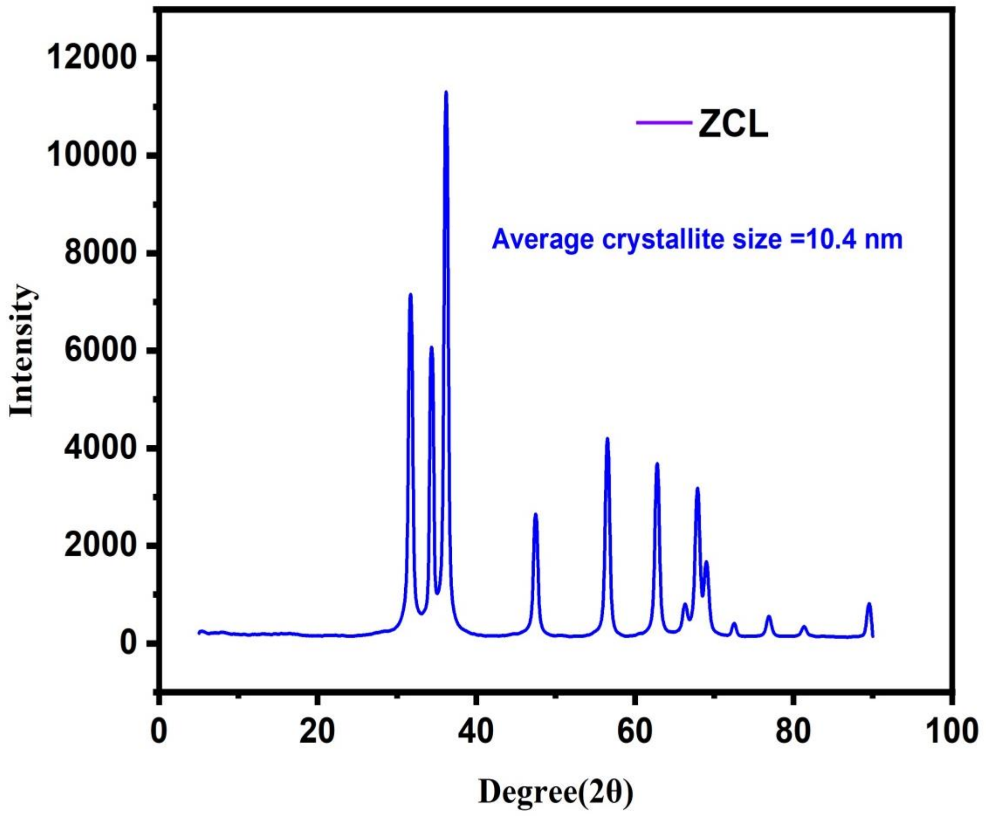

Fig 4.4.1 XRD image of green synthesized ZnO NPs

The strong and narrow diffraction peaks indicate that the product has good crystalline structure. The crystallite size of the nanoparticles was calculated using the Debye Scherrer formula.

$D = (K \times \lambda)/(\beta \times \cos\theta)$

where, K is constant, λ is the wavelength of employed X-rays (1.54056 A°), β is corrected full width at half maximum and θ is Bragg's angle.

Thus, the calculated values for the 2 theta values respectively are 12.6, 12.5,11.8,11.2,10.6,10.1,3.7 which gives the average crystallite size of the synthesized nanoparticles and it was found to be **10.4 nm. (Madan *et al.*,2015) (Ali *et al.*,(2016).**

S.No.	**Degree(2Θ)**	**FWHM(β)**	**Crystallite size (D) nm**
1	31.69	0.64	12.69
2	34.35	0.60	12.59
3	36.17	0.63	11.86
4	47.46	0.64	11.29
5	56.51	0.65	10.61
6	62.78	0.67	10.11
7	68.06	1.73	3.78
Average crystallite size = **10.42 nm**			

Table 4.4.1 Structure and geometric parameters of green synthesized ZnO NPs

4.5 Thermogravimetric Analysis

Thermal gravimetric and differential thermal analysis indicate the thermal stability of nanoparticles.The temperature scale for the measurement was taken from 30 to 800°C.The thermal gravimetric analysis of ZnO NPs was performed on EXSTAR/6300 Thermogravimetric Analyser. TG curve of the synthesized ZnO NPs is shown in Fig.4.5.1 and its derivative curve is shown in Fig.4.5.2.

