DEBRE MARKOS UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE

DEPARTMENT OF CHEMISTRY

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A Thesis on

Green synthesis ,characterization and anti bacterial activity of Zinc oxide nanoparticlesusing *Clutia abyssinica (fiyele feji)*leaf extract

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Declaration

I,the under signed, declare that this thesis is my original work and has not been presented for degree in any other university and that all source of materials used for the thesis have been properly acknowledged.

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APPROVAL SHEET

As fellows of the board of examiners,weexamined this thesis entitled “green synthesis , characterization and anti bacterial activity of zinc oxide nanoparticles using *clutia abyssinica (fiyele feji)* leaf extract”bytamirgodie. we here by certify that the thesis is accepted for fulfilling the requirements for the award master of science in inorganic Chemistryat Debre Markos University.

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ABSTRACT

The increasing demand for environmentally sustainable methods in nanotechnology has led to the development of green synthesis approaches for metal oxide nanoparticles. This study focuses on the green synthesis of Zinc Oxide Nanoparticles using the aqueous leaf extract of Clutia abyssinica,locally known asfiyelefeji,asacapping and stabilizing agent.The green synthesized Zinc nanoparticles were characterized using UV-Vis spectroscopy, Fourier Transform Infrared (FT-IR) spectroscopy and X-ray diffraction (XRD) to determine their optical and structural properties. The results confirmed the formation of zinc oxide nanoparticles. The UV-Vis spectra confirmed the formation of Zinc nanoparticleswith an absorption peaks around 374 nm, 376 nm and 377 nm corresponding to precursor to extract ratio of 1:1, 2:3 and 1:4 respectively. The FT-IR analysis revealed the presence of bioactive functional groups involved the capping and stabilization of the nanoparticles. The XRD patterns of Zinc nanoparticles synthesized at various precursor to extract ratios showed the crystalline nature and hexagonal wurtzite structure of the nanoparticles while the average crystallite sizes calculated using the Debye – Scherer equation are 19.09,15.00 and 13.16 nmcorresponding to (1:4) (2:3) and (1:1, respectively. Furthermore, the antibacterial activity of the synthesized nanoparticles was evaluated against selected gram-positive and gram-negative bacterial strains using the agar well diffusion method. The results demonstrated significant antibacterial effects, particularly against S. aureus and S. epidermidis with inhibition zone of 12.67±0.57nm*and* 13.3±0.57*mm, indicating the potential ofClutia abyssinica-mediated Zinc nanoparticlesas effective antimicrobial agents. Thus, this finding confirmed thatClutiaabyssinicaleaf extract is a green cost-effective capping and stabilizing agent for the synthesis of Zn-O nanoparticles with significant antibacterial properties, which suggests a sustainable route for the fabrication of metal oxide nanoparticles with biomedical applications.*

Key words: *Clutia abyssinica,Phytochemicals, zinc oxide nanoparticles, Antibacterial properties*

Abbreviation and Acronym

FT-IR ……..Fourier Transform Infrared Spectroscopy

XRD ……...X-ray Diffraction

S. aureus …….Staphylococcus aureus

K. pneumonia …….Klebsiella pneumonia

IZ ……Inhibition Zone

S. epidermidis …….....Staphylococcus epidermidis

E.coli …… ….Escherichia coli

LTP……Long-Term Potentiation

RT…….….RoomTemperature

ROS……..…Reactive Oxygen Species

CFU…….Colony Forming Units

TTC…… Tetracycline

UNIT ONE

1. INTRODUCTION

1.1.BackGround of Study

Nanotechnology is an interdisciplinary field that focuses on the study and manipulation of materials at the nanoscale, typically in the range of 1–100 nm [1].This emerging technology has found widespread applications in various industries, including pharmaceuticals, chemicals, and food processing [2]. Green synthesized nanoparticles have found diverse applications in the biomedical field, including drug delivery, tissue engineering, and wound healing, owing to their unique physicochemical properties and biocompatibility. These nanoparticles have demonstrated antimicrobial and anti-inflammatory activities, making them promising candidates for the development of novel therapeutic and diagnostic strategies [3] Moreover, the small size of nanoparticles allows them to readily interact with receptors, nucleic acids, and cell membranes, making them highly useful in the field of medicine [4].

Zinc Oxide (ZnO) is an inorganic metal oxide that consists of nanostructures in a vast range (Raliya et al., 2017) ZnO has a variety of unique qualities that set it apart from other materials, including low toxicity, a broad spectrum of radiation adsorption, high photo stability, high chemical stability, and high electrochemical coupling coefficient. Biocompatibility, biodegradable, and rigidity make ZnO an interesting material in biomedicine field. ZnO can adsorb UV-vis. Nowadays ZnO is used in many sunscreen lotions, ointments, creams, dental pastes, and medicines and it found that ZnO has wound-healing properties which are used in inflammation and itching [5].

Conventionally, metal oxide nanoparticles such as ZnO are synthesized using chemical and physical methods, including sol–gel processes, precipitation, hydrothermal synthesis, and thermal decomposition. However, these techniques often require high energy input, toxic chemicals, and produce hazardous by-products that pose environmental and health risks [6]. As a result, there has been a growing demand for green, sustainable, and eco-friendly synthesis approaches. Green synthesis utilizes biological resources such as plant extracts, microorganisms, and enzymes as reducing and stabilizing agents to produce nanoparticles under mild, non-toxic

conditions [7]. This approach not only minimizes the use of harmful chemicals but also improves biocompatibility and cost-effectiveness, aligning with the principles of green chemistry.

Plant-mediated synthesis of nanoparticles has gained particular importance among green synthesis methods due to its simplicity, scalability, and the wide availability of natural plant materials. Plant extracts are rich in phytochemicals such as alkaloids, flavonoids, terpenoids, tannins, phenolic compounds, and saponins, which can act as stabilizing and capping agents, converting metal ions into stable nanoparticles [8]. These phytochemicals influence the nucleation and growth of nanoparticles, thereby affecting their size, morphology, and surface characteristics, which in turn determine their biological activity [9]. Thus, the selection of a suitable plant species for nanoparticle synthesis plays a critical role in tailoring the physicochemical and biological properties of the resulting nanoparticles.

Clutia abyssinica (locally known as Fiyele Feji in Ethiopia) is a medicinal plant widely distributed in East Africa and traditionally used in Ethiopian ethnomedicine to treat various diseases such as wounds, infections, and gastrointestinal disorders [10]. Phytochemical investigations have shown that *Clutia abyssinica* leaves contain bioactive compounds such as flavonoids, tannins, phenolics, saponins, and alkaloids, which possess strong antioxidant and antimicrobial activities [11]. These bioactive components make*Clutia abyssinica* an excellent candidate for the green synthesis of metal oxide nanoparticles, serving both as a reducing and stabilizing agent. Utilizing *Clutia abyssinica*extract for ZnO nanoparticle synthesis not only enhances the biocompatibility of the nanoparticles but also incorporates the plant's intrinsic medicinal properties into the synthesized nanomaterials.

The characterization of Zinc nanoparticlessynthesized through plant extracts is essential to confirm their formation, morphology, and crystalline nature. Common analytical techniques include ultraviolet–visible spectroscopy, Fourier-transform infrared spectroscopyandX-ray diffraction ,UV–Vis spectroscopy helps identify the characteristic absorption peak of Zinc nanoparticles , confirming nanoparticle formation, while FTIR analysis reveals the involvement of functional groups from the plant extract in the reduction and stabilization processes [12]. XRD patterns determine the crystalline structure and particle size.Together, these techniques establish

the structural, functional groups, and the characteristic absorption peakfeatures of the green synthesized nanoparticles.

Zinc nanoparticleshave been reported to exhibit potent antibacterial activity against a wide range of pathogenic microorganisms, including both Gram-positive and Gram-negative bacteria [13]. The antibacterial mechanism is attributed to several factors, such as the generation of reactive oxygen species (ROS), disruption of bacterial cell membranes, and the release of Zn^{2+} ions that interfere with microbial metabolism [14]. The particle size, surface charge, and crystallinity of Zinc nanoparticlessignificantly influence their antimicrobial efficacy. Green-synthesized Zinc nanoparticles, in particular, have shown enhanced antimicrobial effects due to the synergistic action of the metal oxide core and the bioactive phytochemicals capping the nanoparticle surface [15]. Therefore, the biosynthesis of Zinc nanoparticlesusing *Clutia abyssinica* leaf extract could offer an eco-friendly and effective alternative for developing narrative antimicrobial agents to combat antibiotic-resistant pathogens.

In summary, the green synthesis of zinc oxide nanoparticles using *Clutia abyssinica* (Fiyele Feji) leaf extract represents a sustainable and biologically relevant approach that integrates nanotechnology and traditional medicinal knowledge. This study aims to synthesize Zinc nanoparticlesusing *Clutia abyssinica*leaf extract, characterize the synthesized nanoparticles using modern analytical techniques, and evaluate their antibacterial activity against selected pathogenic bacteria. The research is expected to provide valuable insights into the role of phytochemicals in nanoparticle formation and enhance the development of environmentally benign nanomaterials for biomedical and pharmaceutical applications.

