WALLAGA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

GREEN SYNTHESIS, CHARACTERIZATION AND ANTI-BACTERIAL ACTIVITIES OF COBALT DOPED ZINC OXIDE NANO COMPOSITE USING *Croton macrostachyus* LEAF EXTRACT

Msc. THESIS

BY

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DECEMBER 2025

NEKEMTE, ETHIOPIA

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This thesis was submitted to the college of natural and computational sciences in partial fulfillment of the requirements for the degree of masters of Science in chemistry (Inorganic chemistry)

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APPROVAL SHEET FOR SUBMITTING FINAL THESIS

As member of Board of Final MSc. Thesis open defense, we certify that we have read and evaluated the thesis prepared by **Teferi Gebisa Negeri** under the title"**Green synthesis, characterization and antibacterial activities of cobalt doped zinc oxide nanocomposite using *croton macrostachyus* leaf extract"** and recommended that the thesis be accepted as fulfilling the thesis requirement for the Degree Master of Science in **Chemistry(Inorganic).** .

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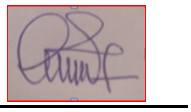
Guta Gonfa (PhD)		
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Certification of the final research

I here by certify that all the correction and recommended suggested by the board of examiners are incorporated into the final thesis entitled" **Green synthesis, characterization and antibacterial activities of cobalt doped zinc oxide nanocomposite using *croton macro stachyus* leaf extract"**by **Teferi Gebisa Negeri**

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DEDICATION

This thesis is dedicated to my wife Gelane Bekare , my child Kayofan Teferi and Lastly to my family for their love, joy, faith, and eternal goodness. I am sure this work will make them happy and proud. May God’s graces and blessings be forever theirs!

DECLARATION

This is to certify that "entitled''G**reen synthesis,characterization and antibacterial activities of cobalt doped zinc oxide nanocomposite using *croton macrostachyus* leaf extract**''is accepted in the partial fulfillment of the requirement for the award of the degree of master of sciences in inorganic chemistry by the school of Graduate studies, Wallaga university through the college of natural and computational science done by **Teferi Gebisa** was a genuine work carried out by him under my guidance. The matter embodied in this thesis work has not been submitted earlier for the award of any degree or diploma. The assistance and help received during the course of this investigation have been dully acknowledged. Therefore, I recommend that it can be accepted as fulfilling the research thesis requirements of M.Sc. Degree in chemistry.

Asnake Lealem (PhD) _____ _____

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STATEMENT OF THE AUTHOR

I Mr.Teferi Gebisa Negeri:I hereby declare and affirm that the thesis entitled**"Green synthesis, characterization and antibacterial activities of cobalt doped zinc oxide nanocomposite using *croton macrostachyus* leaf extract "**is my own work conductedunder the supervision of Asnake Lealem(PhD**)** at Wallaga University. I have followed all the ethical principles of schola rship in the preparation, data collection, data analysis, and completion of this thesis. All the scholarly material that is included in the thesis has been given recognition through citation. I have adequately cited and referenced all the original sources. I also declare that I have adhered to all principles of academic honesty and integrity, and I have not misrepresented, fabricated, or falsified any idea, data, fact, or source in my submission. This thesis is submitted in partial fulfillment of the requirement for a degree from the Postgraduate Studies program at Wallaga University. I further declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate. I understand that any violation of the above will be cause for disciplinary action by the university and can also evoke penal action from the sources that have thus not been properly cited or from whom proper permission has not been obtained when needed.

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BIOGRAPHICAL SCKETCH OF THE AUTHOR

The author was born on May 9, 1995, G.C in Oromia region, West Wallaga Zone, Yubdo Woreda. He attended his primary school at Wora Gutu primary school (1–8), and then he attended his secondary school in the West Wollega Zone at Warke Yubdo secondary school (9–10) and in Bako Tibe Woreda at Bako Preparatory School (11–12). Then he joined Dilla University (DU) in 2015 GC and graduated with a BSc degree in Chemistry in 2017 (GC). Following that, he enrolled and taken post graduate diploma in teaching (PGDT) program at Mekelle University on a regular basis. Finally,he also joined Wallaga University in 2021 to pur sue his MSc degree in Chemistry(Inorganic Chemistry) and now I am teacher at East Wallaga ,Haro Limu Woreda ,in Hunde Haro Special Secondary school and he have 8 years' experience in his work.

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ABBREVIATIONS AND ACRONOYOMS

C. macrostachyus	*Croton macrostachyus*
Co-ZnO NCs	Cobalt doped zink oxide nanocomposite
CVD	Chemical Vapour Deposition
DMSO	Dimethylsulfoxide
DW	Distilled water.
E.coli	*Escherichia coli*
FT-IR	Fourier Transform Infrared Spectroscopy
MONPs	Metal oxide nanoparticles
NCs	Nanocomposites
NIZ	No inhibition zone
NPs	Nanoparticles
P.aeroginosa	*Pseudomonosa aeroginosa*
PNCs	Polymer nanocomposites
PVD	Physical Vapour Deposition
S. aureus	*Staphylococcus aureus*
UV-Vis	Ultraviolet-Visible Spectroscopy
XRD	X-ray diffraction
ZnO NPs	Zinc oxide nanoparticles

TABLE OF CONTENTS

Contents **Pages**

APPROVAL SHEET FOR SUBMITTING FINAL THESIS……i

DEDICATION……ii

DECLARATION……...iii

STATEMENT OF THE AUTHOR……..iv

BIOGRAPHICAL SCKETCH OF THE AUTHOR……v

ACKNOWLEDGEMENT……vi

ABBREVIATIONS AND ACRONOYOMS……..vii

TABLE OF CONTENTS……viii

LIST OF TABLES……xi

LIST OF FIGURES……..….xii

ABSTRACT……xiii

1. INTRODUCTION……..1

1.1. Background of the Study……1

1.2. Statement of the Problem……...3

1.3. Objectives……...4

1.3.1. General Objective……4

1.3.2. Specific Objectives……..4

1.4. Significance of the Study……...4

2. LITEREATURE REVIEW……5

2.1. Bacteria……..……..…….....……5

2.2. Nanomaterials……5

2.2.1. Nanocomposites……...6

2.3. ZnO Nanoparticle……...7

2.4. Metal doping……..7

2.5. Methods Synthesis of Nanomaterials……8

2.5.1. Green synthesis……..10

2.5.2. Synthesis of Nanoparticles by Using Plant……11

2.5.3. Method for synthesis of ZnO NPs and Co-ZnO NCs……12

2.6. Characterization techniques for metal oxide nanoparticles and metal-doped metal oxide nanocomposites……..…..…13

2.7. Medicinal Value of Croton macrostachyus……..…14

2.8. Factors affecting the synthesis of nanoparticles and nanocomposites……16
2.8.1. pH……..16
2.8.2. Temperature……...17
2.8.3. Reaction Time……17
2.8.4. Effect of precursor concentration……..17
3. METHODS AND MATERIALS……18
3.1.Experimental site……...20
3.2. Chemicals and Reagents……...19
3.3.Apparatus and Instruments……19
3.4.*Croton macrosatchyu*s leaf Collection and Preparation…..……..19
3.5. Preliminary Phytochemical Screening…….20
3.6. Optimization of synthesis parameters for Co-ZnO NCs……..22
3.6.1. Effect of concentration of precursor salt solution…….22
3.6.2. Effect of volume of plant extract……...22
3.6.3. Optimization of pH value……..22
3.6.4. Optimization of reaction temperature……23
3.7. Synthesis of ZnO nanoparticles by using Croton macrostachyus leaf extract…….23
3.8. Synthesis of Co-ZnO NCs Using Croton macrostachyus Leaf Extract……...24
3.9. Characterization Technique for ZnO NPs and Co-ZnO NCs……...25
3.9.1. UV-Vis Spectroscopy Studies……...25
3.9.2. Fourier Transform Infrared (FT-IR) Spectroscopy Studies……...26
3.9.3. X-ray diffraction (XRD) Studies……...26
3.10. Antibacterial Activity of Green Synthesized ZnO NPs and Co-ZnO NCs.……..27
3.11.Data Analysis…….……...……..27
4. RESULT AND DISCUSSION…….28
4.1. Phytochemical screening of the Croton macrostachyus leaf extract……...28
4.2. Parameter optimizations for the synthesis Co-ZnO NCs…….29
4.2.1.Concentration of dopant optimization…….……...29
4.2.2.Effect of pH……..……...29
4.2.3.Volume of plant extract optimization……..……29
4.2.4.Effect of temperature…….,…….29
4. 3. Characterization of Synthesized ZnONPs and Co-ZnONCs……...31
4.3.1. UV -Vis analysis……31
4.3.2. FTIR Analysis……..….32

4. 3 3. X- Ray diffraction analysis……..**34**
4.4. Antibacterial activity of green synthesized ZnO NPs and Co-ZnO NCs…….36
5. CONCLUSIONS AND RECOMMENDATIONS……..40
5. 1. Conclusions…….40
5. 2. Recommendations……...40
6. REFERENCES……..41

LIST OF TABLES

Table 1.Different types of nanocomposites.……7
Table 2.Some method of synthesis of ZnO NPs…….12
Table 3.The Recent study conducted by a different types of plants to synthesize ZnO Nanoparticles and Metal doped-ZnO Nanocomposite……13
Table 4.Phytochemical components of *Croton macrostachyus* leaf extract……...28
Table 5.FTIR absorption bands for ZnO NPs ,5% Co-ZnO NCs and 7% Co-ZnO NCs……33
Table 6.XRD peak position, miller indices, FWHM ,Crystalline size and Average crystalline size for ZnO NPs,5% Co-ZnO NCs and 7% Co-ZnO NCs……35
Table 7.Antibacterial activities of biosynthesized ZnO NPs and Co-ZnO NCs…….38

LIST OF FIGURES

Figure 1. Different synthesis method of nanoparticles……...…10
Figure 2. Environment friendly and cheap route for the green synthesis of nanoparticles using plants extract.……..11
Figure 3. *Croton macrostachyus* plant……15
Figure 4. Some use of *Croton Macrostachyus* plant leaf……16
Figure 5. Factors affecting the green synthesis of nanocomposites.……...18
Figure 6. Mechanism representation for the for phytochemical present in *Crotonmacrostachyus* leaf extract synthesis of Co−ZnO nanocomposite……..……...…….18
Figure 7. Schematic preparation of *Croton macrostachyus* leaf extract…….20
Figure 8. Schematic representation of ZnO NPs synthesized by *Croton macrostachyus leaf extract*…….24
Figure 9. Schematic representation of Co-ZnO NCS synthesized by *Croton macrostachyus* leaf extract……..25
Figure 10. Phytochemals screening of *Croton macrostachyus* leaf exract.……28
Figure 11. UV-Vis spectra optimization of a)concentration, b)Volume c)Temperature and d) pH value of Co-ZnO NCs……...30
Figure 12. UV-Vis spectra of Croton macrostachyus leaf extract……..32
Figure 13. UV-Vis spectra of ZnO NPs and Co-ZnO NCs…….32
Figure 14. UV -Vis spectral band gap of ZnO NPs and Co-ZnO NCs……...……32
Figure 15. FTIR results of *Croton macrostachyus* ,ZnO NPs ,5% Co-ZnO NCs and 7% Co-ZnO NCs…….34
Figure 16. XRD results of ,ZnO NPs ,5% Co-ZnO NCs and 7% Co-ZnO NCs…….36
Figure 17. Proposed mechanism for antibacterial activities of Co−ZnO nanocomposites…….37
Figure 18. Zone of Inhibition of ZnO NPs (A), 7%Co-ZnO NCs (B), 5%Co-ZnO NCs (C) against *S.aureus ,E.coli and p.aeroginosa*…….....39

ABSTRACT

Microorganism infections have gained significant attention due to the serious health hazards they pose. Because of the concerning increase in outbreaks of pathogenic strains, antibiotic resistance, new bacterial_mutations, and other problems, there is an urgent need for effective antimicrobials. Numerous nanoparticles made from metals have been shown to be compatible with human cells and to function as micronutrients crucial to the body's fundamental functions. Many bacterial_species, both Gram-positive bacteria and -negative bacteria, are very resilient to the damaging effects of inorganic substances. This study investigated to green synthesis, characterization and antibacterial activity of cobalt doped zinc oxide nanocomposite using Croton macrostachyus leaf extract. The synthesized nanoparticles were characterized by using UV-Vis, FTIR and XRD spectroscopic and tested antibacterial activities. The UV-Visible spectra showed absorbance peaks at 268 nm, 358 nm and 366 nm for Croton macrostachyus leaf extract, ZnONPs and Co-ZnO NCs respectively. The band gap energy, calculated was 3.06 eV and 2.6 eV for ZnO NPs and Co/ZnO NCs respectively. FTIR confirmed the nature of bonds and functional groups in the leaf extract, ZnO and Co-ZnO NCs. The XRD analysis reveals all the synthesized particles have a crystalline with average particle sizes of 10.16 nm,7.19nm and 9.22nm of ZnONPs, 5%Co/ZnONCs, 7%Co/ZnO NCs respectively. The bacterial activity of studies of the synthesize 5% Co-ZnO NCs ,7%Co-ZnO NCs against S,aures, E,coli and p.aeruginosa showed good potential with the reference to the selected standards ciprofloxacin The maximum zone of inhibition activities were found against gram negative(p.areuginosa) for 7% Co-ZnO NCs followed by 5% Co-ZnO NCs whereas minimum inhibition zone were against gram negative (E.coli) for ZnO NPs.And also the determination of the size and composition of the sample is characterized using instruments like SEM,TEM,XPS and EDX.

Keywords: *Antibacterial ,Characterization ,Croton macrostachyus ,Green synthesis, ,*

Nanocomposites,

1. INTRODUCTION

1.1. Background of the Study

Microorganisms play vital role in maintaining human health and involve indifferent infections and diseases.The infections caused by various microbes including bacteria, fungi, and viruses are the greatest challenge to the world (Nwakoby *et al.*,2025). Among all infections,bacteria be eliminated and treated easily but with the advent of antibiotic resistance, management of bacterial infections is very difficult .The processof bacterial infections starts with the initial colonization of bacteria to the host system, adhesion, invasion, and replication inside the host leading to the damage of host system. Antimicrobial resistance is a serious global health threat that has necessitated the exploration of novel approaches.Nanoparticles with anti microbial activity have unique ability to generate hydroxyl radicals and superoxide ions ,with high efficiency indestroying microbialcells **(**Mondal *et al*.,2024).

Nanoparticles are increasingly being explored to control microbial infections as alternatives to antibiotic.They are currentlybeing used as coatings for implantable devices, infection preventi on, detection and diagnostic purposes and in delivery systems.The mechanism of nanoparticle poorlyunderstood however, metal ion release oxidative stress induction and non oxidative mechanisms are widely proposed.To further enhance antbacterial activity of nanoparticles and nanocomposites can be engineered to incorporate different components or surface functionaliti es (Queiroz,& Torresi, 2025).