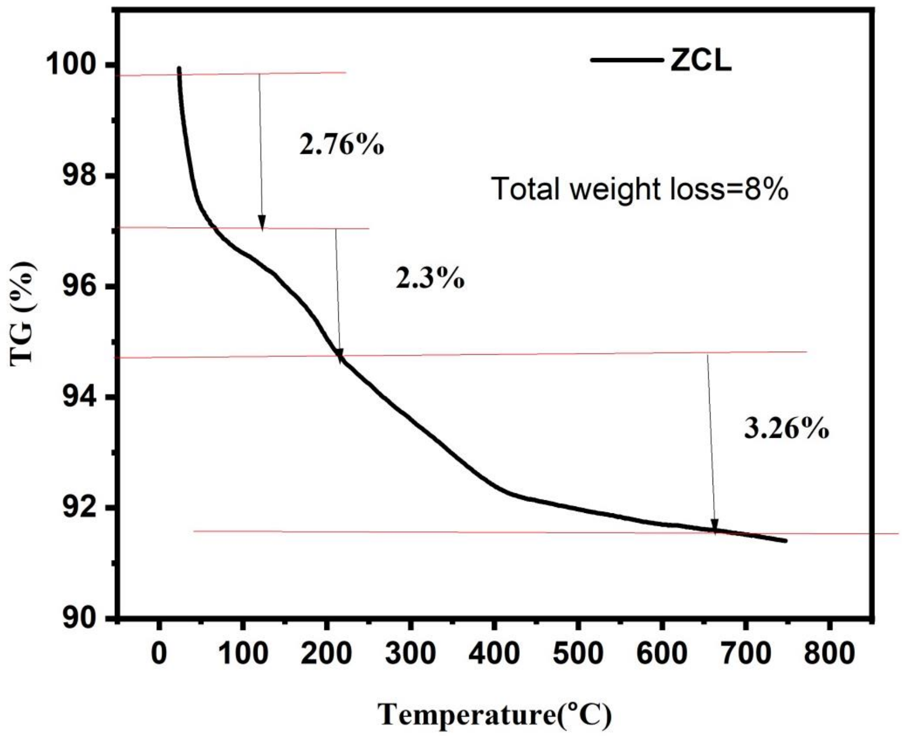

Fig 4.5.1 TG image of green synthesized ZnO NPs

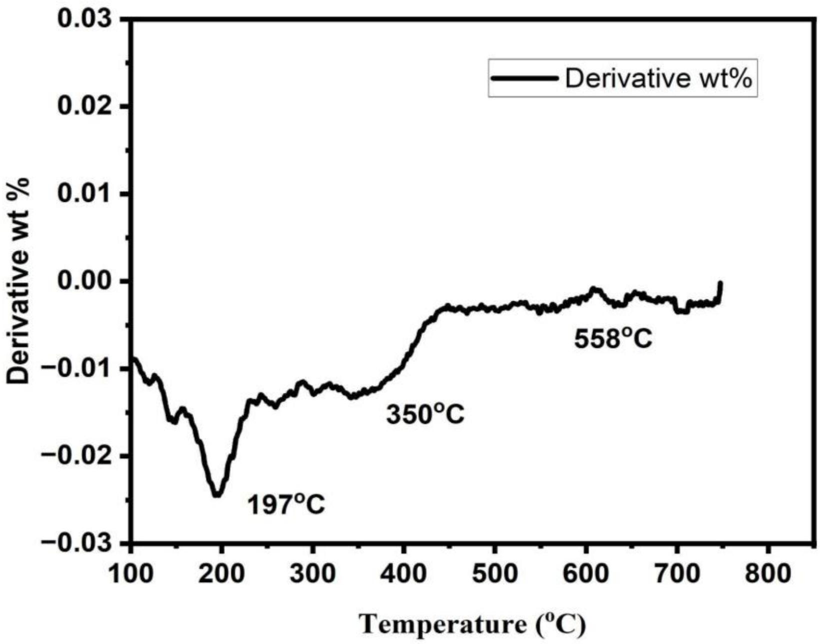

Fig 4.5.2 Derivative TG curve of green synthesized ZnO NPs

The synthesized nanoparticles show that the weight decreased upto 800℃.The total weight decrease was 8% approximately.The weight loss at 100 to 200℃ may be due to thermal desorption of water and carbon dioxide from the surface of ZnO particles.TG curve of ZnO formed at a temperature between 200℃ and 600℃ might indicate the formation of nanocrystalline ZnO **(Prashanth *et al*.,2015;Khalil *et al*.,2015)**.Weight loss between 600 to 800℃ may be because of thermal decomposition of plant bioorganic molecules present on the surface of ZnO NPs. As reported before, at temperatures above 800 °C there may be no weight loss in the TGA curve indicating that the ZnO NPs were stable within this temperature range **(Padalia *et al*.,2017).**

4.6 3-D Optical Profilometer

Optical profilometer studies give us insight about the topography,roughness of nanoparticles.Images clearly demonstrate the smoothness of nanoparticles with capping of phytochemicals over the surface of nanoparticles.**(Santhoshkumar *et al*.,2018)**