1.2.Statements of the problem

Nanotechnology has emerged as one of the most promising scientific fields of the 21st century due to its wide range of applications in medicine, agriculture, and environmental protection. Among various nanomaterials, zinc oxide nanoparticles have gained special attention because of their unique physicochemical properties, low cost, and excellent antimicrobial potential. Traditionally, Zinc nanoparticlesare synthesized using physical and chemical methods, which often require expensive equipment, toxic chemicals, and high energy input. These conventional methods not only pose environmental hazards but also limit their application in biomedical fields due to possible chemical contamination. As a result, there is ancritical need for eco-friendly, cost-effective, and sustainable approaches to nanoparticle synthesis.In recent years, *green synthesis* of nanoparticles using plant extracts has emerged as a safer and more sustainable alternative. Plant-mediated synthesis utilizes natural phytochemicals such as flavonoids, terpenoids, alkaloids, polyphenols, and proteins, which act as both reducing and stabilizing agents during nanoparticle formation. However, the efficiency, stability, size, and biological activity of the synthesized nanoparticles vary greatly depending on the type of plant extract used and its bioactive constituents. Despite the growing number of studies on green synthesis of Zinc nanoparticles, research on the use of *Clutia abyssinica* (locally known as Fiyele Feji) leaf extract remains very limited. This plant is widely distributed in Ethiopia and known in traditional medicine for its anti-inflammatory, antimicrobial, and antioxidant properties, which suggest that its bioactive compounds could serve as effective agents in nanoparticle synthesis and stabilization.Furthermore, the relationship between synthesis parameters (such as pH, temperature, and concentration of precursors) and the structural as well as functional characteristics of Zinc nanoparticlessynthesized using *Clutia abyssinica* is not yet well understood. The size, morphology, and surface chemistry of the nanoparticles play a crucial role in determining their antibacterial efficiency, yet these factors have not been systematically explored in the context of this plant-mediated synthesis. Additionally, bacterial resistance to conventional antibiotics is becoming a major global health concern, and the search for alternative antimicrobial agents is critical. Therefore, evaluating the antibacterial activity of Zinc nanoparticles synthesized through *Clutia abyssinica* leaf extract is not only scientifically important but also highly relevant to addressing the challenges of antibiotic

resistance.Giventhese gaps, the present learn aims to synthesize zinc oxide nanoparticles using *Clutia abyssinica* leaf extract through a green, eco-friendly route, to characterize the obtained nanoparticles using modern analytical techniques, and to evaluate their antibacterial potential against selected pathogenic bacteria. This research will contribute to the development of a sustainable nanoparticle synthesis method, provide insights into the influence of plant-based compounds on nanoparticle formation, and offer potential alternatives for biomedical and environmental antimicrobial applications.

1.3. Objectives

1.3.1. General objective

The main objective of this research is to synthesize and characterize zinc oxide nanoparticles using *Clutia abyssinica* leaf extract and evaluate their antibacterial activity.

1.3.2. Specific objectives

- To identify the phytochemicals present in the leaf extract of *Clutia abyssinica* plant.
- To synthesize Zinc oxide nanoparticles using *Clutia abyssinica* leaf extract as capping agent and stabilizing agent.
- To characterize the synthesized Zinc oxide nanoparticles using different spectroscopic techniques such as UV-Vis spectroscopy, Fourier Transform Infrared spectroscopy and X-ray Diffraction .
- To evaluate the antibacterial activity of the synthesized Zinc oxide nanoparticles against both Gram-positive and Gram-negative bacteria.

1.4. Significance of the study

This study is significant as it promotes an ecofriendly synthesis method for zinc oxide nanoparticles by utilizing *Clutia abyssinica* (locally known as feyele feji) leaf extract, thereby avoiding the use of harmful chemicals commonly employed in conventional nanoparticle synthesis. By harnessing the natural bioactive compounds present in this indigenous plant, the research not only advances sustainable nanomaterial production but also highlights the valuable potential of local flora in high-tech applications. Furthermore, the study demonstrates the practical value-added applications of *Clutia abyssinica* in nanotechnology and medicine,

particularly through the evaluation of the antibacterial activity of the synthesized nanoparticles. This approach encourages interdisciplinary research bridging botany, nanoscience, microbiology, and materials engineering, fostering innovations that can contribute to novel, environmentally safe therapeutic agents and antimicrobial materials. Ultimately, this work supports the integration of traditional plant resources into modern scientific advancements with potential benefits in medical treatment, environmental protection, and advanced material development.

1.5. The Scope of the Study

This study focuses on the green synthesis of Zinc oxide nanoparticles using the aqueous leaf extract of *Clutia abyssinica*. It encompasses the biosynthesis process, physicochemical characterization using Uv-Vis, XRD and FTIR techniques and invitroantibacterial evaluation of the synthesized nanoparticles against selected gram-positive and gram-negative bacterial strains. However, the study does not extend to clinical trials and only one plant species (*Clutia abyssinica*) is used in which comparative plant based synthesis is beyond the scope of this study.

UNIT TWO

2. LITERATURE REVIEW

2.1.Nanotechnology

Zinc oxide nanoparticles a versatile semiconductor material, have garnered significant attention for their unique optical, electrical, and catalytic properties, primarily stemming from their high surface-to-volume ratio and quantum confinement effects. Their biocompatibility and low toxicity have broadened their application scope to include biomedicine (drug delivery, bio imaging, wound healing, and cancer therapy), cosmetics (sunscreen), environmental remediation (catalysis, photo degradation of pollutants), and antibacterial coatings[16]. The synthesis of these nanomaterial's has traditionally involved physical and chemical methods, but recently, green synthesis has emerged as an eco-friendly alternative, utilizing naturally occurring materials, particularly plant extracts, as reducing and stabilizing agents [17] . These methods reduce production cost and toxicity whilst being sustainable. Plant extracts contain a complex mixture of bioactive compounds (e.g., phenols, flavonoids, and terpenoids) that can facilitate the reduction of metal ions and subsequent NPs formation [18]. Kalanchoe petitiana, a succulent plant used in traditional medicines, has been found to contain phytochemicals with promising reducing potential, thus making it a potential candidate for Zinc nanoparticlesgreen synthesis [19].This literature review investigates the influence of pH on the synthesis of Zinc nanoparticles using K. petitiana leaf extract and its subsequent impact on the resulting nanoparticles' size, morphology, and antimicrobial activity. While previous studies have explored the effect of pH on NPs synthesis, few have focused specifically on the unique properties of K. petitiana and its impact on the resulting Zinc nanoparticles [20]

2.2.Nanoparticle Synthesis and Their Applications

Nanoparticles (NPs), typically ranging in size from 1 to 100 nm, have garnered significant attention due to their unique physicochemical properties, including high surface area-to-volume ratio, quantum effects, and tunable morphology. Various synthesis methods are employed to produce nanoparticles, which broadly fall into two categories: top-down and bottom-up approaches. Top-down methods, such as milling and lithography, reduce bulk materials into nanoscale particles, while bottom-up techniques such as sol-gel processes, chemical vapor deposition, and green synthesis construct nanoparticles from atomic or molecular precursors.

Among these, green synthesis using biological resources (e.g., plant extracts, bacteria, or fungi) is gaining popularity for its eco-friendliness, cost-effectiveness, and scalability [21]. Nanoparticles have been extensively applied in numerous fields. In medicine, they serve as drug delivery carriers, contrast agents, and tools for cancer therapy. In environmental science, metal oxide nanoparticles like ZnO and TiO_2 are used for wastewater treatment, photocatalysis, and pollutant detection. In agriculture, nanoparticles enhance fertilizer efficiency and pest control. Their application also extends to electronics, food packaging, and energy storage devices [22]. These wide-ranging applications underscore the importance of developing safe, efficient, and sustainable synthesis methods.

2.3.Plant-Mediated Synthesis of Zinc nanoparticles

Plant-mediated synthesis, often referred to as "green synthesis," has emerged as an eco-friendly, cost-effective, and sustainable approach for the fabrication of metal oxide nanoparticles, including zinc oxide. This method utilizes various plant extracts as reducing and stabilizing agents, leveraging the bioactive compounds naturally present in plants, such as flavonoids, alkaloids, phenolic, terpenoids, and tannins. These phytochemicals play a crucial role in reducing zinc salts (commonly zinc acetate or zinc nitrate) into Zinc nanoparticles, while also stabilizing the formed particles and controlling their morphology and size. Several reports in recent years have successfully demonstrated the use of different plant species for the synthesis of Zinc nanoparticles. For example, [23] reported the synthesis of Zinc nanoparticlesusing *Aloe vera* leaf extract, resulting in spherical nanoparticles with an average size of 20–50 nm and strong antimicrobial properties. Similarly, [24] employed *Citrus sinensis* peel extract to produce Zinc nanoparticles, highlighting their potential use in photocatalytic degradation of dyes due to enhanced surface activity .Another notable study by Sangeetha [25] synthesized Zinc nanoparticles using *Solanum nigrum* leaf extract. The results indicated uniform crystallinity and particle sizes below 40 nm, as characterized by X-ray diffraction (XRD) these nanoparticles demonstrated strong antibacterial activity against both Gram-positive and Gram-negative bacteria, emphasizing the therapeutic potential of plant-derived Zinc nanoparticles. The synthesis parameters, such as pH, temperature, reaction time, and concentration of plant extract, greatly influence the morphology, stability, and yield of Zinc nanoparticles .[26] highlighted that higher pH values during synthesis typically yield smaller-sized Zinc nanoparticles due to rapid nucleation facilitated by increased OH^- concentration. Moreover, the diversity of phytochemicals

in different plant extracts affects the shape producing spherical, rod-like, or hexagonal ZnO structures. Plant-mediated Zinc nanoparticlesare increasingly applied in biomedical fields due to their biocompatibility and reduced toxicity. They exhibit significant antimicrobial, antioxidant, and anticancer properties, as shown in various in vitro and in vivo studies. Furthermore, these nanoparticles are being explored for use in water treatment, cosmetics, sensors, and agriculture, owing to their photocatalytic and UV-blocking properties.

2.4.Synthesisof Zinc nanoparticles Focusing on Various Conventional Synthesis Methods

Zinc oxide nanoparticles have attracted significant interest due to their wide range of applications in electronics, catalysis, sensors, and biomedical fields. Among the many factors influencing the properties and applications of Zinc nanoparticles, the synthesis method plays a crucial role in determining their size, shape, crystallinity, and surface properties. Several conventional methods have been widely employed for the synthesis of Zinc nanoparticles, including sol-gel, precipitation, hydrothermal, Physical method, Chemical method, Biological method and solid-state methods.