Several metal oxide nanoparticles such as AgO, CuO, ZnO, MgO have been explore for their activity against different classes of microbes. Furthermore, in the quest for improved activity, metal ion doping has been observed to significantly alter the physicochemical properties of metal oxide nanoparticles, which alters their antimicrobial activity.Furthermore, metal doping modifies the crystal lattice and alters the electronic band structure, while introducing several crystal defects in the ZnO structure. In addition to the improved antimicrobial activity offered by doped metal oxides, the facile synthesis offered by green synthesis have made them desirable materials for biological activities (Wassie & Yemata , 2025).

Cobalt is often considered as an effective doping specie because of its abundant electronic states compared to other transition metals such as iron and nickel.Cobalt is also well suited for electronic state modification by substituting Co^{2+} into the lattice of the host, without inducing significant distortion (Azam *et a*l.,2025).Furthermore, its ability to stabilize desired defects and increase the surface hydroxyl groups in metal oxides increases the functionality of the host material .Green synthesis of nanoparticles offers numerous advantages over traditional methods such as environmental friendliness and sustainability and reduced toxicity, making them suitable for large-scale synthesis. Green synthesis uses plant constituents such as enzymes, fats, flavonoids, alkaloids and polyphenols as reducing agents (Rautela & Debnath 2019).

Additionally, it provide a facile route in tailoring nanoparticles to varying applications by modifying their morphology and surface properties. Furthermore, plant extract precursors contain a wide range of active substances that aid in the reducing and stabilizing process as well as serve as templates for nanostructure manipulation (Dinah *et al*., 2019). In this study, phytochemical constituents of *Croton macrostachyus* leaves was extracted and the presence of several reducing, stabilizing, and capping agents in the leaf extract was performed qualitatively using standard methods of phytochemical screening. Medicinal Plant extracts are used for the metal ions bioreduction to form nanoparticles. It has been demonstrated that plant metabolites like terpenoids, polyphenols, alkaloids, phenolic acids, and proteins play an important role in metal ions reduction into nanoparticles and in supporting their subsequent stability. The finding of this study contributes to the potential of metal doping in improving the antimicrobial activity of metal oxide nanoparticles obtained through green synthesis (Ogwuegbu *et al.*,2025).

1.2. Statement of the Problem

An antibiotic resistance bacterium is currently at an all-time high and it is the world's major public health issue in the treatment of human patient with infectious diseases.
Bacterial pathogens such as *S.aureus* , *p.aerugenosa* and *E.coli* have acquired drug resistance genes, making them resistant to numerous antibacterial medicines.Due to the various mechanisms, multidrug resistant human pathogenic microorganisms are posing a growing threat to the medical community.However the increasing resistance of diversepathogenic organisms to various antibiotics has prompted a wide range of studies into alternate therapeutic options(MacNair *et al.*,2024).The biological activity of*Croton macrostachyus* plant leaf extract can be further enhanced if supported by metal or metal oxide nanoparticles that helping penetrating to the cells of the pathogen **(**Mondal *et al*.,2024).

Particularly, inorganic nanomaterials, such as metal oxide nanoparticles, are gaining interest in exploring various options to address this problem. Among metal oxide nanoparticles, ZnO NPs and their NCs have advantages over common inorganic and antibiotic compounds since they have lower toxicity, greater durability, and higher stability and selectivity. To produce such nanoparticles, green synthesis using plant extracts is one of the most important areas for the development of novel antibacterial drugs. Thus, C*roton macrostachyus* leaf extract was utilized in this study to synthesize ZnO NPs and Co-ZnO NCs using a green synthesis method and their antibacterial activity was evaluated (Ogwuegbu *et al.*,2025). Therefore, this research is intended to fill the gap in the area of green synthesis of ZnO NPs and Co-ZnO NCs from the leaves of C*roton macrostachyus* and to investigate and compare their effect against three bacterial strains *Staphylococcus aureus* (gram-positive) and *pseudomonas areugenosa* and *Escherichia coli* (gram-negative).

This study addressed the following questions;

- ✓ Is *Croton Macrostachyus* leave extract acted as stabilizing and capping agent for the synthesis of ZnO NPs and Co-ZnO NCs?
- ✓ Does Co-ZnO NCs show more antibacterial activity than ZnO NPs?

1.3. Objectives

1.3.1. General Objective

- To green synthesize and characterize ZnO NPs and Co-ZnO NCs using leaf extract of *Croton macrostachyus* and evaluation of their antibacterial activity.

1.3.2. Specific Objectives

- To prepare and phytochemical screening of *Croton macrostachyus* leaf extract.
- To green synthesize ZnO NPs and Co-ZnO NCs using *Croton macrostachyus* aqueous leaf extract.
- To characterize ZnO NPs and Co-ZnO NCs using UV-Vis, FT-IR and XRD.
- To evaluate the antibacterial activities of *Croton macrostachyus* leaf extract, ZnO NPs and Co-ZnO NCs using disc diffusion method.

1.4. Significance of the Study

In recent years, there is an increasing interest for Synthesizing of ZnO NPs and Co doped ZnO NCs due to their wide applicationThus, the most effective, cheap, and simplest technique of nanoparticles synthesis becomes a much more interesting area of research.

Therefore the development of green methods of synthesis using plant extract is an essential method.In general,the findings of this study have the following significance. Providing further awareness for researchers about antibacterial applications of green synthesis ZnO NPs and Co doped ZnO NCs. And also this study provided baseline information for other researchers who want to work in this area. Additionaly Upgrading the knowledge about the Co doped ZnO NCs regarding its antibacterial activity.

2. LITEREATURE REVIEW

2.1. Bacteria

Bacteria are single celled organisms that live in almost every environment on earth and constitute a large domain of prokaryotic microorganisms. Bacterial size can vary considerably. Escherichia coli inhabiting the intestinal tract, for example, is 2 micrometres long and 0.5 micrometres in diameter. Meanwhile, the largest bacteria, Thiomargarita namibiensis have a diameter of 750 micrometres. The difference in the cell wall constituents is used to distinguish between two large groups of bacteria: gram positive and gram negative bacteria. The staining is named after Danish physician – Hans Christian Gram. The thick layer of peptidoglycan in the cell wall retains the crystal violet during staining, which makes gram positive bacteria stain violet. The thinner peptidoglycan cell wall, which does not retain crystal violet during decolouring, makes the gram-negative bacteria stain red (Razak & Santos , 2025)

There are many different types of bacteria. Most types don't make you sick. Some types are helpful. For example,"good" bacteria in your digestive system can help your body digest food and absorb vitamins and minerals.They can help protect youfrom getting sick. Bacteria are also used in making foods like yogurt and cheese. But some types of bacteria can cause an infection and make you sick. They are called infectious bacteria. These bacteria can reproduce quickly in your body. Many of them give off toxins(poisons) thatcan damage your cells and make you sick.Other types can move into and damage your tissues. Afew examples of bacteria that cause infections include, *P.areuginosa Staphylococcus* and *E. coli* (Nguena dongue *et al*.,2025).

2.2. Nanomaterials

Nanotechnology is a field of science and technology, which deals with production, manipulation and use of materials carrying in nanometers. NPs are wide class of materialsthat include particulate substances, which have one dimension less than 100 nm at least (Saleh , 2020).Nanomaterials having a length scale less than 100 nm have received in creasing inte rest owing not only to their fundamental scientific signifcance but also to the potential applica tions that derive from their fascinating electrical, magnetic, and catalytic properties.

Compared to bulk active electrode materials, the corresponding nanomaterial possess more excellent electrochemical activity, such as higher capacities, larger surface areas and lowe current densities, thereby; nanomaterial have wildly potential application in electrochemistry field.

2.1.1. Nanocomposites

Nanocomposites are materials that are composed of two or more constituents, with different physical and chemical properties, which remain separate and distinct at the microscopic level but collectively comprise single physical material possessing any phase dimension of less than 100 nm(Jahanian *et al*.,2024).

In the broadest sense, the primary motivation of making a nanocomposite is to integrate one or more discontinuous nano dimensional phases into a single continuous macrophase to gene rate synergisticproperties; that is, the physical or chemical properties of the combined entity are inherentldifferent from, andhopefully superior to, those of the individual material constitue nts. In this context, one of the combined material's constituents is generally in much greater concentration and forms a continuous matrix surrounding the others, which assume the role of a nanofiller or reinforcement (Kausar, 2025).

During the nanocomposite formation process, each of the distinct phases is structure- and property-integrated to fabricate hybrid materials that possess multi functionalities in terms of both structures and material properties. The advent of new materials and characterization tools in the nanotechnology domain has been paving the way for the latest design of next-generation nanocomposites that not only are easily controllable but also possess multiple intrinsic engineering functionalities. Based on the different types of matrix materials, nanocomposites can be generally divided into four categories including polymer-, carbon-, metal-, and ceramic-based nanocomposites. Polymers are large molecules or macromolecules composed of repeating structural units, typically connected by covalent chemical bonds; these are generally excellent host matrixes for nanocomposite materials due to their light weight, ease of processing, low-cost manufacturing, and good adhesion to substrates. Polymer nanocomposites (PNCs) are polymer composites using nanostructured materials as reinforcements. Depending on the type of reinforcement material, different properties can be achieved for PNCs (Das *et al*.,2025).

Table 1.Different types of nanocomposites.

Class	Examples	References
Metal	Fe-Cr/Al_2O_3,Ni/Al_2O_3,Co/Cr, Fe/MgO, Al/CNT, Mg/CNT	(Dhinakaran *et al*.,2022)
Ceramic	Al_2O_3/SiO_2, SiO_2/Ni, Al_2O_3/TiO_2, Al_2O_3/SiC, Al_2O_3/CNT	(Gemachu & Bogale, 2024).
Polymer .	Thermoplastic/thermoset polymer/layered silicates, polyester/TiO2, polymer/CNT, polymer/layered	(Bhatia *et al*., 2022.)

2.3. ZnO Nanoparticle

Zinc oxide (ZnO) is one of the very promising inorganic oxides that has recently attracted the attention of many scientists for the biosynthesis of NPs due to its unique properties and multiple applications such as drug delivery, solar cells, photo catalytic degradation, antibacterial and personal care products like sunscreens and cosmetics.Due to their multiple applications in various technical fields, comprehensive investigation into metal oxide nanoparticles has been concentrated in the past decade (Faisal *et al*., 2021).There are several types of Zn nanoparticles, such as ZnS and ZnSe, or quantum dots CdSe/ZnS. Many of them can be modified to have more or better fluorescent properties. Probably the most widespread type of zinc nanoparticles (in practice as well as research) is Zinc oxide nanoparticle. Zinc oxide nanoparticles are very important class of metal oxide nanoparticles. It is environmental friendly and has various set of applications (Afzal *et al*.,2025).It has diverse properties depending upon its morphology .It is widely used in pharmaceutical industry, fabrication of Nano electronics devise, paint industries ,garments and textiles industries and has various biomedical application (Sisodia , 2022).

2.4. Metal doping

Doping is a technique that makes it possible to incorporate substitutional ions into the crystall ine structure of materials, generating exciting properties. The transition metals are elements whose corresponding atoms do not have a more energetic “d” orbital filled or capable of forming cations with an incompleted orbital (Raha *et al*., 2023).

Therefore, the incorporation of these metals in nanocrystals makes it possible to mix properties, such as magneto-optics. In addition to this property, other properties are observed, some of which we will show in this chapter. In particular, we will show results of pure ZnO nanocrystals and doped with transition metals and that depending on the dopant, specific properties may or not be improved. Depending on the doping ion, the nanoparticles' toxicity may be reduced. Cobalt(Co) is one of the transition metals with the lowest toxicity to humans, which allows its application in medicine when used inintrauterine devices, and its antibacterial activity.Thus, in the present work, the ZnO NCs were doped with the ccobalt transition metal (Co doped ZnO) and reduction of toxicity due to doping (Hassaan *et al*.,2024).

To evaluate the biocompatibility of this new nanoparticle, we analyzed the development of the fruit fly (*Drosophila melanogaster*) in a medium containing ZnO or Co doped ZnO.The introduction of metals such as silver, magnesium, and copper provides a synergistic effect with the semiconductor in a wide variety of applications such as wastewater treatment, optical and electronic devices had investigated the effect of cobalt (Co) doping on the ZnO nanoparticles synthesized using Sidarhombi folia leaves via a green approach(Ying *et al*., 2022) .

In terms of morphology, Co doping increased the crystallite size of ZnO nanoparticles from 3.42 nm to 4.21 nm and caused a more agglomerated structure as compared to pristine ZnO nanoparticles In addition, the surface Besides, the surface modification brought to the improvement in the biological activities of ZnO nanoparticles such as antibacterial, antifungal, and anticancer activities (Raha & Ahmaruzzaman, 2022).

2.5. Synthesis Methods of Nanomaterials

Various methods can be employed for the synthesis of NPs, but these methods are broadly divided into two main classes. i.e. (1) Bottom-up approach and (2) Top-down approach as shown in (figure1). These approaches further divide into various subclasses based on the operation, reaction condition and adopted protocols (Bains *et al* ., 2024).

A. Top-down Approach

In this method, destructive approach is employed. Starting from larger molecule, which decomposed into smaller units and then these units are converted into suitable NPs. Examples of this method are grinding/milling, chemical vapour deposition (CVD), physical vapour deposition (PVD) and other decomposition techniques (Singh *et al*.,2025).

