JIMMA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

COLLEGE OF NATURAL SCIENCE

DEPARTMENT OF CHEMISTRY

SYNTHESIS AND CHARACTERIZATION OF ZnO AND Mg-DOPED ZnO NANOPARTICLES USING *Moringa stenopetala* LEAF EXTRACT AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITIES.

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DECLARATION

I, the undersigned, declare that this thesis work is my original work, and to the best of my knowledge, it has never been submitted to any university, college, or institution anywhere for the award of any academic degree, diploma, or certificate and I affirm that I have cited and referenced all sources used in this document.

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JIMMA UNIVERSITY

School of Graduate Studies

Department of Chemistry

Synthesis and characterization of zinc oxide and Mg-doped ZnO nanoparticles using *M. stenopetala* leaf extract and evaluation of their antimicrobial activities.

This is to certify that the thesis prepared by Muluken Alchamo submitted in partial fulfillment of the requirement of the Degree of Masters of Science in Chemistry complies with the regulation of the University and meets the accepted standards for originality and quality.

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DEDICATION

This work is dedicated to my wife Yematawerk Zerihun for her unreserved moral and support throughout my work.

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First of all, let me praise and honor the Almighty God and his mother St. Mary for bestowing up on my health, strength, patience, and protection to realize my hope.

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Table of Content **Page**

LIST OF FIGURE PAGE

LIST OF TABLE **PAGE**

ABBREVIATIONS

1. AgNPs	Silver Nanoparticles
2. ASTM	American Society for Testing and Materials
3. FTIR	Fourier Transform Infrared Spectroscopy
4. ITO	Indium tin oxide
5. LED	Light Emitting Diode
6. M. Stenopetala	Moringa Stenopetala
7. Mg-Doped ZnO NPs	Magnesium doped Zinc Oxide Nanoparticles
8. NPs	Nanoparticles
9. PLAL	Pulsed Laser Ablation in Liquid Media.
10. PLD	Pulsed Laser Deposition
11. SEM	Scanning Electron Microscope
12. UV-Vis	Ultra violate-visible- near Infrared Spectroscopy
13. XRD	X-Ray Diffraction
14. ZnO	Zinc Oxide
15. ZnO NPs	Zinc Oxide Nanoparticles

ABSTRACT

Nowadays, much attention is paid to metal and metal oxide nanoparticles, which exhibit chemical and physical properties owing to their extremely small dimensions and high specific surface area. The main objective of the study was the synthesis and characterization of ZnO and Mg-doped zinc oxide nanoparticles using M. stenopetala leaf extract and the evaluation of their antimicrobial activities. The study was conducted using precipitation method of synthesis of nanomaterial. Characterization of ZnO and Mg-doped ZnO nanoparticles were studied by UV-VIS Spectroscopy, Fourier Transform Infrared Spectroscopy, Scanning Electron Microscope, Cyclic Voltammetry, and X-Ray Diffraction. UV-VIS Spectroscopy analysis indicated the absorption spectrum of ZnO NPs. Fourier Transform Infrared Spectroscopy analysis indicated the functional groups present in the synthesized ZnONPs. The vibrational stretching around 550 cm^{-1} (Zn-O) supports the formation of ZnO nanoparticles. *The particle size and of the synthesized ZnONPs were studied by X-Ray diffraction (XRD) pattern and which confirmed the formation of nanoparticles. The synthesized nanoparticles have a hexagonal wurtzite structure with an average grain size of 27.72 nm and which was confirmed by X-ray diffraction analysis. The morphology of zinc oxide and 3% magnesium-doped ZnO nanoparticles was studied using a scanning electron microscope. The SEM study indicated that the shape and morphology of ZnO and 3% Mg-doped ZnO NPs changes with increasing Mg concentration. The cyclic voltammetry analysis confirmed that ZnO nanoparticles exhibited excellent oxidation-reduction behavior and confirmed that the plant extract were used as efficient reducing agent during the synthesis of the nanomaterials. For antimicrobial activities gram-positive bacteria (S. aureus B. cereus,), gram-negative bacteria (E. coli, S. typhus,) and fungus (C. albicas) were used using the agar plate disc diffusion method. The study also clearly states the antimicrobial activity and proof the leaf extract, ZnO nanoparticles, and 1%, 2%, and 3% Mg-doped ZnO nanoparticles have excellent potential application in antimicrobial activity.*