2.4.1.The sol-gel method

The sol-gel method is one of the most commonly used approaches due to its simplicity, low processing temperature, and ability to produce nanoparticles with high purity and uniformity. In this process, a zinc precursor such as zinc acetate or zinc nitrate is hydrolyzed in a suitable solvent, often under acidic or basic conditions, followed by gelation and subsequent calcination to yield Zinc nanoparticles. Parameters such as pH, temperature, and concentration of precursors significantly influence the morphology and crystallinity of the final product [27] Another widely used technique is the co-precipitation method, which involves the precipitation of ZnO from a solution containing zinc salts using a precipitating agent such as sodium hydroxide or ammonium hydroxide. This method is favored for its low cost, ease of scale-up, and ability to control particle size through the adjustment of synthesis parameters like temperature, stirring rate, and concentration of reactants [28]

2.4.2.The hydrothermal method

The hydrothermal method involves the use of a sealed autoclave to treat aqueous solutions of zinc salts at elevated temperatures and pressures. This approach enables the formation of highly

crystalline Zinc nanoparticleswith controlled morphologies such as rods, spheres, or flower-like structures. The high-temperature and pressure conditions facilitate the growth of well-defined nanostructures and reduce the need for post-synthesis treatments [29]

2.4.3.The solid-state synthesis method

*T*he solid-state synthesis method involves the direct mixing and heating of zinc-containing precursors, typically in the absence of solvents. Although this method is less frequently used for nanoparticle synthesis due to difficulties in achieving uniform particle size, it is advantageous in terms of environmental safety and cost-effectiveness. Solid-state reactions are generally carried out at higher temperatures, leading to the formation of crystalline ZnO particles [30].Each of these conventional synthesis methods has its own merits and limitations, and the choice of method largely depends on the desired properties of Zinc nanoparticlesand their intended application. While sol-gel and precipitation methods are suitable for laboratory-scale synthesis with controlled particle size, hydrothermal and solid-state methods offer better crystallinity and are more adaptable for large-scale production.

2.4.4. Physical method

Physical methods developed for synthesis of ZnO nanoparticles often require specialequipment's or operational control. The physical method often called top-down approach which includes methods like diffusion, irradiation, thermal decomposition, and arc discharge. The thermal de-composition method is one of the significant top-down approaches. It is used for the creation of mono disperse nanoparticles. These approaches use larger initial structures ormacroscopic units which can be externally controlled in the processing of nanostructures.Etching through the mask, ball milling and application of severe plastic deformation are common
examples of physical top-down method of synthesis of nanoparticles. The top-down method of synthesis is a model which generates on a larger scale for further reduction of Nano scale.Mostly the top-down methods are not cheap and quick to manufacture. This method is slow and
not suitable for large scale production [31].

2.4.5. Chemical method

Moderate reaction conditions and convenient synthetic manipulation have rendered chemical methods an attractive option for accessing ZnO nanoparticles. Traditional chemical routes that

are frequently used in recent years include sol-gel approach, chemical precipitation and colloidal Synthesis. Surfactants or polymer based preparative method for ZnO nanoparticles are also quite popular to avoid agglomeration and achieve better control of ZnO nanoparticles. Removal ofsurfactants or polymers, however, poses serious limitations to such methods. Hence, delicate and precise control of experimental factors for the synthesis of nanoparticles with desired morphologies is a cherished goal for future device applications.Homogeneous chemical precipitation method is often considered economically viable for preparation of mono disperse metal oxide particles of different shapes and sizes. The method also provides better control of chemical and morphological characteristics. Chemical methods,including precipitation from inorganic or organic solutions and sol-gel techniques can conveniently provide control of nucleation, growth and ageing of particles in the solution. The methods rely on advanced solution and coordination chemistry theories, enabling the synthesis of the required precursor particles utilizing a variety of parameters to enable control of the solid formation process. A major contribution to the growth of particles is through Ostwald ripening, where small particles with lower solubility product dissolve and re-precipitate on the surface of larger particles in solution. Agglomeration takes place in solution as the particles clog together to minimize surface energy [33]. Mostly the chemical methods of synthesis of nanoparticles are the bottom up approaches. These approaches include the miniaturization of material components (up to atomic level) with further self-assembly processes leading to the formation of nanostructures. During self-assembly the physical forces operating at Nano scale are used to combine basic units into larger stable structures. Typical examples are quantum dot formation during epitaxial growth and formation of nanoparticles from colloidal dispersion. In these methods different metal particles are reduced to form nanoparticles. Various types of metal particles used like Sodium borohydrate and Sodium citrate etc. [34]. Other chemical reagents used are N, Ndimethyl form amide (DMF), Poly (N-vinyl pyrrolidine (PVP), ethyl alcohol and Cetyltrimethyl ammonium bromide (CTAB). This method initiates with a pattern which is generate larger scale, then reduced to Nano scale. This method of synthesis of nanoparticles is cheap and quick to manufacture.

2.4.6. Biological method

Green synthesis method is proved as beneficial over other methods which are implemented for the synthesis of nanoparticles. Green synthesis methods are eco-friendly approach andcompatible for pharmaceutical and other biomedical applications, as the toxic chemicals are not

used in these methods.The use of environmentally beneficial materials like plant leaf extract, bacteria, fungi and enzymes for the synthesis of zinc nanoparticles proposes abundant benefits of eco-friendliness and compatibility for pharmaceutical and many other biomedical applications [35]. Lot of attention has been diverted to the green synthesis of metal nanoparticles using biological material as the reducing and stabilizing agents and due to the usage of eco-friendly, non-toxic and safe reagents during the biosynthesis process, green synthesis has been considered in the field of toxic chemical and physical methods. In the biological method, plant extracts are used for con-trolled and precise synthesis of several metallic nanoparticles. High surface and a large fraction of surface atoms are responsible for the nanoparticles' atom-like behavior [36]. Despite the fact that conventional methods use less time for synthesizing nanoparticles, they contribute to environmental toxicity because they require toxic chemicals as capping agents. Green nanotechnology is an eco-friendly alternative and is cost effective and utilizes proteins as natural capping agents. Synthesis of metal nanoparticles by plants utilizes various secondary metabolites, enzymes, proteins and or other reducing agents [37].

2.5. Chemical Synthesis of Zinc Oxide nanoparticles

Nanomaterials or nanostructures can be synthesized by a variety of techniques such as spray pyrolysis, thermal decomposition, molecular beam epitaxy, chemical vapor deposition, and laser ablation[38].

Table 1: Comparision of nanoparticles synthesis method

Method	Advantages	Limitations
❖ Chemical synthesis	✓ Controlled size ✓ High purity and quality ✓ Nanoparticles are considerably stable	➢ Use of toxic chemicals ➢ Use of organic solvents ➢ Low purity ➢ Hazards ➢ Capping agents and stabilizers are required to control the size and avoid agglomeration ➢ Some of the capping and stabilizing agents might be toxic ➢ Irreversible pollution of the environment
❖ Physical synthesis	✓ No use of toxic ingredients ✓ High-speed method ✓ High production rates ✓ High purity and quality ✓ Uniform size and shape	➢ High cost ➢ High energy ➢ High stability ➢ Irreversible pollution of the environment
❖ Green synthesis	✓ Safe and toxic free ✓ Simple and cost-effective ✓ No expensive equipment ✓ No toxic chemicals ✓ Eco-friendly ✓ Biocompatible ✓ Energy efficient	➢ Difficult to control the particle size and shape ➢ Nanoparticles are relatively less stable

2.6. Medicinal uses of zinc oxide nanoparticles

Zinc oxide nanoparticlesplay some potential role in CNS and perhaps during development processes of diseases through mediating neuronal excitability or even release of neurotransmitters. Some studies have indicated that Zinc oxide nanoparticles affected functions of different cells or tissues, biocompatibility, and neural tissue engineering [39-40] but little

information is present about the influence on CNS and CNS related diseases. Zinc oxide nanoparticles have been suggested to modulate synaptic transmission in vitro and to change the spatial cognition capability via enhancing long-term potentiation (ltp) in rats. it is also suggested that exposure to zinc oxide nanoparticles led to ageno toxic potential mediated by lipid per oxidation and oxidative stress [41-42]. however, due to its targeting potential zinc oxide nanoparticleshave potential utility in the treatment of cancer and/orautoimmunity [43]

2.7.Clutia abyssinica and its medicinal applications

Clutia abyssinica, whose specific epithet directly references its origin, is a medicinal shrub of high cultural and practical significance across various ethnolinguistic groups in Ethiopia, where traditional medicine remains the primary form of healthcare for a majority of the population [44]. Ethnobotanical studies conducted in regions across the country, including Oromia, Amhara, and Southern Ethiopia, consistently document its extensive use, with the roots and leaves being the most frequently employed parts [45]. In Ethiopian traditional practices, *C. abyssinica* is widely used as an anthelmintic to expel intestinal parasites, a remedy for gastrointestinal discomfort such as diarrhea and stomach aches, and a treatment for fever and malaria [46]. Furthermore, it is prepared for ailments specific to the Ethiopian context, including the management of liver problems (e.g., hepatitis and jaundice), skin infections, herpes zoster, and to treat wounds and sores [47]. For instance, a traditional remedy in Ethiopia for herpes zoster involves mixing *C. abyssinica* leaves with other plants like *Calpurnia aurea* and *Datura stramonium* [44]. The plant's use extends to ethnoveterinary medicine, where it is traditionally macerated and given orally to treat animal trypanosomosis [48]. Scientific investigations from Ethiopian institutions and researchers have corroborated some of these claims, demonstrating the plant's antidiarrheal and antitrypanosomal activities in animal models, thereby providing evidence-based support for the deep-rooted indigenous knowledge surrounding this vital medicinal resource [49].