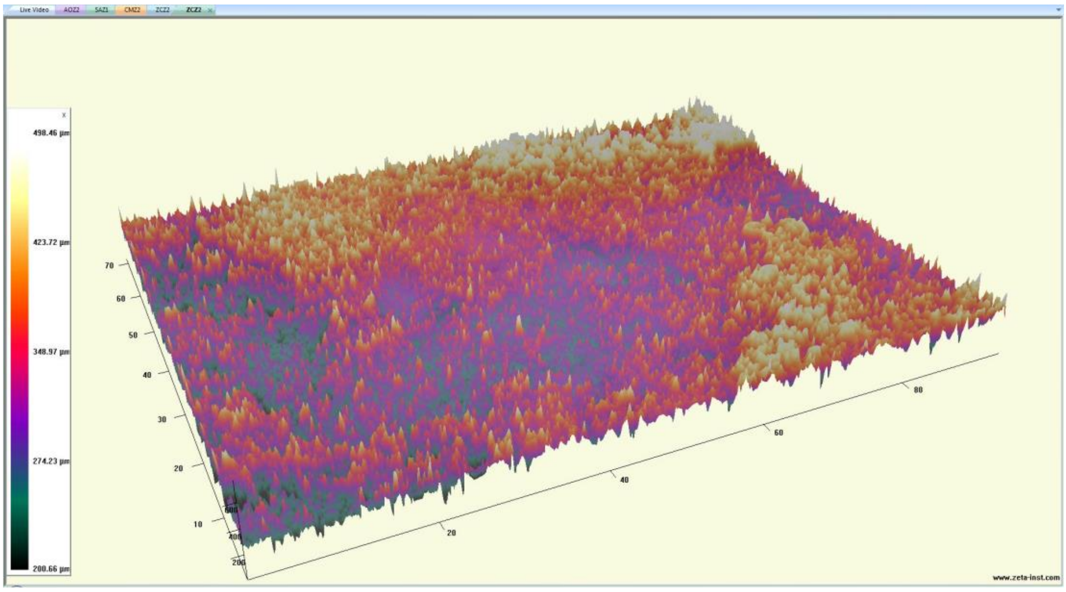

Fig. 4.6.1 3D Optical Profilometer image of green synthesized ZnO NPs

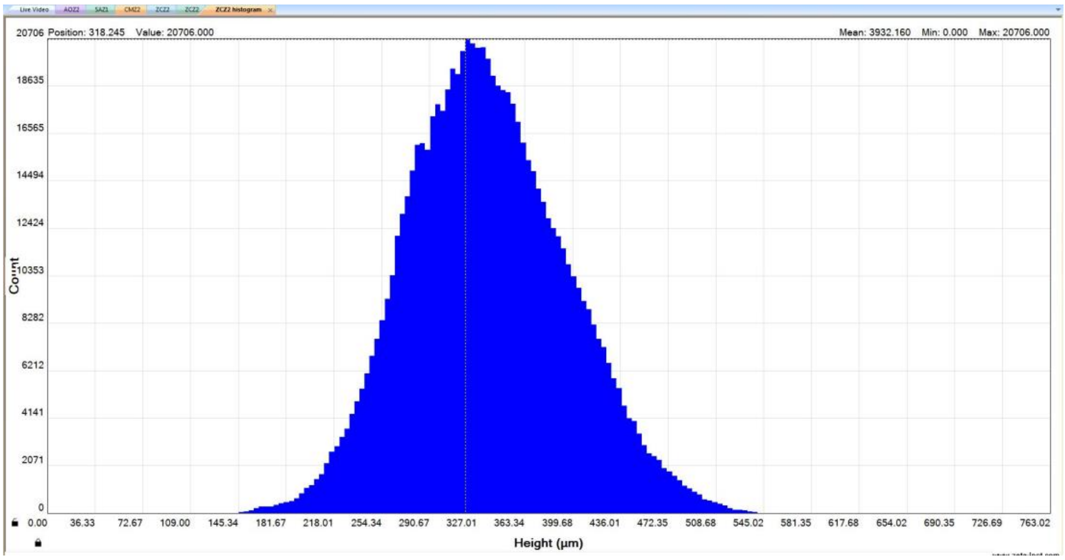

Fig. 4.6.2 Histogram image of green synthesized of ZnO NPs

Sample	Ra	Rq
Green synthesis of ZnO NPs	34.77	44.24

Table 4.6.3 Ra and Rq value of green synthesized ZnO NPs

4.7 Antibacterial activity

The antibacterial activity of green synthesized ZnO NPs was investigated against selected pathogens of gram positive and gram negative bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* by using the Kirby-Bauer method, and the result is shown in Table 4.7.1.

Sample	Zone of Inhibition (mm)			
	Staphylococcus aureus	***Escherichia coli***	***Pseudomonas aeruginosa***	***Bacillus subtilis***
Teicoplanin(Standard)	15	17	16	14
ZCL ZnO NPs	11	16	15	12

Table 4.7.1 Diameters of inhibition zone (mm) of ZnO NPs against bacterial species