B. Bottom-up Approach

This approach is employed in reverse as NPs are formed from relatively simpler substances, therefore this approach is also called building up approach. Examples of this case are sedimentation and reduction techniques. It includes sol-gel, green synthesis, spinning, and biochemical synthesis (Jesus *et al*., 2024).This refers to the construction of a structure atom by atom, molecule by molecule or cluster by cluster. Colloidal dispersion used in the synthesis of nanoparticles is a good example of a bottom-up approach. An advantage of the bottom-up approach is the better possibilities to obtain nanostructures with less defects and more homogeneous chemical compositions. Synthesis of NPs is to reduce the metal ions in the solution using a reducing agent. NPs. are highly reactive due to their high surface energy, and they are aggregated without protection. Lots of natural and synthetic polymers are being used as stabilizing agents to prevent metal NPs from sedimentation, agglomeration, and oxidation. NPs can be synthesized through various methods. Typical methods for synthesis of NPs apply toxic chemicals as reducing agents or as stabilizing agents to preclude NPs from agglomeration.The noble therapeutic applications of NP oblige using environmentally friendly methods. (Naik *et al.* ,2024

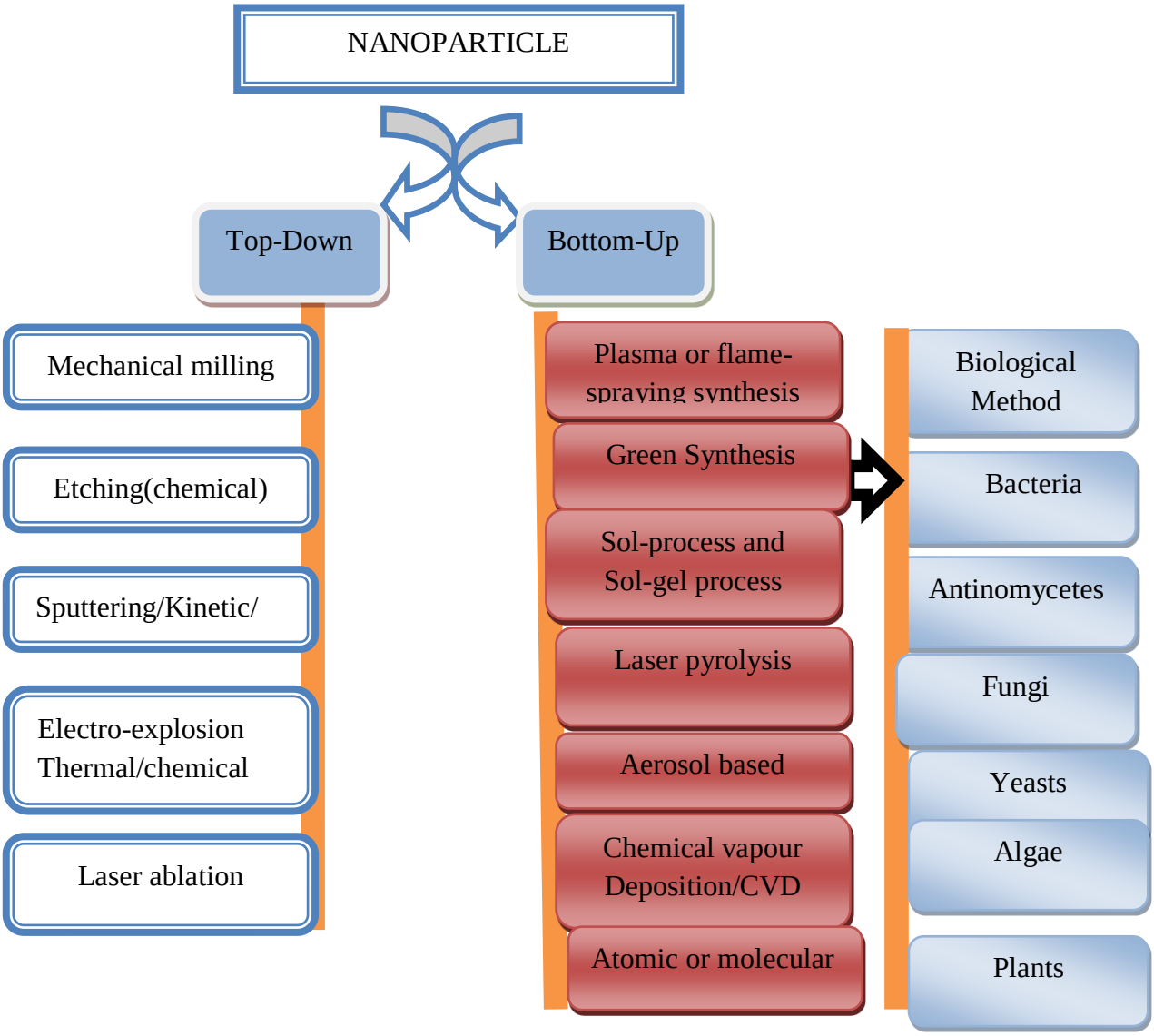


Figure 1: Different synthesis method of nanoparticles (Adeyemi *et al*., 2022)

2.5.1. Green synthesis

Green synthesis is a plant mediated synthesis of metal nanoparticles it is simple easily available,fast and ecofriendly among all the synthesis methods. The bio molecules which are present in the plant materials can increase the rate of nano synthesis or the stability of the product. In this method first of all collect plant extract from boiling leaves of regarding plant in di-ionized water. Further use this extract with precursor and form nanoparticles. Plant materials have an important role in surface morphology and size of the metals. Extracts from plants may act both as capping and reducing agent in nanoparticle synthesis. But the particles formed by this method may have large sizes (Pirsaheb *et al.*,2024).

The use of plants or plant extracts, which act as reducing and capping or stabilizing agents for NP synthesis, is more useful over other biosynthesis procedures. This occurs because in this technique, no need cell culturing and cell maintenance. Bacteria produce metal or metal oxide NPs in nanoscale with excellent morphology by extra-or intracellular methods (Salem & Fouda 2021). The various types of bacteria generally involve an intracellular method, where by the bacterial cell filtrate is treated with a metal solution and maintained in an orbital shaker in the dark at room temperature (RT) and adequate pressure conditions (Aigbe & Osibote,2024). Some of the common method of synthesis of the metal oxide can be; top-down as mechanical milling, etching, electro-explosion, sputtering, Laser ablation or bottom-up as, Green synthesis sol gel process, pyrolysis, chemical vapor deposition, atomic or molecular among many other.

2.5.2. Synthesis of Nanoparticles by Using Plant

The use of plants or plant extracts, which act as reducing and capping or stabilizing agents for NP synthesis, is more useful over other biosynthesis procedures. This occurs because in this technique, no need cell culturing and cell maintenance. Moreover, NP synthesis from plants is more stable, allows the production of NPs with different shapes and sizes, and is cheap (Osman *et al*., 2024)

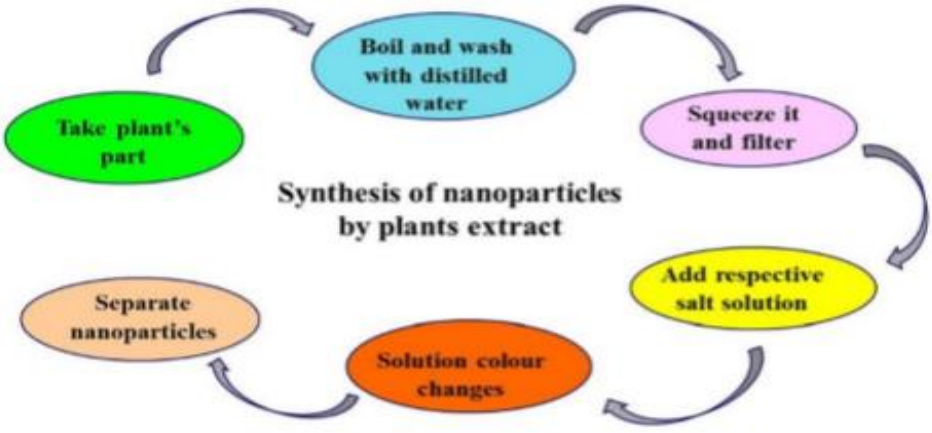


Figure 2**:** Environment friendly and cheap route for the green synthesis of nanoparticles using plants extract (Kandav & Sharma ,2024).

2.5.3. Method for synthesis of ZnO NPs and Co-ZnO NCs

Some of the common method of synthesis of the metal oxide can be; top-down as mechanical milling, etching, electro-explosion, sputtering, Laser ablation or bottom-up as, sol gel process, pyrolysis, chemical vapor deposition, atomic or molecular among many others. Some of the common method of synthesis of the metal oxide can be; top-down as mechanical milling, etching, electro-explosion, sputtering, Laser ablation or bottom-up as, Green synthesis , sol gel process, pyrolysis, chemical vapor deposition, atomic or molecular among many other(Oggu *et al*., 2025)

Table 2: Some method of synthesis of ZnO NPs

Methods	**Process**	**Advantages**	**Disadvantages**	**References**
Chemical synthesis	Spray pyrolysis, thermal breakdown, molecular beam epitaxy, chemical vapor deposition.	It is the most significant process, and it is performed with a variety of precursors and under a variety of variables. The size and geometries of NPs ar e morphologically changed	Hazardous compounds adsorbed on the surface, which could have negative consequences.	(Toma *et al.*,2025)
Vapor transport synthesis	Zinc and oxygen V apors react with each other	It is the most prevalent method and growth temperature is relatively moderate.	Imbalance vapor pressure ratio may affect the ZnO	(Swihart, 2023)
Hydrothema l synthesis	Low temperature process	The use of simple equipment, catalyst free growth, low cost, homogeneous production, and Ecofriendliness,	May require high temperatu re to initiate.	(Liu & Zeng, 2024)
Green synthesis	Plant components such as the leaf, and other parts	This is a very environment friendl y, low-cost method that does not require the use of intermediate base groups.		(Kaningini et al., 2022)

Table 3: The Recent study conducted by a different types of plants to synthesize ZnO Nanoparticles and Metal doped-ZnO Nanocomposite

NO.	Plants	Part Used	Nanoparticle	Size(nm)	Studies	References
1.	Alotropis gigantean	Leaves	ZnO	30-35	Antibacterial	(Pirsaheb *et al*.,2024)
2	Aloe vera	Leaves	ZnO	40	Antibacterial	(Wu *et a*l.,2024)
3	Seaweeds	Leaves	ZnO	45	Photocatalytic	(Bai *et al*.,2024)
4	Cassia auriculata	Flower	ZnO	36	Antibacterial	(Seshadr ,2021)
5	Salvadora persica	Leaves	ZnO	80-90	Photocatalytic	(Dhabbe *et al*.,2023)
6	Allium stavium	Bulbs	ZnO	30-50	Antioxidant	(Ali *et al*.,2023)
7	Vitis vinifera	Leaves	Ag –ZnO	20-30	Antibacterial	Saravanadevi *et al*.,2022)
8	Carica papaya	Leaf	Mn-ZnO	25-40	Photocatalytic	(Subbiah *et al*.,2021

2.6. Characterization techniques for metal oxide nanoparticles and metal-doped metal oxide nanocomposites

UV-Vis spectroscopy uses light in the visible range. The principle of UV-vis spectroscopy is based on the absorption of ultraviolet light or visible light by chemical compounds, which results in the production of distinct electronic spectra. UV-Vis spectroscopy was used to show the band gap energy of synthesized nanoparticles. The band gap increases with decreasing particle size, and the absorption edges are shifted to higher energy (blue shift) with decreasing particle size (Goktas *et al*.,2022).

The plots of variation of $(\alpha h\nu)^2$ versus hν for the ZnO and Co-ZnO NCs were presented. These plots are known as Tauc's plots, and they are used to find out the accurate optical band gap value by the extrapolation of the linear part, which is used to calculate an optical band gap of the. The tauc plot has the photon energy (hν) on the X-axis and a quantity $(\alpha h\nu)^2$ on the Y-axis, and extrapolating the linear portion of the curve to the X-axis yields the band gap energy of the material (Parajul *et al*.,2023).

FT-IR spectroscopy analysis is a method based on the principle of infrared spectroscopy. Functional groups can be associated with characteristic infrared absorption bands, which correspond to the fundamental vibrations of the functional groups .

For phase identification, XRD patterns were collected on an X-ray diffractometer. The atomic planes of a crystal cause an incident beam of X-rays to interfere with one another as they leave the crystal (Sinha *et a*l., 2023)

XRD is a non-destructive technique used to identify crystalline phases and orientations, measure the thickness of thin films and multi-layers, and determine the atomic arrangement and structure of the NPs. The XRD technique is also used to get information like lattice parameters, the crystallite size of crystals, defects, and strains, and the average crystallite size of the nanoparticles, which was calculated using Scherer's formula Where D is grain size, K is an empirical constant of 0.94, (λ is the wavelength of the incidence beam (λ=1.5406 Å), β is the full-width half maximum (FWHM) in radian, and θ is the angular of the peak position(Nadjia & Abdelkader,2025).

The **scanning electron microscope** is a very useful instrument to get information about topography, morphology, and composition information of the sample The morphologies of the phytomolecules coated ZnO nanoparticles and Co-ZnO nanostructures were studied using SEM .The SEM instrument is based on the principle that the primary electrons released from the source provide energy to the atomic electrons of the specimen, which can then release secondary electrons (SEs) and an image can be formed by collecting these secondary electrons from each point of the specimen (Datye & DeLaRiva ,2023).

2.7. Medicinal Value of *Croton macrostachyus*

Green synthesis of zinc oxide (ZnO) nanoparticles using different plant extracts of Azadirachta indica, Hibiscus rosa-sinensis, Murraya koenigii, Moringa oleifera, and Tamarindus indica for biological applications has been reported(Prasad, *et al.,* 2021).C*roton macrostachyus* is commonly known as; rush foil (English), Bakkaniisa (Afan Oromo) and Bisana (Amharic). It belongs to the Euphorbiacea with 300 genera and 8,000- 10,000 species and most abundant plant in the tropics (Abdisa ,2019). It is native to Ethiopia, Eretria, Kenya, Tanzania, Uganda and Nigeria which is a medium-sized deciduous tree of East Africa particularly wide spread between 200-2500 m. The name of *Croton* comes from a Greek word '*Kroton'* which means ticks, because of the seeds' resemblance to ticks, the specific name "macrostachyus" is a contraction of two words, the Greek word "macro" meaning large and "s*tachyus*" relating to the spike, hence, a species characterized by large spikes *croton macrostachyus* is regarded as a multipurpose tree by subsistence farmers in Ethiopia, Kenya, and Tanzania and the species has potential in playing an important role in the healthcare.

The bark, fruits, leaves, roots, and seeds of *croton macrostachyus* are reported to possess diverse medicinal properties and *Croton macrostachyus* is used as herbal medicine for at least 61 human and 20 animal diseases and ailments. (Asfaw *et al*., 2022). Traditionally *croton macrostachyus* used for treatment of malaria, rabies, gonorrhea, wound, diarrhea, hepatitis, jaundice, scabies,too thache, abdominal pain, cancer, typhoid, pneumonia and gastrointestinal disorders and as ethnoveterinary medicine. Pharmacological studies on C*roton macrostachyus* indicate that it has a wide range of pharmacological activities suchas anthelmintic, antibacterial,antimycobacterial,antidiarrheal,antifungal,sedative,antidiabetic,anti inflammatory ,antileishmanial ,antioxidant,antiplasmodial,and larvicidal effects. The leaves and shoots of C*roton macrostachyus* are used to treat fever and oedema and also mashed leaves used for treatment of hemorrhoids. Moreover, the maceration of *croton macrostachyus* stem bark is used as abortifacient and uterotonic to expel retained placenta. *Croton macrostachyus* have the activities of against diarrhea; traditional healers in Ethiopia use a wide range of medicinal plants with antidiarrheal properties .The chemical constituent in the C*roton macrostachyus*, Terpenoids such as abietic acid and steroids like phytosterols have been showed to inhibit production of prostaglandin E2 which have a crucial role in stimulation of intestinal secretion, therefore it has antidiarrheal activities.

Figure 3**.** *Croton macrostachyus* plant **(**Ayele *et al.*,2022).