2.8.Research Breach on *Clutia abyssinica*for ZnO Nanoparticle Synthesis

Clutia abyssinica offers a promising green-route biological platform for the synthesis of zinc oxide nanoparticles, yet research on its use remains nascent and fragmented a gap this study seeks to address. As a plant rich in diverse phytochemicals (phenolics, flavonoids, terpenoids and other stabilizing biomolecules commonly found in medicinal flora), Clutia abyssinica has the

biochemical potential to reduce zinc precursors and to cap forming nanoparticles, producing ZnO with distinct size, shape and surface chemistry under mild, eco-friendly conditions; however, systematic investigations into optimal extraction methods, reaction parameters (pH, temperature, precursor concentration, plant extract dose, time) and the mechanistic roles of specific phytoconstituents are largely missing [50]. Existing green-synthesis reports frequently emphasize proof-of-concept formation and basic characterization (UV–Vis, XRD, FTIR, SEM/TEM), but few studies thoroughly correlate synthesis conditions with particle morphology, colloidal stability, crystallinity and surface functionalization attributes that directly influence photocatalytic, antibacterial and biomedical performance. Moreover, the antibacterial efficacy, cytotoxicity, and environmental fate of Clutia abyssinica -derived Zinc nanoparticlesrequire rigorous, standardized evaluation against clinical strains and mammalian cell lines to confirm safe, application-ready profiles. Scalability and reproducibility are additional unresolved challenges: process up-scaling, batch-to-batch consistency, and green waste management must be demonstrated if this route is to transition from laboratory novelty to practical use. Finally, leveraging Clutia abyssinica ’s local availability in the Horn of Africa could create sustainable, low-cost nanomaterials for water purification, antimicrobial coatings, and agricultural uses but realizing that promise demands an integrated research program that couples phytochemical analysis, controlled synthesis optimization, comprehensive physicochemical and biological characterization, and preliminary environmental and economic assessments [51].

2.9.Antibacterial Activity of Zinc oxide Nanoparticles

The biological method of the Zinc oxide nanoparticles synthesis is gaining importance due to its simplicity, eco-friendliness and extensive antimicrobial activity. the main factor for the increase of the re-sistance pathogenic bacteria is over use of antibiotics and this has led to the emergence and spread of resistant pathogens and resistant genes in them . the studies about toxicity have shown that zinc ions do not cause any damage to the dna of human cells. the antibacterial activity of zinc oxide nanoparticles is due in part to their electrostatic interaction with cell surfaces which shows that on contact with bacteria, the cytotoxic actions of zinc oxide nanoparticles ruptures the lipid bilayer of bacterium, resulting in leakage of the cytoplasmic substances[52].nanoparticles as antimi-crobial agents are their better efficiency on resistant bacteria, less toxicity and heat resistance. Among metal oxide nanoparticles , zinc oxide have many significant features such as chemical and physical stability, high catalysis and effective

antibacterial activity [53]. Synthesized zinc oxide nanoparticles exhibit strong antibacterial activity through various mechanisms, including oxidative stress, membrane disturbance, and DNA damage. Characterization techniques such as X-ray diffraction confirm the crystalline structure and phase purity of the synthesized Zinc oxide Nanoparticles , while Fourier Transform Infrared spectroscopy provides insights into the presence of functional groups and potential interactions with biomolecules. UV-Vis spectroscopy allows for the determination of the optical properties and bandgap of the Zinc oxide Nanoparticles . While numerous studies have investigated the antibacterial properties of Zinc oxide Nanoparticles , this research aims togreensynthesis,characterization and antibacterial activity of zinc oxidenanoparticlesusing *clutiaabyssinica*leaf extract[54]investigating the effect of specific synthesis methods on antibacterial activity, exploring the mechanism of action in detail, developing novel formulations for targeted distribution, assessing the environmental impact of Zinc oxide Nanoparticles .This research was contribute to a deeper understanding of Zinc oxide nanoparticles antibacterial activity and pave the way for the development of more effective and sustainable antimicrobial strategies [55]. The possible mechanism for the bactericidal activity, explained as follows, it involves the factors including mainly reactive oxygen species (ROS) and the release of Zn ion. It has been found that the activity depends up on crystallite size, morphology, composition, specific surface and phase of crystalline material. Because of larger surface area than the bulk and distinguishable porosity as the particle size reduces, greater number of reactive oxygen species (ROS), namely O^{2-}, hydro-gen peroxide (H_2O_2), $OH^{-\cdot}$ and organic hydroperoxides are generated due to small crystallites, which in turn are considered responsible for the damage of cellular composition such as lipids, phosphorus-containing elements (DNA) and disabling of proteins by oxidative stress. It should be noted that oxidative stress results when reactive oxygen species production surpasses the capability of antioxidant defense of the cell. Zinc oxide nanoparticles provide enhanced particle surface reactivity and adherence with the microbial pathogens, and so surface properties also affect the interactions with the cell walls of the bacteria by allowing easy penetration into the bacteria. the exposure of nanoparticles to the surface of bacteria or accumulation of nanoparticles in the cell or even in the cytoplasm inhibit several functions in the cell thereby inducing structural abnormalities such as distraction of cellular function or deformation n or rupture or blebs in membranes, leading eventually to death of bacterial cells [56].

UNIT THREE

3. MATERIALS AND METHODS

Chemicals and Solvents

In this study, the following chemicals and solvents were used: Zn $(NO_3)_2.6H_2O$ (96% purity), sodium hydroxide , ethanol pure (99%) and distilled water. All compounds were analytical grade and were utilized without further purification.

Apparatus and Equipments

The following laboratory equipments were used for this work: XRD, FTIR, UV-Vis, beaker Conical flasks, funnel, volumetric flasks, borosilicate glass, scissor mortar, Whatman filter paper No. 1, crucible, cuvette, spatula, analytical balance, magnetic stirrer, aluminum foil, drying oven, and Muffle furnace.

3.3.Collection and identification of Plant material

Fresh leaves of *Clutia abyssinica* were collected from East Gojjam zone DebreMarkous town spacificallyfrom Genet Parkgarden area. The identity of the plant sample was realexperienced botanist in Debre Markos University, College ofComputational and Natural Science, Department ofBiology. The leaves were thoroughly washed and rinsed with distilled water to remove the dust particles and then dried at room temperature[57].

3.4.Preparation of Clutia abyssinica Leaf Extract

Fresh leaves of *Clutia abyssinica* 'feyele feji' were collected from East Gojjam zone Debre Markus town specifically from Genet Park garden area Ethiopia. The collected leaves were thoroughly washed three times using distilled water to remove the dust particles [58] and then dried to remove the residual moisture. The dried leaves were cut and ground into powder. Then, the ground powder was sieved by using 150 μm size sieves. The aqueous extract of sample were prepared by boiling 20 g of fine powdered leaves in 500 ml glass beaker along with 400 ml of distilled water, at 80 ℃ for 30 minutes while stirring using magnetic stirrer at 5000 r.p.m until the color of the aqueous solution changed from green to brown [59] (Figure 1). The extract was cooled to room temperature and filtered using Whatman filter paper. The extract was stored in a refrigerator at 4 ℃ in order to be used for further experiments. The filtrate aqueous extract were

used as *stabilizing*and capping agent for synthesis of Zinc Nanoparticles [60] .Figure.1. Shows a step-by step process of the leaf extract preparation.

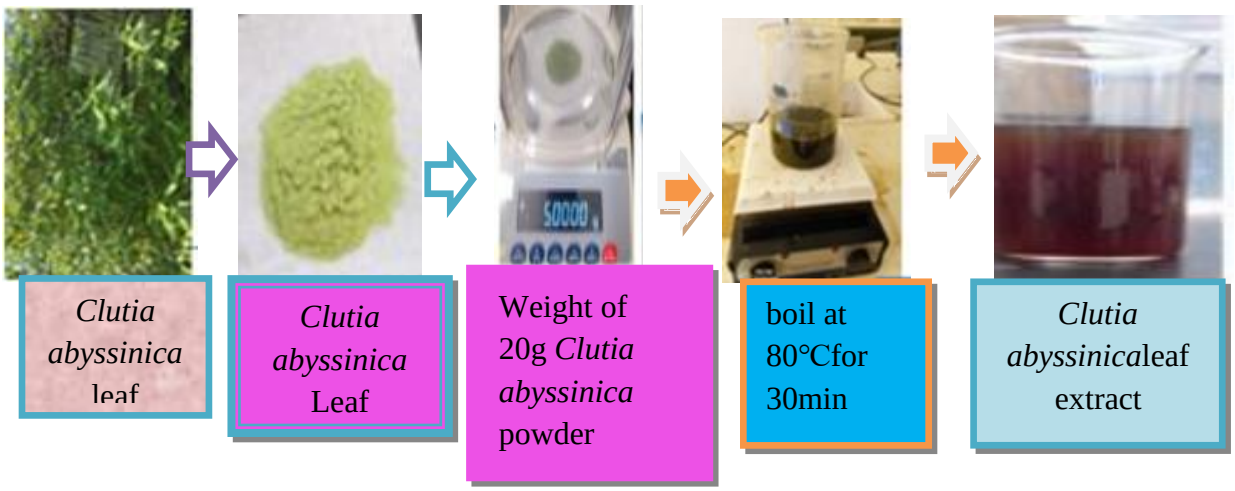


Figure1**:** the general process of preparations of aqueous leafextract

3.5 .Phytochemical test of leafs extracts

The crude leaf extract was subjected to preliminary qualitative phytochemical screening to detect the presence of major secondary metabolites including alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, and glycosides, using standard procedures [60]. As described below

3.5.1.Test for phenol:2ml of plant leaf extract was treated with a solution of ferric chloride ($FeCl_3$).The presence of phenol compound is confirmed by the presence of bluish black changes in the solution.