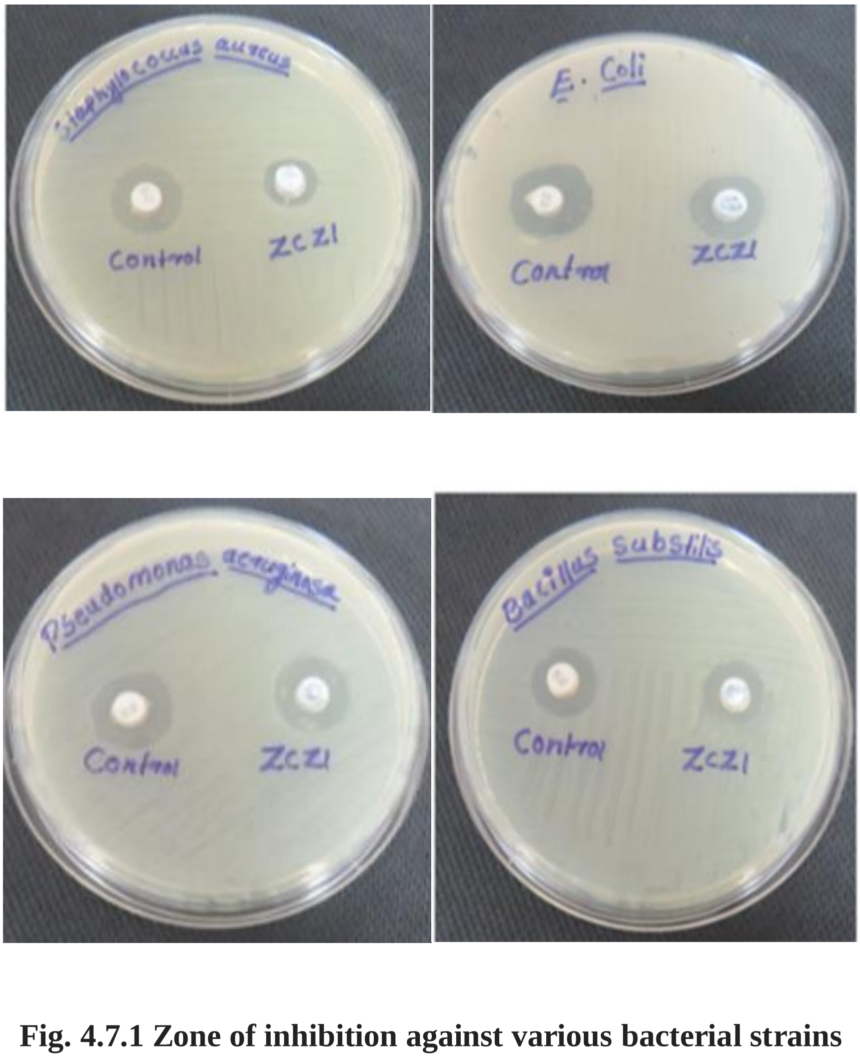

Fig. 4.7.1 Zone of inhibition against various bacterial strains

The results indicate that the ZnO NPs have a significant antibacterial activity with minimum inhibition zones of 11mm ,15mm,16mm,12mm for *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis* respectively.It is seen from the result that the inhibition zone was only slightly lower than the standard disc Teicoplanin.

The mechanism by which the nanoparticles were able to penetrate the bacteria is not completely understood but study suggest that the mechanism for the antibacterial activity of the ZnO NPs can be ascribed to many factors including: including: (1) The accumulation of nanoparticles on the surface of the bacteria, which leads to the destruction of cell envelope and the release of intracellular components; (2) the formation of Zn ions and their electrostatic interaction with bacterial cell wall. This interaction enhances the penetration of the nanoparticles through the cell membrane and causes damage to the bacterial cell components; (3) the generation of reactive oxygen species (according to the mechanism described in the previous section), which have the ability to damage DNA and proteins, resulting in bacterial cell death**.(Aldeen et al.,2022)**

Thus, the results prove the toxicity of green synthesized ZnO NPs against the bacterial strains studied and therefore, they can be used as an effective antibacterial agent in industrial and pharmaceutical applications, such as food preserver packaging.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