Keywords: ZnO nanoparticles, Mg-doped ZnO, FTIR, UV-Vis, XRD, CV, SEM, Antimicrobial activity

1. INTRODUCTION

1.1 Background of Study

In the modern research era of science, nanotechnology has found immense interest. Nanomaterial play an essential role as building blocks of nanotechnology. Nowadays, nanotechnology is widely applied in different fields mainly in sensor, electronic, antibacterial, water purification, cosmetic, biomedical, pharmaceutical, environmental, catalytic, and material applications. The size, crystallinity, and morphology of the nonmaterial can greatly influence their catalytic, magnetic, electronic, and optical properties [1].

The major advantages of nanoparticle synthesis at room temperature and from plant extracts are partly to fulfill the green synthesis [2].The metal and metal oxide nanoparticles are synthesized by physical, biological, chemical, and very recent green approaches. Recently, green synthesis has become a popular way to synthesize NPs due to its low cost, environment compatibility, synthesizable in ambient atmosphere, and non-toxicity [3]. The noticeable importance of Zinc oxide and ZnO NPs is found as the best semiconductor in nature with a wide range of band gaps (3.37) having room temperature-dependent high excitation binding energy (60 meV) [4]. Zinc oxide and ZnONPs have excellent thermal and chemical stability with exceptional optical behavior [5]. Nowadays considerable interest was seen in the biosynthesis of different nanoparticles as well as their antimicrobial activities. Different plant extracts are the main source of large-scale synthesis of NPs via green way which includes the synthesis of several noble metal nanoparticles like gold (Au), silver (Ag), and palladium (Pd) [6].

Medicinal plants and traditional preparation with antimicrobial activities have been used extensively in the African regions. These plants of medicinal importance have been proven to be very effective even where treatments with antibiotics failed [7]. The Moringa plant has 13 varieties of species. These are *M. stenopetala, M. oliefera, M. aborea, M. hildebrandtii, M. ovalifolia, M. douhardii, M. peregrine, M. borziana, M. arivea, M. pymaea, M. concansis, M. ruspoliana, M. longituba, M. stenopetala*, commonly known as African moringa or cabbage tree, Shiferaw in Amharic belongs to Moringaceae family and native to Kenya and Ethiopia [8-9]. A drought-resistant species is characterized by its bottle-shaped trunk, long twisted seed pods, and edible leaves likened to cabbage, from which its common name is derived. *M. stenopetala* is extirpated in the wild in Ethiopia, though still grown there as a crop on the

terraces of the Ethiopian highlands, mainly in the south region. *M. stenopetala* is known under various vernacular names throughout its native regions, including aleko in the (Gidole, Gamogofa) language, shelagda or telchada in the (Konso) languages, and Shiferaw in (Amharic). In English, it is the most commonly known as the African Moringa or cabbage tree, though these names may be shared with other Moringa species from Africa. Like it is widely cultivated relative *M. oleifera*, *M. stenopetala* is a multipurpose tree: the leaves, pods, and flowers are edible and nutritious; the seeds contain aromatic oil with culinary and cosmetic applications, and the seed press cake or powdered bark can be used for water purification. It is featured in various dishes and has a history of uses in folk medicine throughout its native range [10-13].

Medicinal plants, especially those with anti-infective properties are extensively used in Africa due to their availability and ease of access; furthermore, the lack of access to modern medicine in rural communities has made traditional medicinal practice the only option in combating diseases. Such plants could also serve as sources of new antimicrobial and other drugs [14]. In fulfilling the immediate needs of Africa, such plants can be developed into phytomedicines as is the case in some world countries. Even in modern medicine; a considerable number of drugs used are derived from plants. It is therefore likely that plants will continue providing templates for the development of new drugs [15]. No studies were conducted regarding to synthesis and characterization of ZnO and Mg-doped ZnO nanoparticles using *M. stenopetala* leaf extract and evaluation of their antimicrobial activities.