3.5.2.Check for terpenoids:4 mL of plant leaf extract was mixed with 2 mL of chloroform, and then 3 mL of concentrated sulphuric (H_2SO_4) acid was carefully added. The presence of Terpenoids is indicated by a layer of reddish brown coloration**.**

3.5.3. Test for Flavonoids: 2 mL of plant leaf extract was treated with a 10% NaOH solution. The presence of Flavonoids is indicated by the formation of an intense yellow color.

3.5.4. Test for Tannins (Ferric chloride test):3ml extract was stirred with 5ml ferric chloride; the presence of a greenish-black precipitate was checked for the presence of tannins

3.5.5. Test for alkaloids:A 3 mL of concentrated plant leaf extract was placed in a test tube, and 1 mL of hydrochloric (HCl) acid was added. After that, the mixture was gently heated for 20 minutes before being cooled to room temperature. The resulting mixture was then filtered

through filter paper. The presence of alkaloids was indicated by the formation of a reddish brown color when the filtrate was treated with Wagner reagent.

3.5.6. Test for glycosides:2ml of each extract was dissolved in 1 ml of water and the aqueous 5% NaOH solution was added. Formation of reddish color indicates the presence of glycosides.

3.5.7.Test for Saponins: One milliliter of plant leaf extract was slowly added to three milliliters of distilled water. The mixture was then vigorously shaken. Finally, the presence of Saponins in the leaf extract is confirmed by the formation of foam.

Synthesis of zinc oxide nanoparticles

A 0.45 M(Zn $(NO_3)_2.6H_2O$) Zinc nitrate hexahydrate solution was prepared by dissolving 40.9 g of Zinc nitrate hexahydrate in 400 mLdistilledwater using a 500 mL volumetric flask and 0.45M NaOH solution was prepared using distilled water to be used as precipitating agent.According to previous this letrature of "synthesis of Zinc nanoparticles usingleaf extract of Kalanchoae petitiana and evaluation of its antibacterial activities" Three different samples were prepared by taking different ratios by volume of 0.45 M of Zn $(NO_3)_2.6H_2O$ solution and *Clutia abyssinica*leaf extract sample by volume of (50 ml: 50 ml; 20 ml:80 ml and 40 ml: 60 ml).

1. Zn $(NO_3)_2.6H_2O$ with *Clutia abyssinica*leaf extract (1:1) ratio (50mL:50mL) labeled as Sample A.
2. Zn $(NO_3)_2.6H_2O$ with *Clutia abyssinica*leaf extract (1:4) ratio (20 mL:80mL) labeled as Sample B.
3. Zn $(NO_3)_2.6H_2O$ with *Clutia abyssinica*leaf extract (2:3) ratio (40 mL:60mL) labeled as Sample C.

For sample A (1:1 Ratio)

A 50 mL of 0.45 M of Zn $(NO_3)_2.6H_2O$, 50 mL of 0.45 M of NaOHwas added to the mixture drop wise and 50 mL of*Clutiaabyssinica*plant leaf extract wasmixed with a (1:1) ratio. After that, the mixture was continually mixed at room temperature for two hours,usinga magnetic stirrer. The three liquids were constantly swirled for two hours, causing yellow precipitate to develop using a glass filter. The precipitates were cleaned of contaminants by repeatedly washing them in ethanol and distilled water. Finally, the precipitate was dried in an oven at 80 ˚C overnight and grinded to fine powder using agate mortar. The yellow powder obtained from the above method

was calcined at 380 ˚C for one hours and made ready for further characterizations [61].The same procedures were also used for the preparations of samples 2 and 3 using different precursor to plant extract ratios.Figure 4shows the synthesized of Zinc oxide nanoparticles from*Clutia abyssinica* leaf extract.

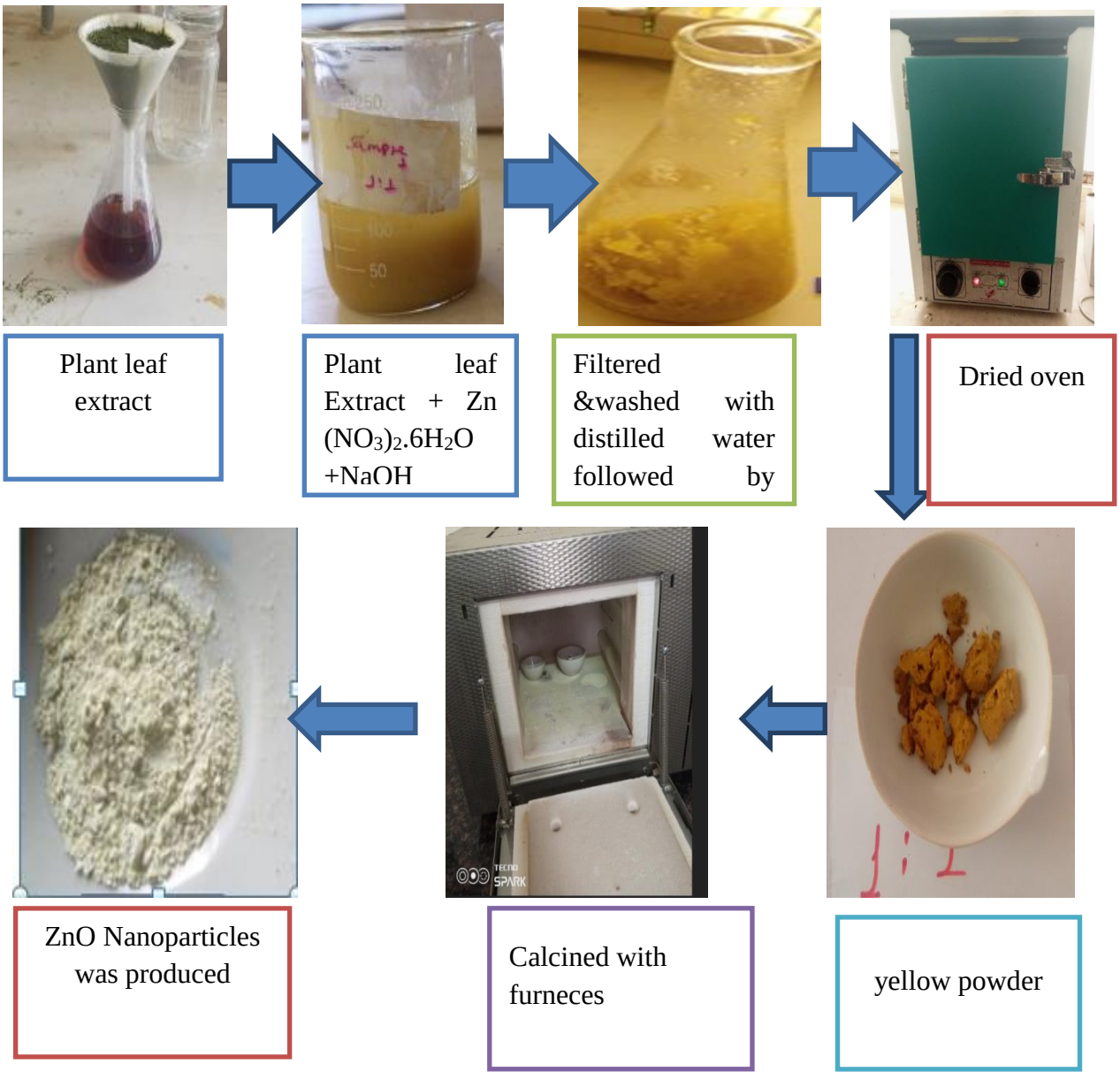


Figure 2synthesis of zinc oxide nanoparticles using leaf of *clutia abyssinica*

Characterization of green synthesized zinc oxide nanoparticles .

The Crystalline structure and the average crystalline size of the synthesized Zinc oxide nanoparticles were characterized using an X-ray diffractometer (XRD-7000, Shimadzu Co.,

Japan) equipped with a Cu target for generating a CuKα radiation with λ = 1.54056 ˚ A. XRD spectra were recorded from 10° to 80° with 2θ angles using CuKα radiation operated at 40 kV and 30 mA[62]. The crystallite sizes (*D*) of the powders were calculated by using Scherrer's equation:

$$D = \frac{0.94\lambda}{\beta\cos\theta}, \quad \ldots\ldots\ldots\

UNIT FOUR

4. RESULTS AND DISCUSSION

4.1.Phytochemicals

The qualitative phytochemicals analysis was tested for flavonoids, tannin, Phenol, flavonoids, terpenoids, alkaloid, and glycosides. Table 2 and Figure 3, below present the findings of the qualitative phytochemicals analysis of the leaf extract of *Clutia abyssinica*togetherwith other polyphenolic substances including tannins and flavonoids, which are employed as reducing and capping agents during the synthesis of zinc oxide nanoparticles , it contains the alkaloid. Thus, the phytochemicals found in *Clutia abyssinica*leaf extract have the ability to capping and stabilizing agent the resultant nanoparticles . For instance, because of the hydrogen abstraction caused by the OH groups in the reductant molecules, the poly phenolic compounds present in plants, such as*Clutia abyssinica* are highly significant plant ingredients. The primary cause of poly phenolic compounds' antioxidant qualities is their redox ability, which enables them to function as capping agents[64]