This study focused on the green synthesis of Zinc oxide nanoparticles using *Zephyranthes candida* leaves extract.The plant extract acts as capping as well as reducing agents. The metal ions are reduced by the plant extract, resulting in the formation of nanoparticles. The reducing agents involved in the reduction process include the various water-soluble natural products such as flavonoids,glycosides and alkaloids.

Further,this green synthesized nanoparticles formation has been confirmed through various characterization techniques including Fourier- transform infrared spectroscopy, X-Ray Diffraction, UV Visible spectroscopy, 3 D Optical profilometer, Thermo gravimetric analysis and Cyclic Voltametry.

UV spectroscopy confirmed the formation of ZnO nanoparticles by showing an absorbance peak at 251 nm.FT-IR spectra also revealed the presence of Zn-O stretching vibration which is observed at 563 cm^{-1} and also showed other characteristic peaks.Average crystallite size of the synthesized nanoparticles were found to be 10.4 nm by using XRD results.Thermogravimetric analysis showed approximately 8 % of weight loss .

Also,the synthesized NPs was studied for antibacterial activities against the microorganisms of both Gram positive (S. aureus,B.subtilis) and Gram negative (P.aeruginosa, E. coli) bacteria.The present study is so helpful and useful to the scientific community for using the ZnO NPs as the potent applications in biomedical field. In addition, it is inexpensive, stable and nontoxic, safe and eco-friendly without side effects to human beings. Therefore,this present study is designed to prepare eco-friendly, stable and nontoxic ZnO nanoparticles from *Zephyranthes candida* leaf extract.

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