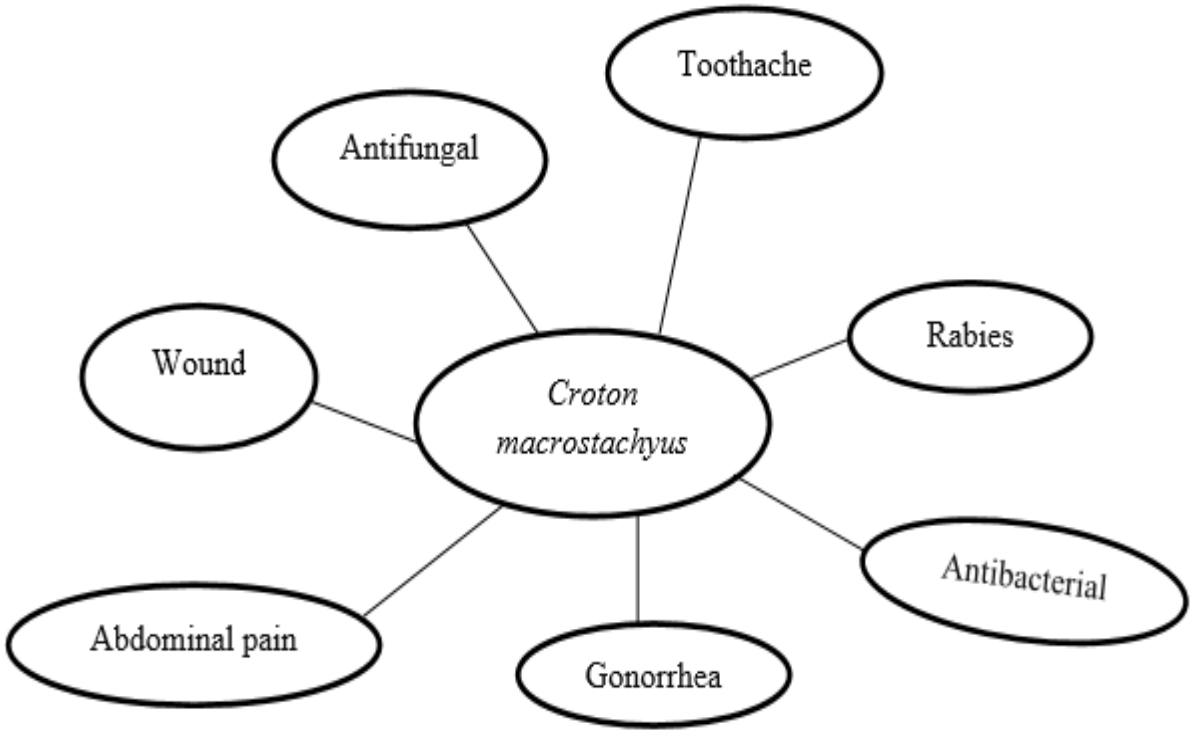


Figure 4.Some use of *Croton Macrostachyus* plant leaf (Asfaw *et al*., 2022).

2.8. Factors affecting the synthesis of nanoparticles and nanocomposites

There are several factors that affect the synthesis, characterization, and application of nano composite. Many researchers have reported the change in nature of the synthesized nanoparticles with the type of the adsorbate and the activity of the catalysts used in the synthesis process . Some of them have reported the dynamic nature of the synthesized nanoparticles with different types of symptoms and implications by change with time and environment,and so forth. There are several other important factors that affect the synthesis of nanoparticles, including pH of the solution, temperature, concentration of the extracts used, concentration of the raw materials used, size, and above all the protocols that are followed for the synthesis process. Some dominant factors that affect nanoparticle biosynthesis are described below (Azad *et al*.,2023).

2.8.1. pH

pH is an important factor that influences the synthesis of nanocomposites by green technology methods. Researchers have discovered that pH of the solution medium influences the size and texture of the synthesized nanoparticles. Therefore, nanocomposites size can be controlled by altering the pH of the solution media.

The effect of pH on the shape and size of the synthesized silver nanoparticle was demonstrated by(Liaqat *et al* .,2022).Changing the shape and size of metallic NPs can greatly improve their efficiency. These morphological parameters can be controlled by adjusting experimental conditions, such as reaction time, reactant concentration, pH, temperature, and the concentration of plant extracts and salts. Proper control of these parameters is crucial for optimizing the synthesis of metallic NPs. pH specifically plays a significant role in determining NP morphology. In an acidic pH environment, larger NPs tend to form.

2.8.2. Temperature

Temperature is another important parameter that affects the synthesis of nanoparticles using all three methods. The physical method requires the highest temperature (>350°C), whereas chemical during long storage; they may have shelf life, and so forth, that affects their potential methods require a temperature less than 350°C.In most cases, the synthesis of nanoparticles using green technology requires temperatures less than 100°C or ambient temperature. The temperature of the reaction medium determines the nature of the nanoparticle formed (Iqbal *et al*.,2021)

2.8.3. Reaction Time

The quality and type of nanoparticle synthesized using green technology are greatly influenced by length of time for which the reaction medium is incubated .Similarly the characteristics of the synthesized nanoparticles were also altered with time and greatly influenced by the synthesis process, exposure to light, and storage conditions, and so forth. The variations in the time may occur in many ways such as aggregation of particles due to long time storage; particles may shrink or grow (Eker *et al*.,2025).

2.8.4. Effect of precursor concentration

The concentration of the precursor is responsible for the morphology and size of nanoparticles. Increasing precursor concentration generally leads to larger nanoparticle sizes and can alter their shape (morphology) and crystallinity, often growth or different crystal facets, impacting optical/magnetic properties; higher concentrations mean more building blocks, accelerating nucleation and growth, but sometimes leading to aggregation or different growth directions (e.g., spherical to rod-like) (Sedefoglu ,2024).

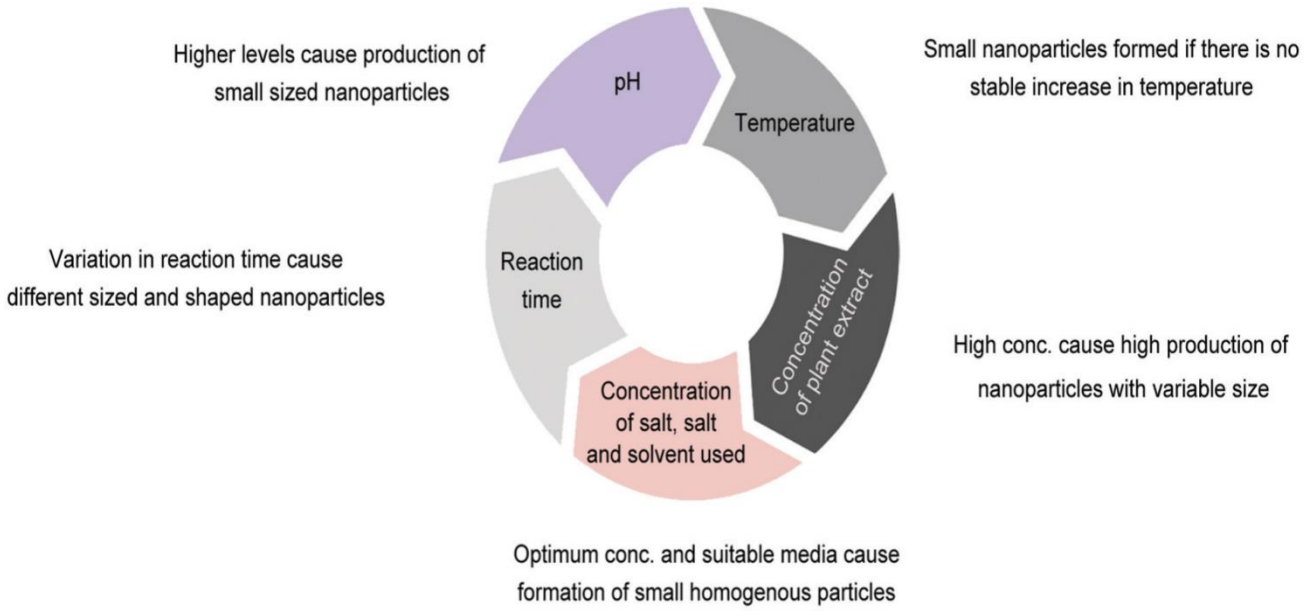


Figure 5: Factors affecting the green synthesis of nanocomposites (Azad *et al*.,2023).

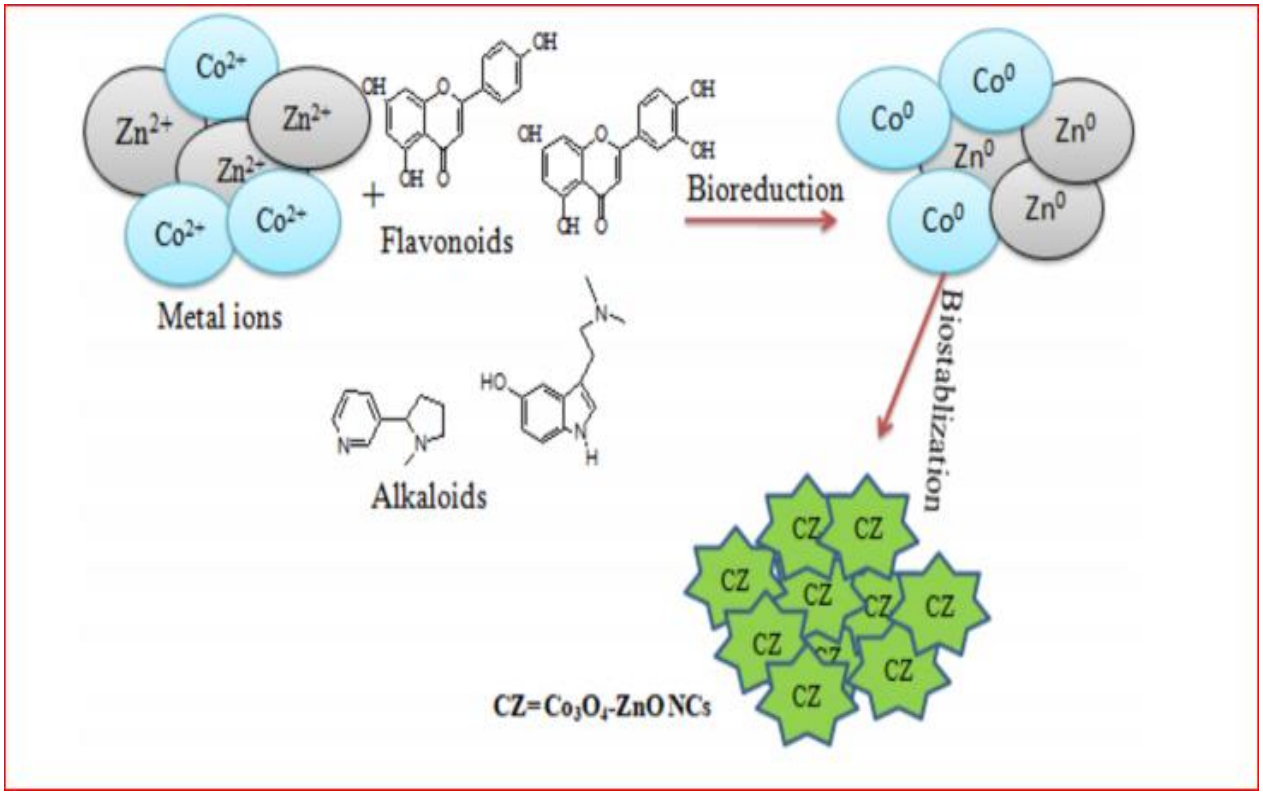


Figure 6**:** Mechanism representation for the for phytochemical present in *Croton macrostachyus* leaf extract synthesis of Co−ZnO nanocomposite **(** Alemu & Zeleke ,2024).

3. METHODS AND MATERIALS

3.1.Experimental site

The study was carried out at Wallaga University main campus, Chemistry Research Laboratory which is located on the verge of Nekemte town, which is 310 km away from Finfine westwards, with beautiful scenery of landscape and spectacular view of Mount Komto. It is situated on an area of 150 hectares.

3.2. Chemicals and Reagents

All the chemicals and reagents used in this study were of analytical grade. Cobalt nitrate hexa hydrate ($Co(NO_3)_2.6H_2O$) (99 %), Sodium hydroxide (NaOH,99%) Zinc Nitrate hexahydrate ($Zn(NO_3)_{2.}6H_2O$(99%),Sulphuric acid (H_2SO_4), Dimethylsulfoxide (DMSO) , Nutrient agar,), potassium iodide (KI, 98.5%), Iodine (I_2, 99%), Ethanol (CH_3CH_2OH, 98%), Muller Hinton Agar (MHA, Merck chemical company), Ferric chloride ($FeCl_3$,99% from Loba Chemie, India),acetic anhydride($(CH_3CO)_2O$,99.5%),DMSO(dimethylsulfoxid $(CH_3)_2SO$,99%),hydroch loric acid (HCl,37%India),*Croton macrostachyus* leaf extract and D -ionized water (D. H_2O).

3.3. Apparatus and Instruments

Mortar and pestle, digital balance (Adam, AFP-110L, UK), magnetic stirrer plate (Staffordshire ST15OSA, UK), refrigerator (BekoRRN2650,Turkey), beakers, test tubes, -droplets, graduated cylinder cuvettes, eryemer flasks, electrical grinder, test tube rack and Oven (model: 158005/12, Gallen Kamp, England)and pH meter was used in this study.Double beam UV-Vis Spectrophotometry (UV-Vis: SPECORD 200 PLUS -223, Germany X-Ray Diffractometer (XRD 700 Drawell, Artist of Science, Shimdazu, Japan), Fourier Transform Infrared Spectroscopy (FT IR:Perkins Elmer,Spectrum Two LITA, L1600300, UK) was used for characterization of ZnO NPs and Co-ZnO NCs.

3.4. *Croton macrostachyus* leaf Collection and Preparation

Fresh leaf of *Croton macrostachyus* was collected from University of Wallaga's Botanical Garden in April 2025. Its scientific name was identified and the sample was authenticated at Wallaga University Department of Biology by Doctor Tashome Gemechu. The collected fresh leaves was thoroughly washed by tap water followed distilled water to remove any dirt particles.

The washed leaves was air dried and the dried *croton macrostachyus* leaf was grinded using electrical grinder. Then, 50 g dry *Croton macrostachyus* leaf was weighed using electronic balance and transferred to 1000 mL beaker; 500 mL of distilled water was added into the beaker. The mixture was heated for 30 minutes with stirring using a magnetic stirrer at 60 °C and the extract was chilled for 30 minutes at room temperature (Masho *et al*., 2024).Then, the clear extract was collected by filtration using What-man no.1 filter paper and then stored in the refrigerator at 4 °C for further uses (Figure 7) (Masho *et al*.,2024).