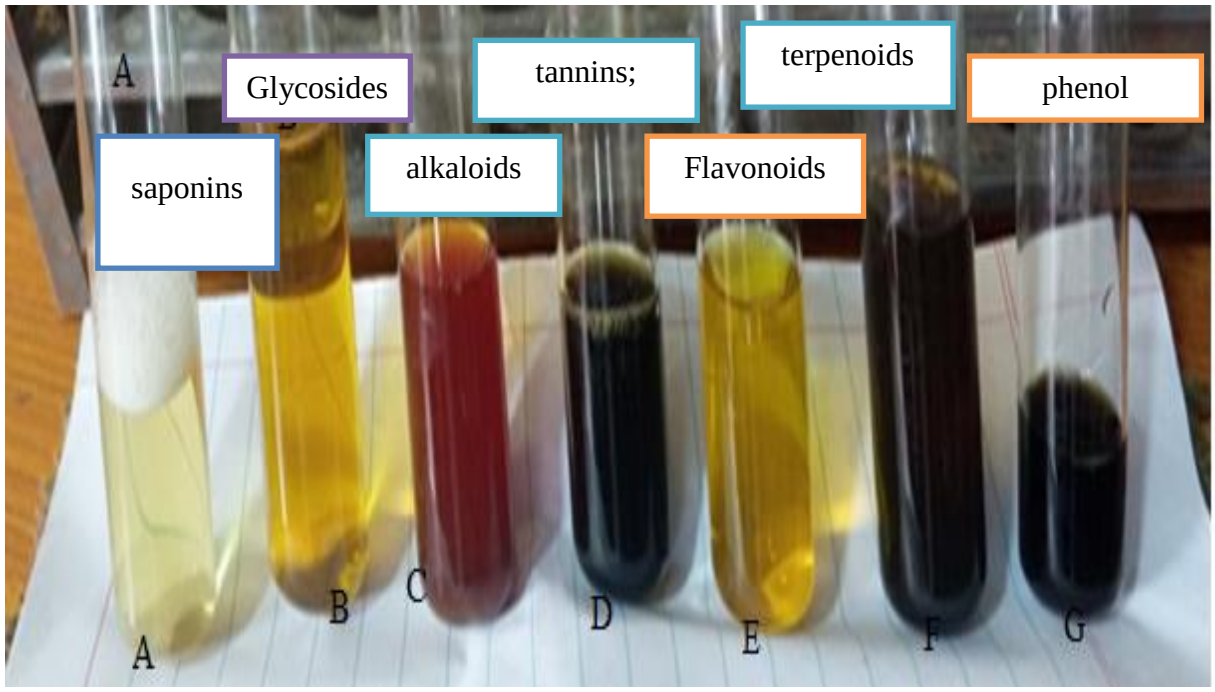


Figure 3. the colours observed in phytochemical screen test of *clutia abyssinica* leaf extract.

Table 2: Phytochemicals test of *Clutia Abyssinica*Leaf Extract

No	Phytochemical	Chemical test	Results	Colors observed
1.	Saponins	Distil water	++	Formation Of Foam
2.	Glycosides	NaOH Solution	+	Reddish
3.	Alkaloid	Wagner's Test	+	Reddish Brown
4.	Tannins	Ferric Chloride Test	+	Greenish-Black
5.	Flavonoids	NaOH Solution	+	Yellow
6.	Terpenoids	Chloroform Test	+	Reddish Brown
7.	Phenol	Ferric Chloride Test	+	Bluish Black

Key :++ more detected +: detected

From the above table 2 the phytochemical screening of*clutia abyssinica*extract. The results of the qualitative phytochemical analysis of the*Clutia abyssinica*extract are shown in table 2. The result represented in table 2 indicates the presence of favonoids, phenols, alkaloids, and saponins confrming the availability of polyols which serve as the capping agent..

4.2. Uv-Visible spectroscopy analysis

The formation of Zinc oxide Nanoparticles was initially confirmed by a noticeable color change in the reaction mixture from pale yellow to milky white upon heating, indicating the reduction of zinc ions and the successful synthesis of Zinc oxide nanoparticles .thisobservation aligns with previous reports on plant-mediated synthesis methods. asillustrated in figure 4, the uv-vis absorption spectrum of the synthesized zinc oxide nanoparticles displays the absorption bands within the range of 374-377 nm which is characteristic of zinc oxide nanoparticles and attributed to surface plasmon resonance [65]. the absence of additional peaks in the spectrum further confirms the purity of the synthesized zinc oxide nanoparticles . moreover, the sharp and well-defined absorption peak suggests that the nanoparticles are of uniform size and within the nanoscale range [66].The wavelength of (374-377nm) absorption peak confirms the occurrence of blue shift absorption with respect to the bulk value (386 nm) of the Zinc oxide Nanoparticles , due to the quantum confinement effect, which is in good agreement of the previous reports [67]. The spectrum shows the characteristic absorbance peak of Zinc oxide nanoparticles , which confirmed the presence of Zn-O nanoparticles .

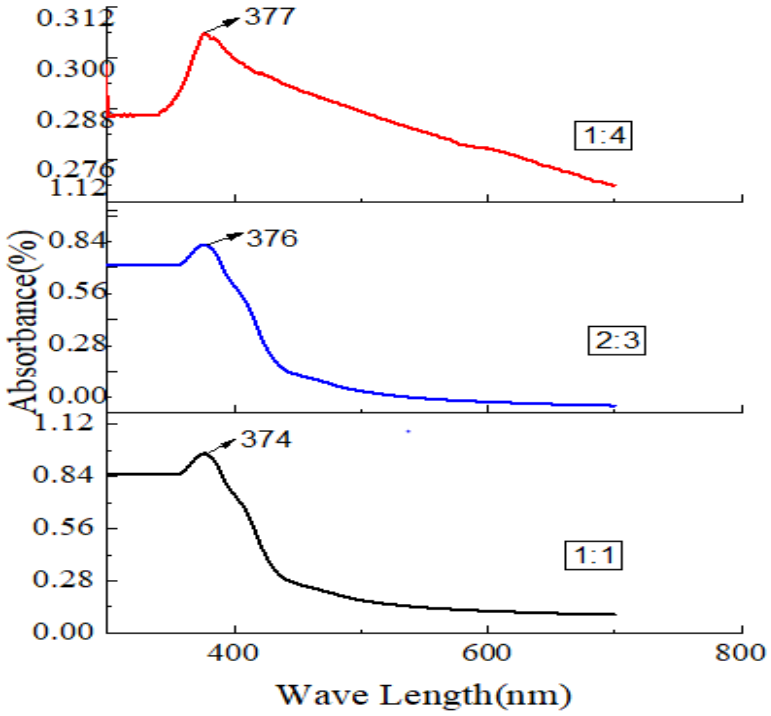


FIgure4**:**UV-Visspectral result of green synthesized zinc oxidenanoparticles

The band gap energy (E_g) of Zinc oxide was obtained from the wavelength value corresponding to the intersection point of the vertical and horizontal part of the spectrum, using the equations:

$$Eg = \frac{1240}{\lambda} \text{ eV} \quad \text{----------(2)}$$

The band gap energy corresponds to the absorption limit and can be roughly evaluated by the above relation. where, Eg is the band gap energy (eV) and λ is the wavelength (nm). From figure4,The absorption edges are positioned at 374 nm, 376 nm and 377 nm respectively for 1:1, 2:3 and 1:4 ratio with the corresponding band gap value of 3.32 eV, 3.29eV and 3.28eV and from this result it is observed that the variation in Eg for the different ratios could be due to variation in average crystal size of the Nanoparticles .From this result it is inferred that the band gap energy decreases with an increase in leaf extract concentration.

4.3.Fourier Transform Infrared Spectroscopy Analysis

The FT-IR spectroscopy gives information on the vibrational and rotational modes of motion of a molecule and hence an important technique for identification and characterization of a substance. The Infrared spectrum of an organic compound provides a unique fingerprint, which is readily distinguished from the absorption patterns of all other compounds; only optical isomers absorb in

exactly the same way. Hence FT-IR is an important technique for identification and characterization of molecules. The vibrational phonon and the quality of Zinc oxide nanoparticleswere characterized by Fourier Transformed Infrared in the wave number range of 400-4000 cm^{-1} .Measurements of Zinc oxide nanoparticles were performed by putting the powder of Zinc oxide nanoparticleson KBr plate

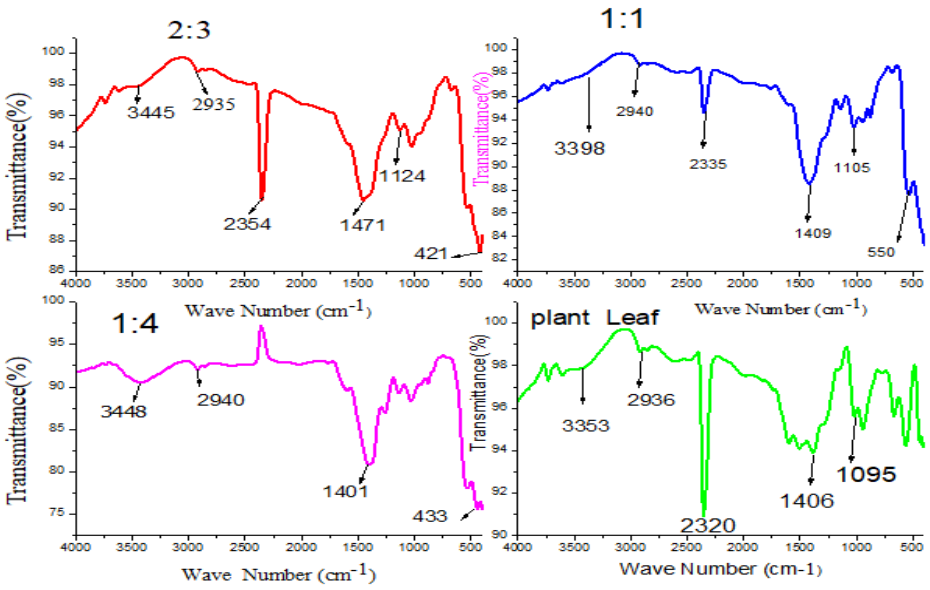


Figure 5**:**ft-ir spectral results of green synthesized zinc oxide nanoparticles

The FT-IR results for green-synthesized Zinc oxide nanoparticles are presented in Figure 5, where different bands were observed for zinc oxide obtained at varying ratios. For the 1:1 ratio, peaks appeared at 3398 cm^{-1}, 2940 cm^{-1}, 2335 cm^{-1}, 1409 cm^{-1}, 1105 cm^{-1}, and 550 cm^{-1}. At the 2:3 ratio, absorption bands were recorded at 3445 cm^{-1}, 2935 cm^{-1}, 2354 cm^{-1}, 1471 cm^{-1}, 1124 cm^{-1}, and 421 cm^{-1}, while the 1:4 ratio showed peaks at 3448 cm^{-1}, 2940 cm^{-1}, 1401 cm^{-1}, and 433 cm^{-1}. The plant extract exhibited characteristic peaks at 3353 cm^{-1}, 2936 cm^{-1}, 2320 cm^{-1}, 1406 cm^{-1}, and 1095 cm^{-1}. In general, the FT-IR spectra showed broad peaks around 3445 cm^{-1}, 3398 cm^{-1}, 3448 cm^{-1}, and 3353 cm^{-1} in the higher energy region, which correspond to O–H stretching vibrations of alcohols and phenols [68]. Absorption bands at 2354 cm^{-1}, 2335 cm^{-1}, & 2320 cm^{-1}, were attributed to the presence of CO_2 molecules in the environment [69]. Strong intensity peaks at 1471 cm^{-1}, 1409 cm^{-1}, 1401 cm^{-1}, and 1406 cm^{-1} were due to α-CH_2 bending vibrations of aldehydes and ketones. Finally, the peaks observed in the region between 400 and 600 cm^{-1} were assigned to Zn–O stretching vibrations, confirming the successful synthesis of

Zinc oxide nanoparticles using *Clutia abyssinica* extract as both a stablizing and capping agent [70].

4.4.X-Ray Diffraction (XRD)

Figure 6 shows the XRD pattern of Zinc oxide nanoparticles synthesized usingZinc nitrate hexahydrateand*Clutia abyssinica* leaf extract using three different ratios by volume.

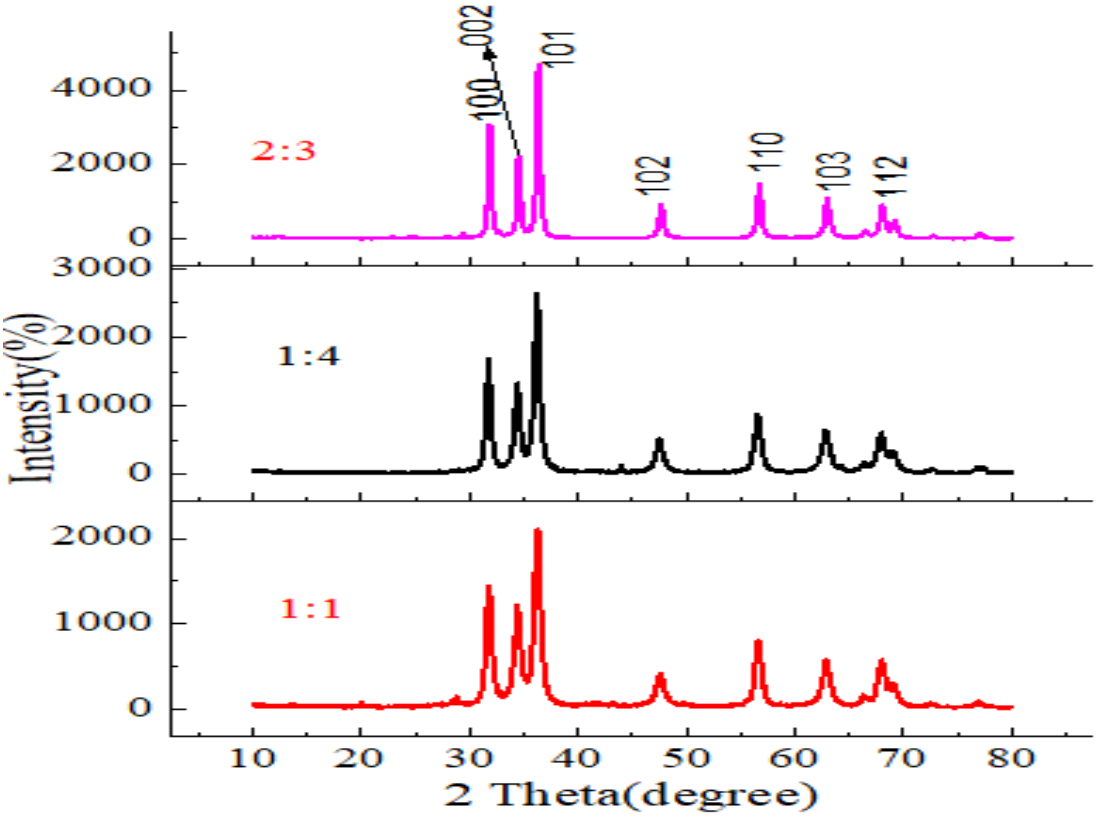


Figure 6:xrdof zinc oxide nanoparticles

Table 3: Miller indices, d-spacing (dhkl), crystalline sizes, and FWHM values of Zinc oxide Nanoparticles

Samples	2Θ(degree)	d-spacing	I/I1	FWHM	FWHM in Radian	Cos θ in Radian	Crystalline size	Miller indices
1:1	31.7571	2.81543	67	0.51180	0.00892	0.27699	16.75	100
	34.4391	2.60207	49	0.58940	0.01028	0.30038	14.53	002
	36.2374	2.47696	100	0.58120	0.01013	0.31607	14.75	101
	47.5337	1.91134	19	0.67720	0.01181	0.414599	12.65	102
	56.5517	1.62608	30	0.66250	0.01155	0.493256	12.94	110
	62.8591	1.47723	21	0.79140	0.01380	0.548271	10.83	103
	67.9501	1.37841	19	0.88330	0.01540	0.59267	9.70	112
	Average	size		13.16nm				
	31.7349	2.81735	66	0.46080	0.00803	0.27679	18.61	100
2:3	34.4118	2.60407	46	0.52770	0.00920	0.30014	16.26	002
	36.2164	2.47834	100	0.50860	0.00887	0.31588	16.85	101
	47.5152	1.91204	20	0.60170	0.01049	0.41443	14.24	102
	56.5375	1.62645	31	0.58040	0.01012	0.49313	14.76	110
	62.8318	1.47780	22	0.71190	0.01241	0.54803	12.04	103
	67.9160	1.37902	19	0.69830	0.01218	0.59237	12.27	112
Average	Size			15.00nm				
1:4	31.6970	2.82063	64	0.359300.	0.00626	0.27646	23.87	100
	34.3803	2.60638	44	41840	0.00729	0.29987	20.50	002
	36.1815	2.48065	100	0.39460	0.00688	0.31558	21.72	101
	47.4777	1.91346	19	0.47870	0.00835	0.41411	17.89	102
	56.5099	1.62718	31	0.44680	0.00779	0.49289	19.18	110
	62.8081	1.47830	22	0.54780	0.00955	0.54782	15.65	103
	67.8860	1.37956	19	0.57830	0.01008	0.59211	14.82	112
Average	Size				19.09nm			

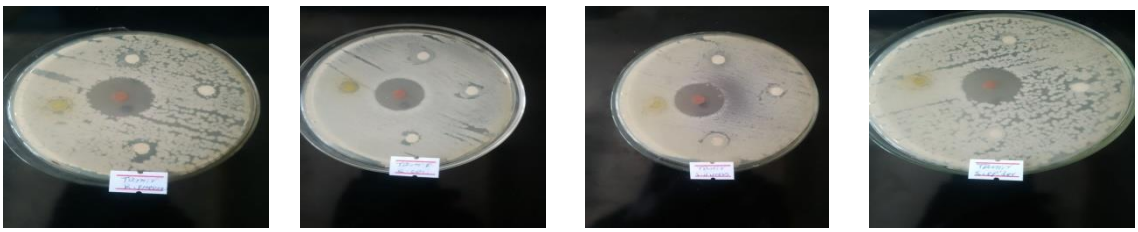
As indicated in Table 3 the Zinc oxide nanoparticlesdiffraction peaks observed at 2θ of(1:1)were 31.7571, 34.4391,36.2374,47.5337, 56.5517,62.8591 and67.9501 for (1:4) 2θ value peaks

were31.7349, 34.4118,36.2164,47.5152, 56.5 375,62.8318, and 67.9160 for (2:3)2θ value peaks were31.6970, 34.3803, 36.1815, 47.4777, 56.5099, 62.8081 and 67.8860 corresponding to (100), (002), (101), (102), (110), (103),and (112) which are in a good agreement with the literature report obtained [71]from the international center of diffraction Data card (JCPDS-36-1451), and confirmed the presence of zinc oxide particles. As observed from the XRD peaks no extra diffraction peaks were detected, indicating the phase purity of Zinc oxide nanopowder[72].The sharp and narrow diffraction peaks suggest that the synthesized product has good crystallinity with relatively larger crystallites, and signifies the effects of experimental conditions on the nucleation and growth of the crystal. The XRD pattern is identical to the hexagonal phase with Wurtzite structure with unit cell parameters a = 3.249 Å and c = 5.2 Å. The average crystalline size of nanoparticles was estimated using the Debye-Scherer formula. Accordingly the calculated value of crystalline size was found to be (14.23- 23.87nm), (12.21 - 18.61nm) and (9.70- 16.75 nm) for 1:4, 2:3, and 1:1 ratios respectively. As compared to Zinc oxide nanoparticles synthesized in different ratios, XRD result showed that the crystalline size of Zinc oxide nanoparticles in 1:1 ratio has a smaller particle size than 2:3.and 2:3has a smaller particle size than 1:4.This difference may be due to the greater amount of leaf extract used during synthesis process resulting in more capping agents that in turn effectively stabilize the synthesized nanoparticles. In addition, in Table 4, it is clearly seen that the peak width of the diffraction line at half of the maximum intensity (FWHM) of (2:3),(1:4) ratio Zinc oxide nanoparticles is less than that of 1:1 ratio. The broadening of peaks in the XRD patterns of solids is attributed to smaller particle size. This is due to the reason that, from Scherer's formula, the particle size increases with decreasing of the peak width of the diffraction line at half of the maximum intensity. As it can be observed from the data's given above in Table 3, the inregularities or lack of regularity in size of naoparticles were observed.This might havedependedon physical and chemical parameters which mainly include temperature, pH, time to substrate and the substrate concentration [73] Other authors explained the inconsistencies in the results of the nanoparticles size due to the sample preparation[74]