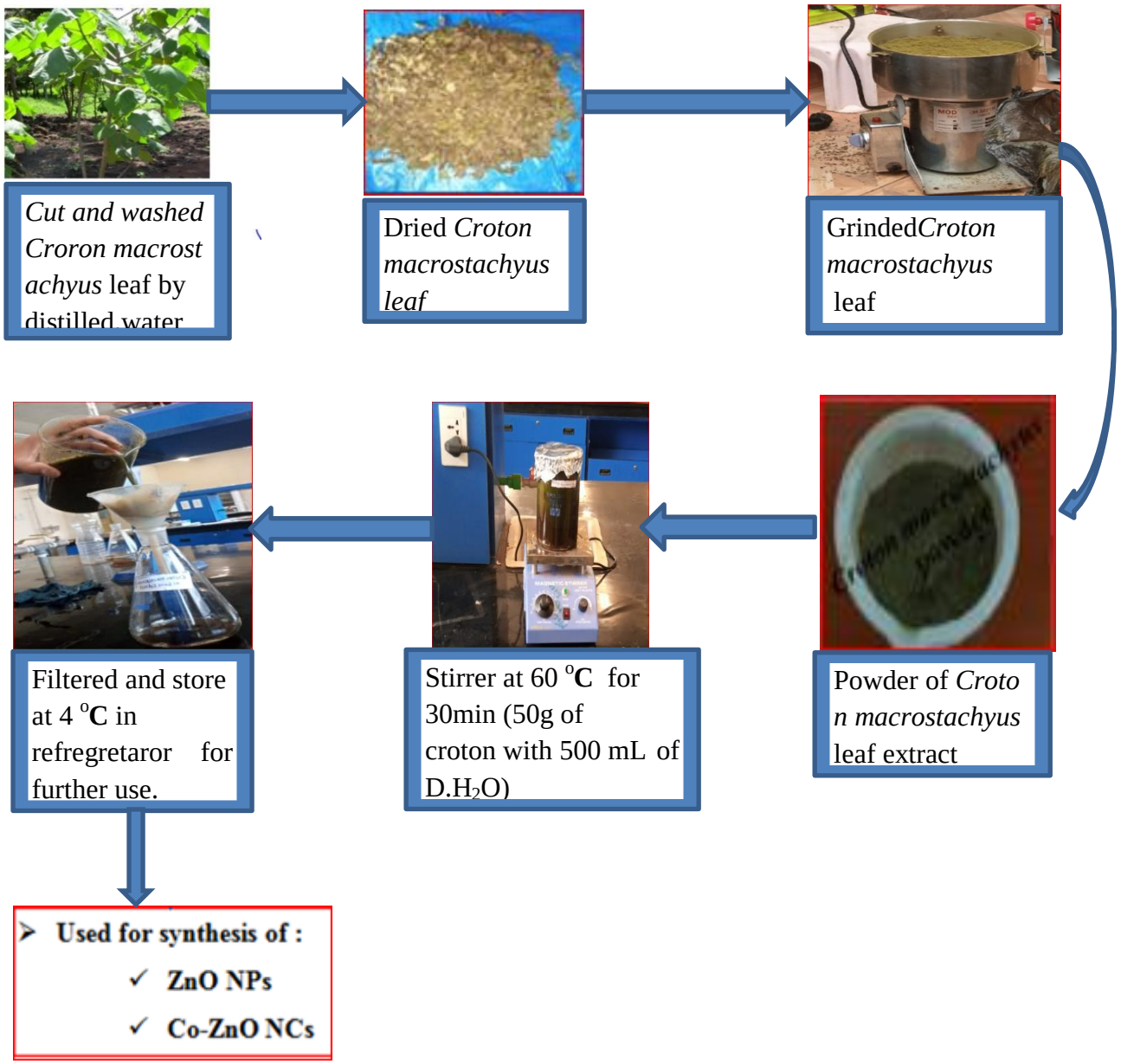


Figure 7.Schematic preparation of *Croton macrostachyus* leaf extract

3.5. Preliminary Phytochemical Screening

The qualitative phytochemical investigation of aqueous solution extract of *Croton macrostach yus* leaf was carried out.Phytochemicals,non nutritive plant substances with anti inflammatory and disease-fighting properties, were identified in *C. macrostachyus* leaf extract through secondary metabolite qualitative analysis. The *C. macrostachyus* leaf extract was tested for alkaloids, flavonoids, tannins, saponins, and phenols . A reddish-brown precipitate was found in Wagner's test, while a yellow-colored precipitate was found in the lead acetate test. Tannins and phenolic compounds were detected through the ferric chloride test, while saponins were detected through the foaming test

Flavonoids (Alkaline reagent test). when 3 mL of the extract was treated with 3 mL of NaOH solution. During this test formation of yellow color was indicated the presence of flavonoids in *Croton macrostachyus leaf* extract (Masho *et al*., 2024).

Tannin (Ferric Chloride test): During this test 3 mL of *Croton macrostachyus* leaf extract was mixed with 2 mL of distilled water and few drops of $FeCl_3$ solution (5% w/v) was added. Formation of a green precipitate was indicated the presence of tannins (Malgwi *et al*., 2024).

Saponins (Foaming test) : During this test 3 mL of *Croton macrostachyus* leaf extract was introduced into a tube containing 5 mL of distilled water and agitated in a measuring cylinder for 15 min. The formation of 1 cm layer of foam shows the presence of saponins in the *Croton macrostachyu*s leaf extract (Masho *et al*., 2024).

Alkaloids(Wagner's test): During this test 3 mL of *Croton macrostachyus leaf* extract was stirred with 1% hydrochloric acid (HCl), and then Wagner reagent was added. Then , formation Reddish-brown precipitate was formed which show the presence of alkaloids *Croton macrostachyus leaf* extract (Gendo *et al*.,2024)

Phenolic(Ferric Chloride test): During this test 4 mL *Croton macrostachyus* leaf extract of the was treated with 4 drops of ferric chloride ($FeCl_3$) solution.Then, the formation of bluish black precipitates was confirmed the presence of phenolic compounds in Crotonmacrostachyus leaf extract (Malgwi *et al*., 2024).

3.6. Optimization of synthesis parameters for Co-ZnO NCs

3.6.1. Effect of concentration of precursor salt solution

The effect of precursor salt concentration was optimized for the optimum synthesis of the metallic nanoparticles by increasing the concentration of $Co(NO_{3)2}.6H_2O$ solutions from 0.03(3%) , 0.05 (5 %) and 0.07 (7 %) with keeping the 20 mL volume of the plant extract ,pH 10 , temperature 60 ^{0}C and time 1hrs constant. 3% ,5% and 7% Co doped ZnO nanoparti cles were synthesized using thesame procedure of the synthesized ZnO nanoparticles using the same volume of plant extract and 0.089g ,0.148g and 0.210g of $Co(NO_{3)2}.6H_2O$ were added to 2.975g of $Zn(NO_3)_2{\cdot}6H_2O$ and 20 mL *Croton macrostachyus* leaf extract were added to three separate 500mL beaker to obtain 3% Co/ ZnO NPs ,5% Co/ ZnO NPs and7% Co/ZnO NPs .

After the addition of 1 M NaOH, the solution was stirred for 1 h at 60 ^{o}C and the yellowish color solution was obtained. Then, the precipitate was collected, centrifuged for 5 min, and washed with diionized water three times followed by ethanol. Next, the product was dried in an oven at 60 ^{0}C for 4 hrs Then, absorbance of Co/ZnO NCs wasmeasured in the range of (200 to 800nm by UV_Vis Spectroscopy(Ogwuegbu *et al* 2025). During this study 5%Co ZnO NCs was preferred as optimum value depending on UV –Vis spectroscopy result obtained.

3.6.2. Effect of volume of plant extract

The other optimizations was an optimization of the volume of plant extract. In doing so, different volumes (10 mL, 15 mL, 20 and 25 mL) of plant extract was mixed with a constant concentration of precursor(5% Co(NO3)2.6H2O with 2.975 g of $Zn(NO_3)_2{\cdot}6H_2O$ at the pH value of 10 , 60 ^{0}C and 1 hrs constant and the UV-Vis result was recorded. During the experiment, the sharpest peak with the highest absorbance was preferred. During this optimization 20 mL of volume of plant was taken as optimum value (Bekele *et al.*,2024).

3.6.3. Optimization of pH value

The pH of the solution influences the size, shape, and rate of synthesis in the plant mediated biosynthesis of nanoparticles. The ability of the reaction (pH) to vary the electrical loads of biomolecules, which may affect their ability to cap and stabilize, and, as a result, the formation of nanoparticles, is a major influence .

In this study the pH of the solution was varied from (4,6.8,10 to 12)for CoZnONs at constant concentration of metal salt precursors 5% Co/ZnONCs ,60 ^{0}C .volume of *Croton macrostac hyus* leaf extract (20mL) and time 1 hrs to obtain the optimum pH value.During this study p H 10 was taken as optimum value (Singaravelu *et al.*,2025).

3.6.4. Optimization of reaction temperature

At constant pH 10, volume of Croton macrostachyus leaf extract 20 mL and cobalt salt con centration of 5% Co/ZnO NCs various temperatures (room temperature 50,80 and 100 ^{0}C) was used to obtain the optimum temperature for synthesis of Co ZnONCs (Oluwaniyi *et al.*,2025).In this case 80 ^{0}C was taken as optimum temperature**.**

3.7. Synthesis of ZnO nanoparticles by using C*roton macrostachyus leaf* extract

The synthesis of ZnO nanoparticles was carried out using the method with (Endeshaw *et al*., 2023) Accordingly, 2.975 g of Zn $(NO_3)_2.6H_2O$ was prepared in a 250 mL flask which contains 100 mL of distilled water and it was shaken until dissolved well and transferred to a beaker. After that, 20 ml of C*roton macrostachyus* leaf extract was added into the solution. The pH of the solution was adjusted by adding 1 M NaOH until pH was adjusted to 11.It was heated for 2 hours at temperature of 60^{o}C. And then, it was cooled at room temperature for filtered by filter paper whatman no.1 then,washed using distilled water and ethanol to remove impurities The product was collected and dried in Oven at 60 ^{o}C for 3 hours .It was powdered by mortar and pestle and then calcinated in muffle furnance for 2 hours at 500 ^{o}C then,stored in desiccators for further characterization.

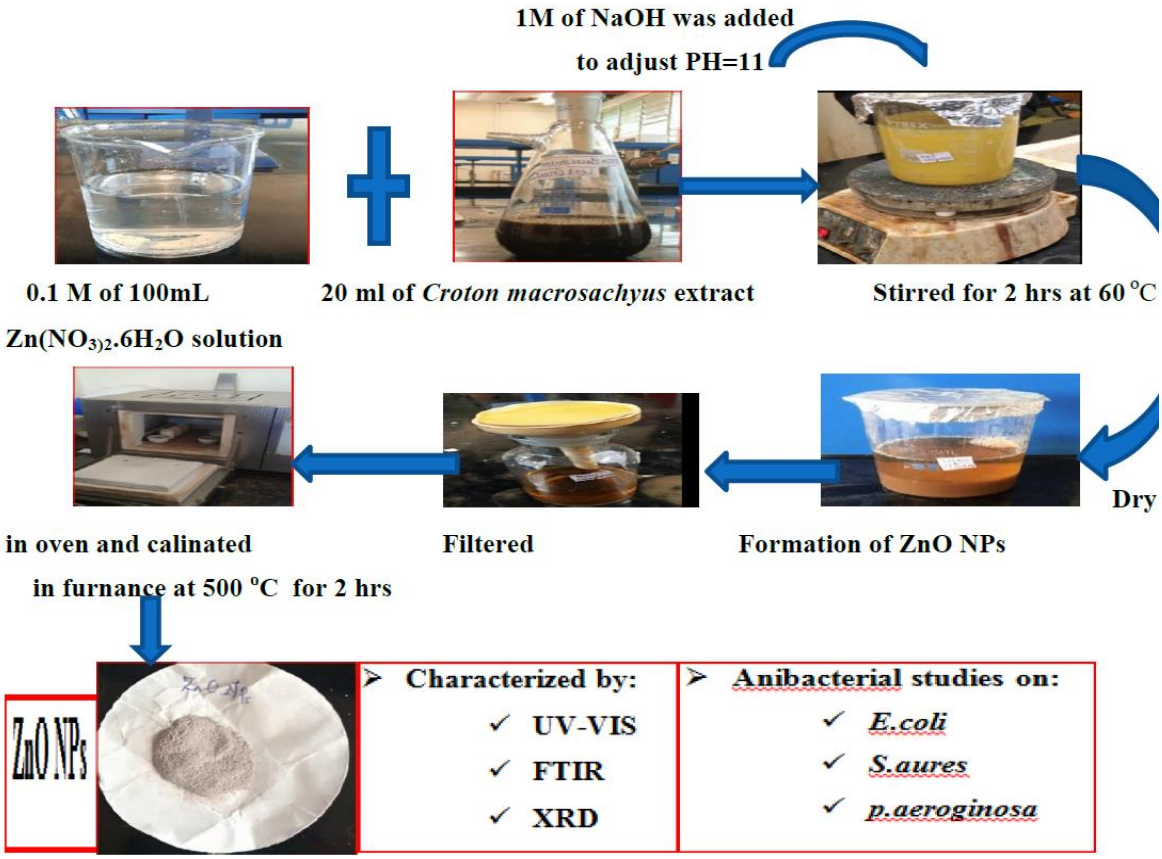


Figure 8: Schematic representation of ZnO NPs synthesized by *Croton macrostachyus leaf extract*

3.8. Synthesis of Co-ZnO NCs Using *Croton macrostachyus* Leaf Extract

Synthesis of Co-ZnO NCs using *Croton Macrostachyus* leaf extract was carried out based on the literature with slight modifications (Ogwuegbu *et al* 2025). 2.975 g Zinc Nitrate hexa hydrate ($Zn(NO_3)_2.6H_2O$) solution was prepared in 100 mL of distilled water.

After its complete dissolution,0.05 (5%) (0.148 g) of cobalt nitrate ($Co(NO_3)_2.6H_2$ was added together.Then,20 mL of *Croton macrostachyus* leaf extract was mixed into the mixture of the solution and the reaction mixture was heated with stirring using magnetic stirrer at 80 ^{0}C for 2 hour until the color was changed which indicates the formation of Co/ZnONCs.

The precipitate was washed with di_ionized water three times followed by ethanol to obtain pure Co/ZnO NCs and dried in oven at 60 ^{0}C for 3 hours.Finally, it was ground in amortar and pestle to give the final nanocomposite, then calcinated in muffle furnance for 2 hours at500 ^{0}C which was used for different characterizations.