4.5.Antibacterial activities of Zinc oxide nanoparticles

The goal of the current investigation is to determine whether ZnO Nanoparticles have antibacterial action on both gram positive and gram-negative bacteria. The microorganisms employed for testing were Gram-positive (Staphylococcus aureus and S. epidermidis) and Gram-

negative (Escherichia coli and Klebsiella pneumonia). Using the disc diffusion method, ZnO nanoparticles antibacterial activity was evaluated.Using this technique, the effects of ZnONpsAntibacterial activity on precursor ratio, plant extract concentration, and particle size were investigated. Standard procedures were followed for conducting these tests in nutrient broth and nutrient agar. Standard zones of inhibition have been established for sensitive and resistant values in this well-documented approach. Table 4 shows zone of inhibition (mm) of the tested samples of ZnO NPs using Zn $(NO_3)_2.6H_2O$ (99% purity) and *Clutia abyssinica* leaf extract at different ratios, plant extract alone, and against four different bacterial strains were also gave The. Concentration of 30 mg/ml against Staphylococcus aureus, S. epidermidis and Escherichia coli .Klebsiellapneumonia.Findings suggest that dose plays a dangerous role, and the selected concentration demonstrated strong inhibition with minimal toxicity risks reported in previous studies [75]



A. K. Pneumonia B. E. coli C. S. Aureus D. S. Epidermidis

Figure 7:Zone Of Inhibition OfZinc oxide Nanoparticles Againstgram-positive &gram-negative bacteria

Table4:Zone of inhibition (mm) of Zinc oxide Nanoparticles

Bacteria strains		Diameter of zone of inhibition in diameter (mm)			
	30 mg/ml	30 mg/ml	30 mg/ml	30mg/ml	TTC standard30mg /ml
	(1:1) Nanoparticles	(2:3) Nanoparticles	(1:4)Nanoparticles	Plant	
E.coli	11.33±0.57	10.33± 0.57	10±1	8.33±0.57	20.67±0.57
K.pneumonia	12.67±0.57	11.67±0.57	10.33±0.57	7.330±.57	20.33±0.57
S.aureus	12.67±0.57	12.33±057	11.33±0.57	9±1	21.67±0.57
S.epidermidis	13.3±0.57	12.33±0.57	11± 1	9±1	21±1

According to Table 4 Zinc oxide nanoparticles using Zinc nitrate hexahydrate are sensitive to gram-positive bacteria (*S. aureus* and *S. epidermidis*) and gram-negative bacteria (*K.pneumonia* and *E.coli*). The study assessed the zone of inhibition at a concentrationof 30mg/ml for three nanoparticlesratio(1:1,(2:3)and (1:4) near crude plant extracted and positive control (Tetracycline,TTC).Across all bacterial strains ,Tetracycline showed the highest zone of inhibition,ranging from 20.67± 0.5mm to 21±1mm. The results showed that Zinc oxide nanoparticles have antibacterial inhibition zone of 11±1, and 13.3±0.57mm for gram positive bacteria against *S. aureus* and *S. epidermidis* and 10.33±0.57, and 12.67±0.57 mm for gram negative bacteria at the same concentration of 30 mg/mL against *K.pneumonia*and*E.coli* bacteria. The presence of an inhibition zone clearly indicated the antibacterial effect of Zinc oxide nanoparticles. This indicates that Zinc oxide nanoparticles are more sensitive to gram positive bacteria.From the above results,Table 4 the size of Zinc oxide nanoparticlesusing zinc nitrate hexahydrate have smaller particle size based on the concentration, combining ratio and calcination temperature.

The smaller size of Zinc oxide nanoparticlesshows greater zone of inhibition indicating that they have greater antibacterial effect on gram-positive bacteria and less on gram negative bacteria but, Gram-negative bacteria seemed to resistant to Zinc oxide nanoparticles than Gram-positive bacteria. It was found that the antibacterial activity of Zinc oxide nanoparticles increased with

decreasing particle size and increasing powder concentration.*Clutiaabyssinica*leaf extracts have synergistic antibacterial action with the (1:1) calcinated Zinc oxide nanoparticles.Furthermore, when compared to Gram-negative bacterial strains, the (1:1) calcined Zinc oxide nanoparticles showed higher antibacterial activity against Gram-positive bacteria [76]. Furthermore, a prior study found that Zinc oxide nanoparticles had more bactericidal effect against Gram-positive bacteria than Gram-negative bacteria. The morphological constitutions of these microorganisms may be the cause of the differences in sensitivity between Gram-positive and Gram-negative bacteria. Because gram-negative bacteria lack an exterior lipopolysaccharide membrane, antimicrobial chemicals cannot pass through their cell walls.However ,because gram-positive bacteria only have an exterior peptidoglycan layer which is ineffective as permeability Barrie they are more vulnerable .As a result, Gram-negative bacteria have more complexly arranged ready cell walls than Gram-positive bacteria, which serves as a diffusion barrier and reduces their susceptibility to antibacterial drugs. Furthermore, it has been reported that the plant extract *Clutia abyssinica* exhibited effective antibacterial activity against strains of Gram-positive bacteria[77]. A few potential bactericidal mechanisms for Zinc oxide nanoparticles interactions with bacteria have been proposed by scientist's .Some proposed that smaller Nanoparticles have greater surface reactivity and easier cell penetration that released Zn^{2+}. The release of Zn^{2+} from Zinc oxide nanoparticles is one of the main propositions in antibacterial mechanisms which are known to inhibit several bacterial cell activities including active transport ,bacteria metabolism, and enzyme activity leading to bacterial cell death due to the toxicity properties of Zn^{2+}[78]. The other proposed antibacterial activity is caused by the formation of reactive oxygen species (ROS) which leads to oxidative stress and subsequent cell damage or death. The formation of ROS is a common antibacterial activity adopted by Zinc oxide Nanoparticles Another possible mechanism for the antimicrobial activity of Zinc oxide nanoparticlesis through the attachment of Nanoparticles to the bacteria cell membrane via electrostatic forces. This interaction may distort the membrane plasma structure and damage the bacterial cell integrity, resulting in the leakage of intracellular contents and ends with cell expiry.

UNIT FIVE

5.CONCLUSION AND RECOMMENDATION

5.1.Conclusions

In summary, Green synthesis of Zinc nanoparticlesis known to be much safer and more environmentally friendly than chemical synthesis. In this study, Zinc nanoparticleswere successfully synthesized from leaf extract of *Clutia abyssinica* for the first time through a simple, cost-effective, eco-friendly and green approach. This showed *Clutia abyssinica*could potentially be used as an effective reducing and capping agent for biological synthesis of Zinc nanoparticles. The biosynthesized Zinc nanoparticleswere characterized using techniques such as XRD and FTIR. From the crystalline of the green synthesized Zinc nanoparticles was proved from XRD analysis, and the analysis showed that all the diffraction peaks fit well with the hexagonal wurtzite structure. Furthermore, XRD analysis showed that the average crystal sizes ofZinc nanoparticles synthesized from 1:1, 2:3, and 1:4 ratio by volume were found to be (9.70-16.75 nm), (12.21 - 18.61nm) & (14.23-23.87nm) respectively. The FT-IR analysis shows the interaction between the capping agents and the synthesized ZnO nanoparticle. As a result, the smaller size of Zinc nanoparticlesallowed for easier entry into the bacterial cell membrane and effective inhibition of the bacterial strains. In general, the results of this study show that Zinc nanoparticlesare effective against both gram-positive Gram positive (*S. aureus* and *S. epidermidis*) and Gram-negative (*E. coli* and *K. pneumonia*) indicating that green synthesized Zinc nanoparticles have a strong action against biological systems.

5.2.Recommendation

As this study, there are some unresolved issues, such as particle size, and understanding of the mechanisms involved in producing metallic nanoparticles using biological entities, even though using green chemistry-based biological synthesis has advantages over traditional methods in terms of the environment. when it comes to extract planning, different plant species have different ways for producing nanoparticles . therefore, additional study is required to assess and fully understand the true mechanisms that are dependent on plants. to fully utilize the green synthesis of metallic materials, a substantial amount of additional funds for research is needed in this largely unexplored field. particularlyzinc oxide nanoparticles reduce bacterial viability albeit it is yet unclear how exactly they work against bacteria further investigation is needed to

enhance the antibacterial action mechanism of zinc oxide nanoparticles . Because green synthesis of Zinc oxide nanoparticles has the best antibacterial activity and many other uses, it is inexpensive, easy to handle, and safe for the environment to produce.

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