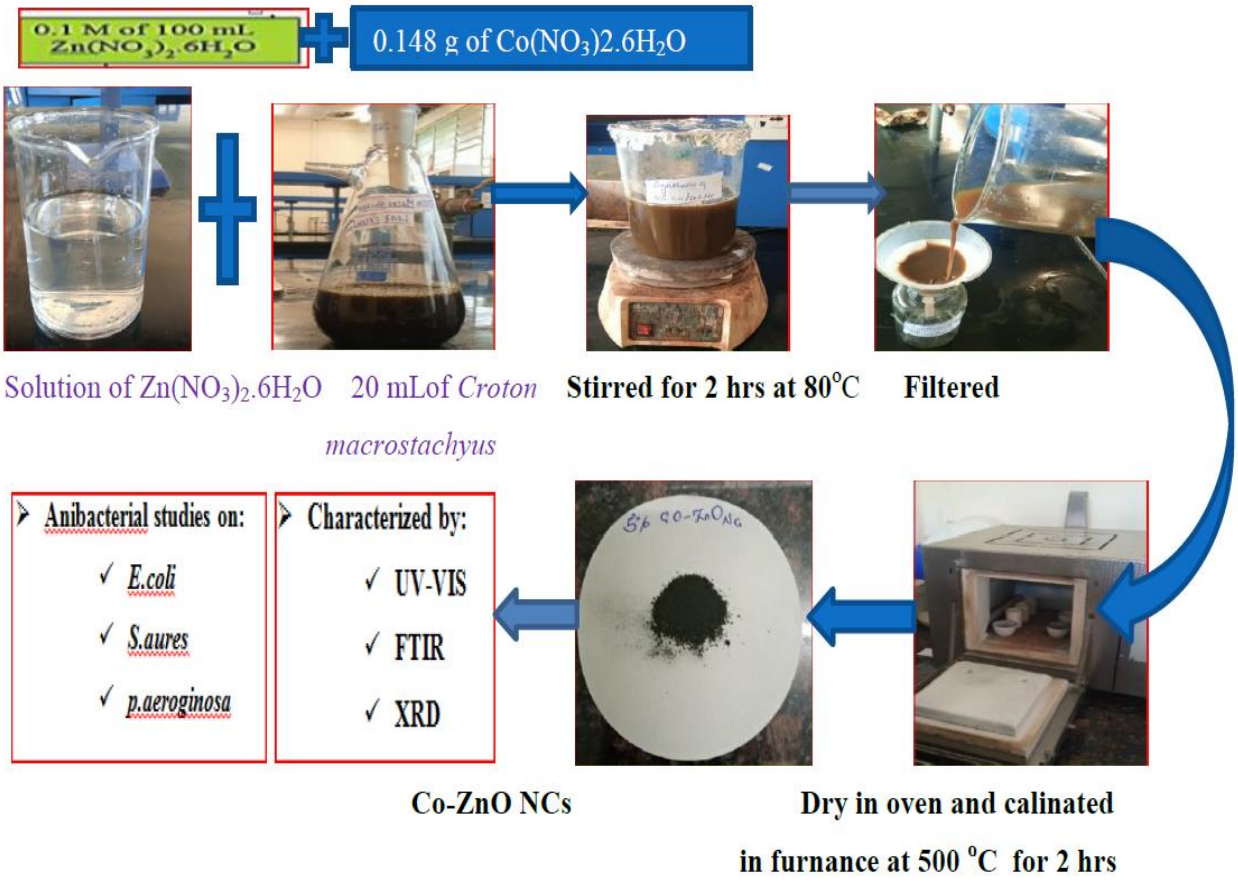


Figure 9: Schematic representation of Co-ZnO NCS synthesized by *Croton macrostachyus* leaf extract

3.9. Characterization Technique for ZnO NPs and Co-ZnO NCs

3.9.1. UV-Vis Spectroscopy Studies

The ZnO NPs and Co-ZnO NCs was dissolved in distilled water sonicated and transfer into cuvette inserted into UV/Visible spectrophotometer.The absorbancewas measured at the range of the200-800 nm (Riaz *et al*.,2023).A UV−vis study was conducted to evaluate the visual properties of ZnO NPs, and Co−ZnO NCs. The absorbance was recorded in a quartz cell at room temperature, and the band gap energy was calculated using the Tauc/David-Matt model.The band gap energy (*E*g) of ZnO NPs and Co−ZnO NCs was calculated from the Tauc plot by using the Tauc/David-Matt model (eq 1).

$$Ahv = (hv - Eg)^{n/2} \quad \text{.....(1)}$$

where *A*, *h*, *v*, *α*, and *E*g are ,Absorbance, Planck's constant, light frequency, absorption coefficient, and energy band gap,respectively.

3.9.2. Fourier Transform Infrared (FT-IR) Spectroscopy Studies

The KBr was grounded into finer particles and then,0.2 g of KBr was then weighed into a sampler holder.The lids of the sample holder were then kept in place and the powdered KBr crashed (molded)into transparent plate for analysis.

The transparent plate was then placed between IR souce of the instrument and the spectrum obtained.The septrum obtained from KBr was set as background reference for theanalysis.(i.e The spectrum for KBr is substracted from any subsequent analysis). After calibration was completed 0.2 g of KBr was weighed and mixed with 0.002 g of each sample(i.e. *Croton macrostachyus* leaf extract ,ZnO NPs ,5% Co-ZnO NCs and 7% Co-ZnO NCs).The mixture was then placed in the sample holder and molded into a transparent plate .The plate obtained was then placed between the IR source and a spectrum was obtained when the IR lght turn on to scan four times. Then, the obtained spectrum was then converted into transmittance for analysis of the varius functional group present in the range of 4000 – 400 $cm^{-}1$(Rayya *et a*l.,2025).

3.9.3. X-ray diffraction (XRD) Studies

X ray diffraction was conducted to study the phase formation, size, purity, and crystallinity of the synthesized nanoparticles and nanocomposites .The diffraction patterns were recorded from 10 to 80 with 2θ angles using Cu−Kα (λ =1.54 A) radiation operated with accelerating voltage and applied current of 40 kV and 30 mA, respectively, and the average crystallite size was calculated using the Debye−Scherrer equation (eq 2)((Bhattacharje *et al*.,2023)

$$\chi = \frac{k\lambda}{\beta\cos\theta} \quad \text{.....eq (2)}$$

Where, D is crystallite size in nanoparticle, K is Scherer constant with value from 0.94, β is the full width at half maximum (FWHM) in radian, λ is the wavelength of X-ray source (0.15406 nm) and θ is the Bragg angle.

3.10. Antibacterial Activity of Green Synthesized ZnO NPs and Co-ZnO NCs.

The Müller–Hinton agar (38 g) and Nutrient agar (28 g) was dissolved in 1000 mL of deionized distilled water to prepare bacteria culture media (Al-Enazi *et al*., 2023). Then it was sterilized in an autoclave for 15 min at a temperature of 121^{o}C. At last, it was poured into sterile plates. To ensure the absence of any contamination, it was placed in an incubator at 37 ◦C for 24 h. In order to prevent the entry of any microbial contamination into the culture medium, all these steps was performed next to the flame and under sterile conditions. The diameter of the halo of non-growth of bacteria was investigated by different of Co-doped ZnO-NPs on *Staphylococcus aureus*, *pseudomonas aeroginosa* and *Escherichia coli* cultured for 24 h at 37 ◦C by welling in agar or spreading wells in agar.For this purpose, 24 h after making a well on the plate and transferring 25 mL of Co-doped ZnO-NPs in different concentrations into each well, the diameter of the halo of non-growth of bacteria by Co-doped ZnO-NPs on the plates was measured and reported in milimeters with a precise ruler.

In the MIC evaluation, first, dissolve 0.2 g of Co-doped ZnO-NPs in 1 mL of sterile deionized water and prepared the original stock. In the next step, after preparing the concentrations of 0–100 μg/mL, take 50 mL from the leading stocks of the desired substance using a sterile sampler. Take 50 mL from the leading stocks of the desired substance (Basseem *et al*.,2023).

Then added it into 1000 mL of Mueller Hinton Broth culture medium.An additional tube containing only the liquid culture medium and the desired bacteria was considered a control. Then, all the tubes was transferred to a shaker incubator at 37 ◦C and 150 rpm and heated for 24 hrs. After this period, the tubes was removed from the incubator, and their MIC was measur eed by direct eye observation and concentration measurement with a nanodrop device.A disk impregnated with ciprofloxacin (10μg/disk) and DMSO(10%) was employed as a positiveand negative control, respectively,totest the effect of the standard and solvent on the pathogens, respectively.

3.11. Data Analysis

All data obtained from UV-Visible, XRD, FT-IR spectroscopy characterization of synthesized NPs and NCs was analyzed using Origin Pro 64-bit and IBM SPSS software was used to interpret the antibacterial activities of synthesized NPs and NCs and aslo Origin pro 64-bit was used for sketching graph of the sample depending on the result displayed.

4. RESULT AND DISCUSSION

4. 1. Phytochemical screening of the *Croton macrostachyus* leaf extract

Croton macrostachyus leaf extract were contain secondary metabolites, such as phenols, saponins,flavonoids, tannins, and alkaloids, among others and also it was used as reducing ,capping, and stabilizing agents in the formationof nanoparticles and nanocomposites.

The phytochemical screening of *Croton macrostachyus* leaf extract with very intense color and with intense color was observed as shown in (Figure 10) and as shown in (Table 4) also the sign "+" indicates the presence of phytochemicals, whereas "++" indicate high intensity ,respectively.This result was good agreement with (Masho *et al.*,2024), in which it was tested for the presence of phytochemicals in the *Croton macrostachyus* leaf extract. .

Table 4: Phytochemical components of *Croton macrostachyus* leaf extract

S/No	Secondary metabolites	Test used	Color observed	Results
1	Alkaloids	Wagner`s	Redish brown	+
2	Flavonoids	Alkaline reagent	Yellow color	+
3	Phenols	Ferric chloride	Bluish black	++
4	Tannins	Ferric chloride precipitate	Green	+
5	Saponins	Frothing test	Foam	++

Note : The "+" and "++" indicates the occurrence of Phytochemicals in *Croton macrostachyus* Leaf more and most respectively.

Figure 10: Phytochemals screening of *Croton macrostachyus* leaf exract**.**

4.2. Parameter optimizations for the synthesis Co-ZnO NCs

4.2.1. Concentration of dopant optimization.

In this study red shift was observed in the wavelength from 366 nm to 375 nm while the concentration of the dopant increase from 5% to 7% in which resulted in a change in the optical band gap value and therefore, (5%) was taken as optimum dopant concentration to synthesize Co/ZnO NCs, because it gave a sharp peak at maximum intensity compared to others shown on (Figure11a). A similar result was reported in the literature that studied the effect of concentration of Co dopant on the surface Plasmon peak resonance of ZnO NPs (Ogwuegbu *et al* 2025).

4.2.2. Effect of pH

During the optimization of Co-ZnO NCs the result with highest absorbance with sharpest peak or high intensity was preferred to get the optimizied value of pH. As shown (Figure11d) pH 10 was taken as the optimum value to synthesize Co/ZnO NCs because it gave a sharp peak at maximum wavelength (358 nm), as observed on o UV/Vis spectra result, and this result was in close agreement with (Singaravelu *et al.*,2025).

4.2.3. Volume of plant extract optimization

During the optimization of Co-ZnO NCs the result with highest absorbance with sharpest peak or high intensity was preferred to get the optimized value During this investigation when the volume of the *Croton macrostachyus* leaf extract was increased from 10 mL,15 mL,20 mL to 25 mL, and the peak formed was also different (Figure 11 b) (Nagajyothi *et al*., 2019).
In this case the phytochemicals present in the *Croton macrostachyus* leaf extract was use for the bio reduction and stabilization of the Co-ZnO NCs .During this study 20 mL was taken as optimum value due its high intensity when compared with other volume optimized as shown on UV-Vis spectra result.

4.2.4. Effect of temperature

During the optimization of Co/ZnO NCs the result with highest absorbance with sharpest peak or high intensity was preferred to get the optimizied value , temperature on the UV/Vis result shown on(Figure 11c), 80 ^{o}C was taken as optimized value because it have high absorbance with high intensity when compared to others. Co/ZnO NCs showed a small absorption peak at room temperature as observed on UV Vis spectra result.

Additionally at 80 ^{o}C a sharp peakwith a wave length of 356 nm was formed. And aslo there was a shift in wavelength at 100 ^{0}C observed from UV/V is spectra result (Oluwaniyi *et al.*,2025).

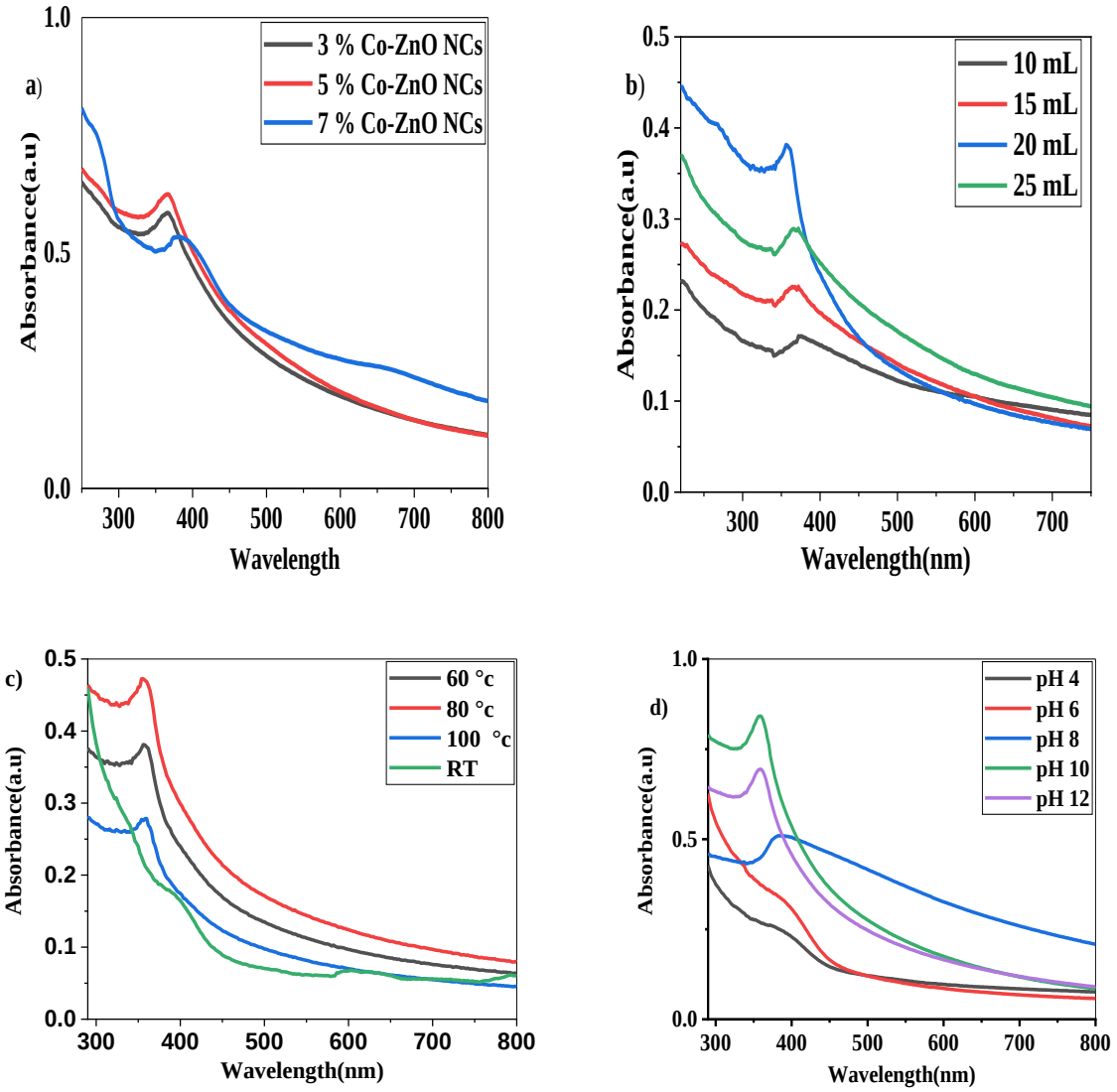


Figure 11**:** UV-Vis spectra optimization of a)concentration, b)Volume c)Temperature and d) pH value of Co-ZnO NCs .

4. 3. Characterization of Synthesized ZnO NPs and Co-ZnO NCs

4.3.1. UV -Vis analysis

The synthesized samples were characterized by UV/Vis spectroscopy at Wallaga University main campus. The absorption peak appeared at 268 nm for *Croton macrostachyu*s which showed on (Figure 12),358 nm and 366 nm (Figure 13) which confirms the formation of ZnO NPs and Co/ZnO NCs,respectively and which was closed agreement with (Gendo *et al.*, 2024).

The observed sharp peak indicated as the zinc ion is efficiently reduced by the *C.macro stachyus* leaf extract. The band gap energies of these synthesized nanoparticles were obtained by plotting absorptivity $(\alpha h\nu)^2$ as a function of energy (hv). Extrapolating the linear portion of the curve to the absorption axis gave band gap energies of 3.06 e V and 2.6 eV for ZnO NPs and Co-ZnO NCs respectively (Figure 14).There was a decrease in bandgap energies from ZnO NPs to Co-ZnO NCs.

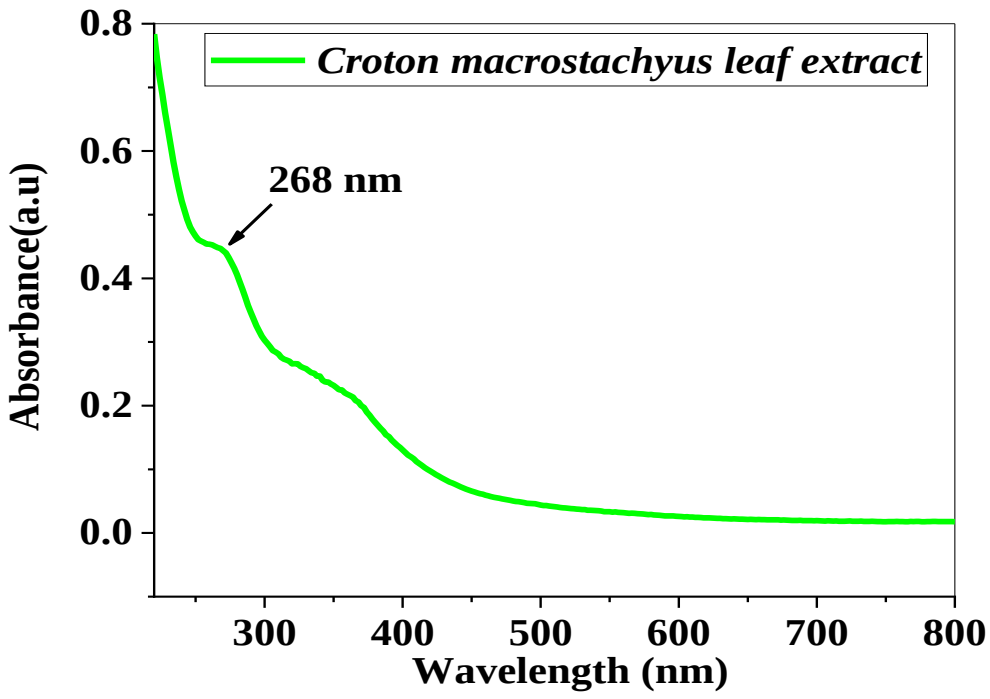


Figure 12: UV-Vis spectra of *Croton macrostachyus leaf* extract

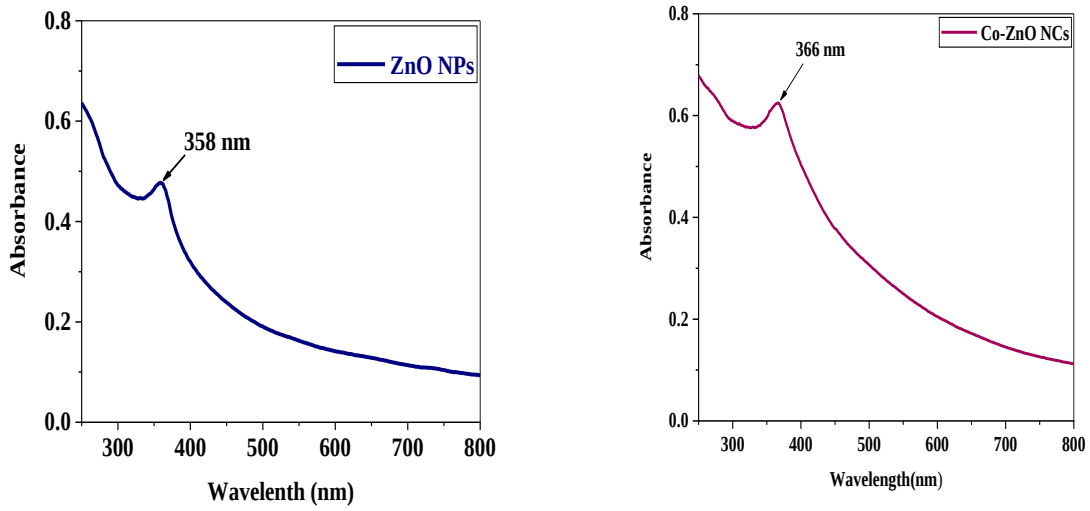


Figure 13: UV-Vis spectra of ZnO NPs and Co-ZnO NCs

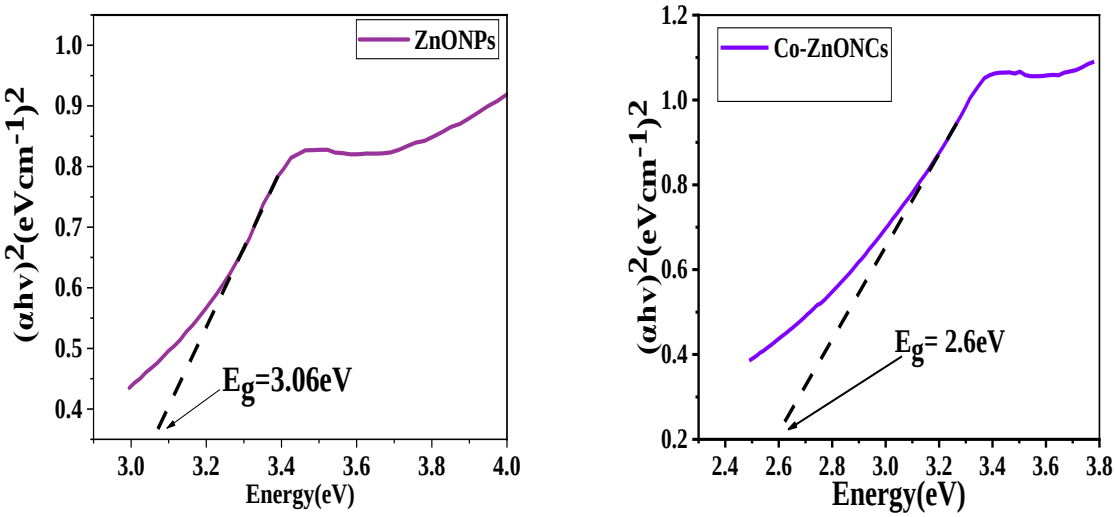


Figure 14: UV -Vis spectral band gap of ZnO NPs and Co-ZnO NCs

4.3.2. FTIR Analysis

The FTIR spectra of ZnONPs 5 % and 7 % of Co-ZnO NCs samples were showed in(Figure 15) The absorption bands formed was discussed in(Table 4). The weak intensity bands at around 872 cm^1 and 873 cm^1 are characteristics of metal oxygen stretching vibrations of the Zn-Ogroup well matched with (Raza *et al.*,2024) especially in the spectrum of raw ZnONCs which almost shifts in the case of Co-ZnO NCs.

Overall, the increase of the intensity of some peaks, the appearance of others, and the shift of some band positions Co-ZnO NCs may be attributed to the differences in the symmetry of molecules and their molecular dipole moments by the effect of green synthesis.

Table 5: FTIR absorption bands for ZnO NPs ,5% Co-ZnO NCs and 7% Co-ZnO NCs

Absorption bands				Explanation of the bending, vibration, and stretching bonds
Croton macrostachyus extract (cm^{-1})	ZnO NPs(cm^{-1})	5% Co-ZnO NCs(cm^{-1})	7% Co-ZnO NCs(cm^{-1})	
3261	3303	3392	3360	For *Croton macrostachyus* OH stretching vibration due to the presence of Phenols (Masho *et al*.,2025) and The band of hydroxyl groups (alcohols, phenols) and N–H (primary (1°) amines, amides) for Co-ZnO NCs
2926	1410	1409	1410	H bonded alcohols, and OH stretch for *Croton macrostachyus* and for Co-ZnO NCs there is C-O or C - N bond (Faramawy *et al*., 2024)
1601	1044	1048	1041	N–H bend amines for *Croton macrostachyus and* C-N stretch amines for Co-ZnO NCs(Sultan *et al*.,2025)
1387	873	872	873	C─H bend alkanes (Masho *et al*.,2025)for *Croton macrostachyus* leaf extract and metal oxygen stretching vi

		brations of the Zn/O group on CoZn O NCs((Raza *et al.*,2024
1064	-	Due to the C–O stretching vibration (Negash *et al.*,2023).

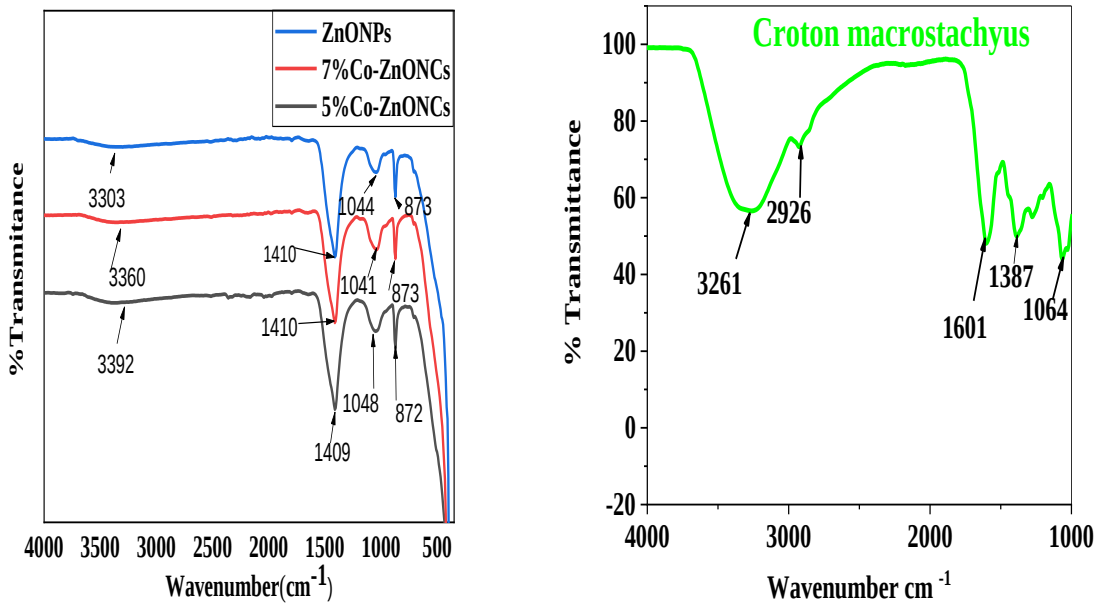


Figure 13**:** FTIR results of *Croton macrostachyus* ,ZnO NPs ,5% Co-ZnO NCs and 7% Co-ZnO NCs

4. 3. 3. X- Ray diffraction analysis

As shown (Figure 16) ZnO NPs were observed at 2θ values 29.41477, 31.7801, 34.53248, 3 6.23783,42.91347,56.6195,62.5363,68.18357 were indexed as (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202) planes, respectively. The observed diffraction peaks in the diffraction pattern of ZnO agreed well with the hexagonal wurtzite ZnO structure with (JCPDS card no 36 1451). For Co doped ZnO peaks, peaks due to the diffraction peaks appeared at 2θ values of 28.4385,31.7296,34.5088,36.2478,42.9588,47.673 4,56.5547,62.6254 and 68.122 ,which were indexed as (111), (220), (311), (400), (222), (511) , and (440), respectively, and the synthesized material was confirmed to be Co_3O_4 NPs, and JCPDS 042−146 (220) and (311) planes of Co_3O_4 nanoparticles were observed alongside the characteristic peaks of ZnO, which confirms the formation of solid solution in the Co doped ZnO nanoparticles.which was confirmed with (Asaulyuk *et al.*,2021).

For Co doped ZnONPs peaks, were observed alongside the characteristic peaks of ZnO NPs which confirms the formation of solid solution in the Co doped ZnO nanoparticles. As the result showed in the (Table 6) ,ZnO NPs have high average crystalline than Co-ZnO NCs.

Table 6: XRD peak position, miller indices, FWHM ,Crystalline size and Average crystalline size for ZnO NPs,5% Co-ZnO NCs and 7% Co-ZnO NCs

ZnO NPS			5% Co-ZnO NCs			7% Co-ZnO NCs		
2θ deg.	FWHM	Crystaline Size	2θ deg	FWHM	Crystaline Size	2θ deg	FWHM	Crystalline Size
29.41477	0.26857	29.8823	29.4385	0.3434	23.3640	29.41476	0.27113	29.6001
31.78401	0.83736	9.53023	31.7296	1.0366	7.69929	31.78469	0.85671	9.31496
34.53248	1.05092	7.53956	34.5088	1.0366	7.69929	34.52935	1.08752	7.2856
36.23783	0.83785	9.41209	36.2478	1.0139	7.77736	36.23927	0.84473	9.33540
42.91347	1.03593	7.45447	42.9588	1.1444	7.77736	42.91258	1.0628	7.26602
56.6195	0.83231	8.77674	47.6734	1.4293	5.30968	47.72399	1.52618	4.97197
62.5363	0.83231	3.75612	56.5547	1.0759	5.30968	56.62494	0.88825	8.22379
68.18357	1.88816	4.94992	62.6254	1.9023	3.72637	62.52964	1.97904	3.58376
			68.122	1.672	3.72637	68.20115	1.97904	3.47172
Average cyrstaline size 10.16n				**7.91nm**				**9.22 nm**

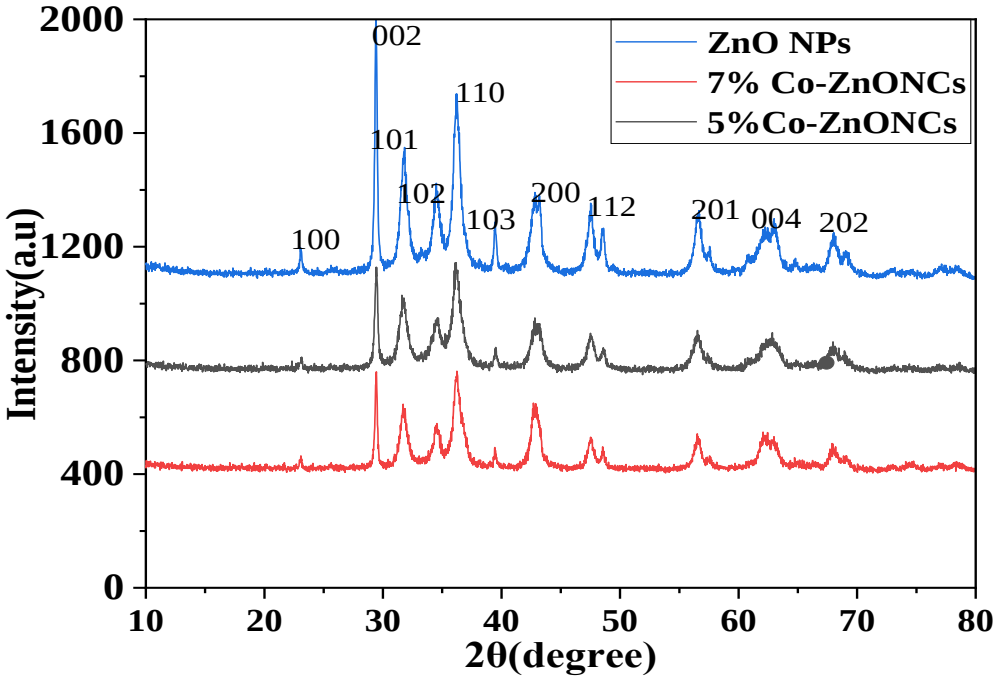


Figure 14: XRD results of ,ZnO NPs ,5% Co-ZnO NCs and 7% Co-ZnO NCs

4.4. Antibacterial activity of green synthesized ZnO NPs and Co-ZnO NCs

The antibacterial properties of, ZnO NPs, 5% Co-ZnO NCs and 7% Co-ZnO NCs at concentrations ranging from (100,75,50 to 25 mg mL−1 varied as shown (Table 7) . Bacterial growth inhibition reduces as the concentration of the NPs and NCs lowers for all samples, and there was no inhibition at 25 mg mL−1 on ZnO NPs and Co/ZnO NCs In this study, the an.tibacterial activity of Co/ZnO NCs was good when compared with ZnO NPs. The antimicrobial activity of Co /ZnO NCs is due to the high affinity of Co ions to the sulfur thrill protein of bacteria. The diffusion disk results of the antibacterial activities of the green synthesized nanoparticles and nanocomposite are given in (Figure 17). Green nanoparticles and nanocomposites were found to be effective against Gram-positive (*S. aureus*) and Gram-negative (*E. coli and p.areoginosa*) bacteria. The ratio of 5% Co−ZnO NCs and 7% Co-ZnO NCs inhibited *S. aureus and p.areoginosa* growth the most, with inhibition values of 12± 1 and 14± 0.1 mm,respectively.This affinity leads to the breakage of disulfide bonds in thiols, which results indisruption of protein tertiary structure and cell death in bacteria(Chowdhary *et al.*,2025).The band gap energy of ZnO NPs and 5%Co-ZnO NCs was 3.06 eV and 2.6eV. In this case the smaller the size of the particles, the higher the oxide content due to the larger surface area.

Generally, the antibacterial effectiveness of ZnO NPs and Co-ZnO NCs has been enhanced by the development of highly reactive oxygen species like, H_2O, O2-, OH radicals on the surface of Co-ZnO NCs and ZnO NPs, which cause the death of bacterial cells (Riaz,2025)

Nanoparticles can cause various bactericidal and bacteriostatic effects, including improved contact with microbes, metal ion binding to DNA molecules, disruption of metabolic processes, and the formation of reactive oxygen species (ROS). These mechanisms can lead to cell wall damage, increased membrane permeability, internalization, and the uptake of toxic metal ions (Mouzakis *et al*.,2025). Microparticles (NPs) attach themselves to bacterial cells through electrostatic interactions. Because of these interactions, bacteria experience oxidative stress due to alterations in the integrity of their cell membranes and the production of harmful free radicals. Particles with varied physical, optical, chemical, and electrical properties are produced as particle size reduces and the number of constituent atoms around the particle's surface increases, resulting in highly reactive particles (Figure 17).

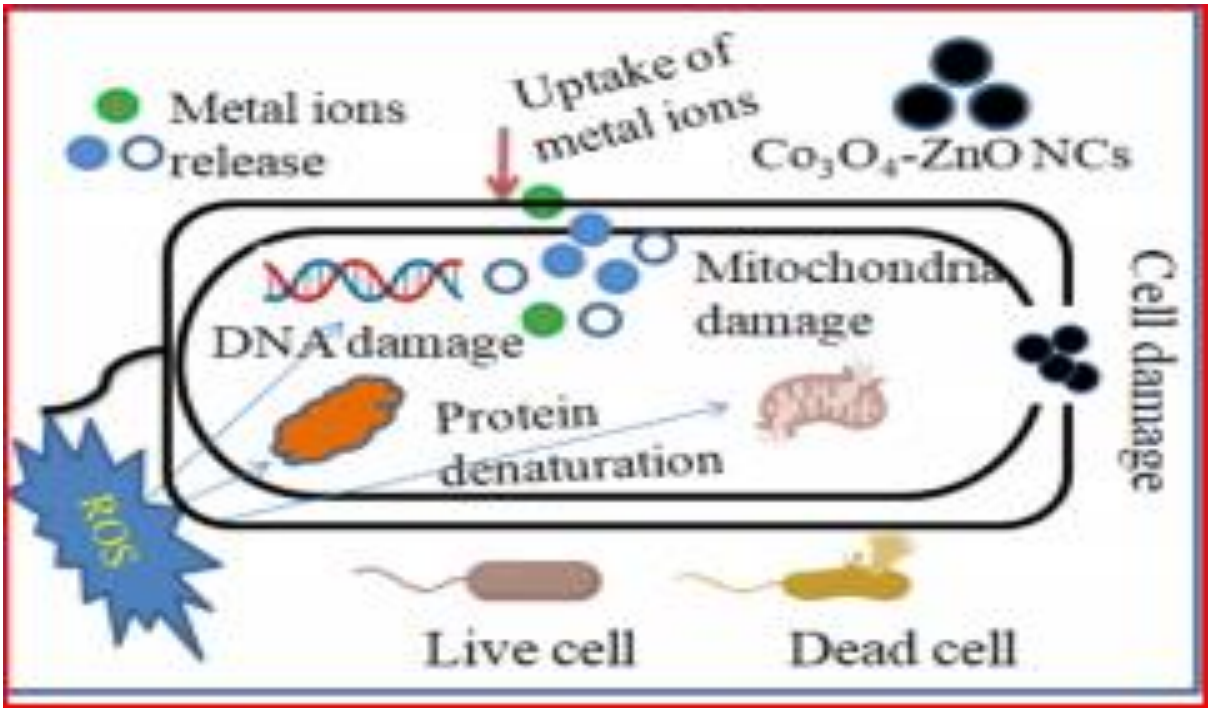


Figure 17. Proposed mechanism for antibacterial activities of Co−ZnO nanocomposites. (López *et al*.,2025)

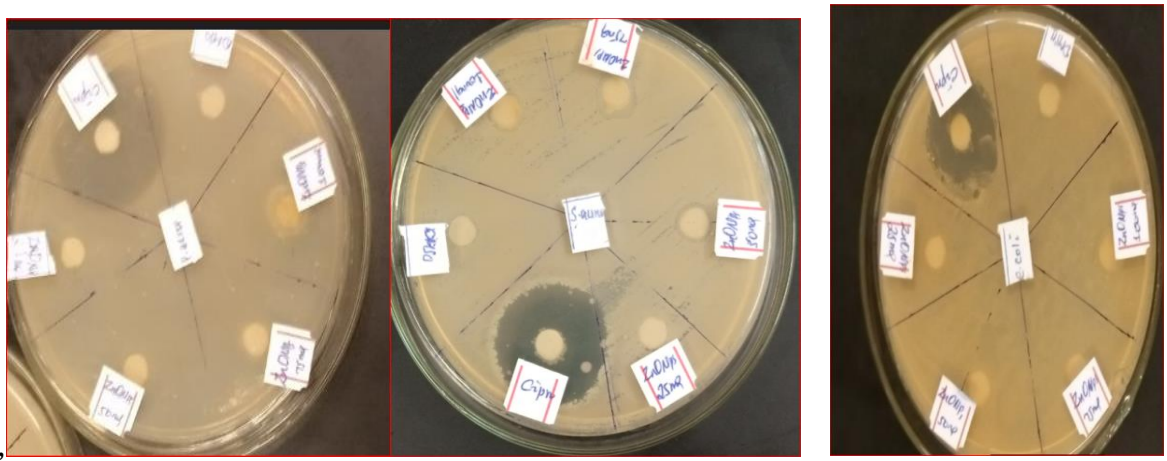
Table 7: Antibacterial activities of biosynthesized ZnO NPs and Co-ZnO NCs

Concentration mg/L	ZnO NPs zone of Inhibition (mm)			5% Co-ZnO NCs zone of Inhibition (mm)			7% Co-ZnO NCs zone of Inhibition (mm)		
	S.aures	*E.coli*	*P.areruginosa*	*S.aures*	*E.coli*	*P.areruginosa*	*S.aures*	*E.coli*	*P.arerugin osa*
100	12 ± 1^{cd}	9 ± 0.9^{def}	11 ± 1^{de}	9 ± 0.9^{fg}	8 ± 0.9^{efg}	14 ± 1^{c}	9 ± 1^{efg}	9 ± 1^{fg}	12 ± 1^{cd}
75	9 ± 0.9^{fg}	7 ± 1^{g}	8 ± 0.9^{fg}	8 ± 0.9^{fg}	7 ± 0.9^{h}	12 ± 1^{c}	8 ± 0.8^{fg}	8 ± 0.9^{fg}	10 ± 1^{g}
50	7 ± 1^{h}	NI	NI	NI	NI	9 ± 0.9^{fg}	7 ± 0.9^{g}	7 ± 0.9^{g}	8 ± 1^{fg}
25	NI	NI	NI	NI	NI	NI	7 ± 0.9^{g}	7 ± 0.9^{g}	8 ± 1^{fg}
Cipro	25 ± 1^{b}	24 ± 1^{b}	30 ± 1^{a}	25 ± 1^{b}	24 ± 1^{b}	30 ± 1^{b}	25 ± 1^{b}	24 ± 1^{b}	30 ± 1^{a}
DMSO (10%)	NI	NI	NI	NI	NI	NI	NI	NI	NI

Note: **NI** indicates No Inhibition

In the table above the the letters a,b,c,d,e,f.g and h Columns means with different superscripts for each organism between the different concentrations and Values of the inhibition zone are superscripted by the same letter across the row are significantly different from each other at $\alpha=0.05$ different(n=3).The Codoped ZnO NPs showed a higher antibacterial efficiency against *pseudomonas areginosa* with a zone of inhibition of 14.±1mm.

Therefore, Co doping significantly enhances the nanoparticles antimicrobial efficacy making them promising candidates for combating microbial infections. The Codoped ZnO NP also showed enhanced antibacterial activity, with a minimum inhibitory concentration of 7±1mm observed for ZnO NPs. Therefore, Co doping significantly enhances the nanoparticles' antimicrobial efficacy, making them promising candidites for combating microbial infections. Antibacterial activity was found to be more effective on *S. aureus* than on *E. coli* due to their thick cell walls and cytoplasmic membrane, which prevent certain medicines and antibiotics from entering their cells.

A, 

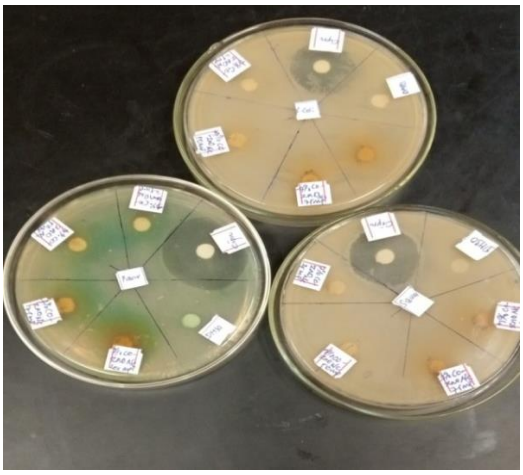
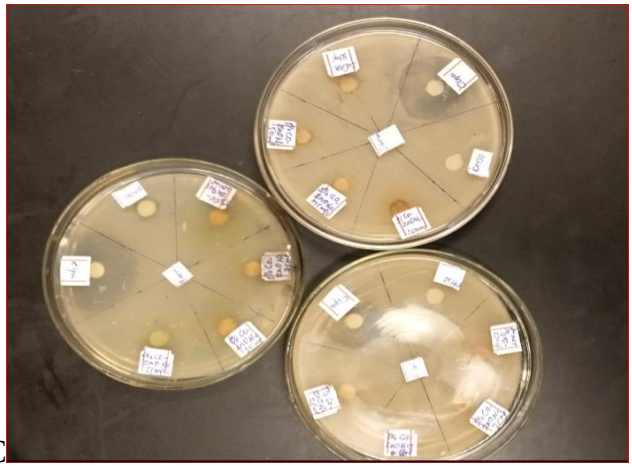
B  C 

Figure 18**:** Zone of Inhibition of ZnO NPs (A), 7%Co-ZnO NCs (B), 5%Co-ZnO NCs (C) against *S. aureus E. coli and p.aeroginosa*

5. CONCLUSIONS AND RECOMMENDATIONS

5. 1. Conclusions

The green synthesis of ZnO NPs and Co- ZnO NCs using *Croton macrostachyus leaf* extract provides an environmentally friendly approach to producing nanomaterials with enhanced antibacterial properties.The synthesized ZnO NPs and Co/ZnO NCs from $Zn(NO_3)_2.6H_2O$ and $Co(NO_3)2.6H2O$ were characterized by UV/Vis, FTIR,and XRD. The FTIRspectroscopy revealed the presence of possible functional groups in the synthesized ZnO NPs and Co/ZnO NCs *Croton macrostachyus leaf* extract that were used as capping and stabilizing agents . The XRD result showed the synthesized NCs have a crystalline structure.The synthesized ZnO NPs and Co/ ZnO NCs have exhibited significantly greater antibacterial activity against gram positive bacteria than gram negative bacteria due to differences in their cell structures. The synthesized Co/ZnO NCs have greater antimicrobial activity than ZnO NPs.In this study *Croton macrostachyus* leaf extract synthesis of ZnO NPs and Co/ ZnO NCs has significant applications for antibacterial activity**.** The Codoped ZnO NPs showed a high er antibacterial efficiency against *pseudomonas areginosa* with a zone of inhibition of 14.±1 mm and Co doped ZnO NPs also showed enhanced antibacterial activity, with a minimum inhibitory concentration of 7±1mm observed for ZnO NPs. Therefore, Co doping significantly enhances the nanoparticles' antimicrobial efficacy, making them promising candidates for combating microbial infections. This study underscores the potential of doping and green synthesis techniques in advancing multifunctional nanomaterials for sustainable applications.

5. 2. Recommendations

For further work, the following points were forwarded as a result of the recommendations:

- Further characterization is required for the determination of the size and composition of the sample using instruments like SEM, TEM, Zeta Potential, XPS, and EDX.
- Further research work is needed on some other metal-doped metal oxide NCs by using *Croton macrostachyus* leaf extract with an antibacterial activities.
- The antbacterial activity of ZnO NPs and Co-ZnO NCs against some other microbial strains should be studied.

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