This study presented ZnO and Mg-doped ZnONPs from Zinc acetate and magnesium nitrate metal salt precursors by using *M. stenopetala* (Shiferaw) leaves as a natural stabilizing agent. The main objective of this was to synthesize ZnO and Mg-doped ZnONPs by using *M. stenopetala* (Shiferaw) leaves as reducing and capping agent and evaluate their antimicrobaial activities.

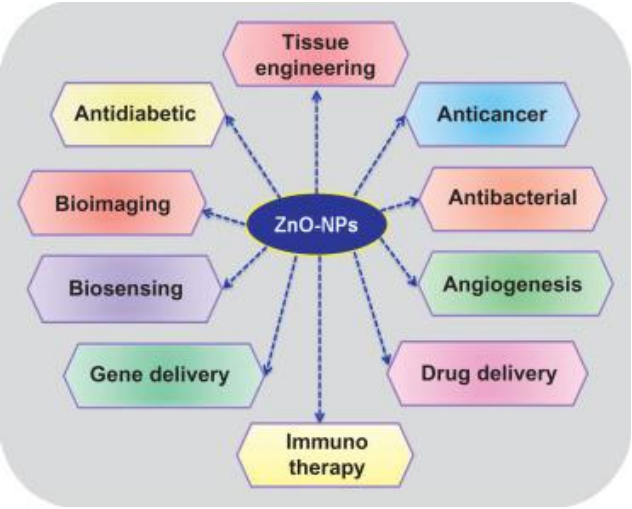


Figure 1. Applications of zinc oxide nanoparticles.

1.2 Statement of the Problem

Many research works had been concerned with the synthesis of nanomaterials for antimicrobial applications which is the recent research focus. However, no studies have been conducted to evaluate the antimicrobial effects of zinc oxide nanoparticles and Mg-doped zinc oxide nanoparticles on gram-positive and gram-negative bacteria and fungus. A rapid increment in the development of new antimicrobial materials has been observed as a consequence of the spread of antibiotic-resistant infections during the last decades which has become a major issue in health care. The emerging infectious diseases and the development of drug-resistant bacteria at an alarming rate is a matter of serious concern and an increasing public health problem. New strategies for controlling microbial activities are urgently needed. A possible alternative is the synthesis of new inorganic compounds with antimicrobial properties.

. This study was intended to determine the degree to which zinc oxide and Mg-doped ZnO nanoparticles can be manipulated using *M. stenopetala*. Also considering that other methods of synthesis of nanoparticles; physical, chemical, etc. are considered more toxic. In this work, the study was focused to perform the green synthesis which is less toxic and eco-friendly.

1.2.1. Research Question

- Does the plant leaf extract of *M. stenopetala* effective as reducing agent in the nanomaterials (ZnO and Mg-doped ZnO NPs) Formations?
- Do the utilized characterization techniques confirm the formation of the targated Nanomaterials?
- Are the synthesized Mg-doped ZnO NPs potentially applicable to antimicrobial activity?

1.3 Objectives of Study

1.3.1 General Objective

The main objective of the study was to synthesize and characterize ZnO and Mg-doped ZnO nanoparticles using *M. stenopetala* leaf extract and to evaluate their antimicrobial activities

1.3.2 Specific Objectives

- To prepare leaf extract of *M. stenopetala*.
- To synthesize ZnO and Mg-doped ZnO NMs.
- To characterize ZnO and Mg-doped ZnO nanoparticles using UV-Vis Spectroscopy, XRD, CV, SEM, and FTIR techniques.
- To evaluate the antimicrobial activity of ZnO and Mg-doped ZnO nanoparticles.

1.4 Significance of the Study

Critical studies for characterization and controlling bacterial activity of nanoparticles are urgently needed and very promising approach. A possible alternative is the synthesis of new inorganic compounds with characterization, antimicrobial properties, and other different applications. Zinc oxide nanoparticle has the advantage of durability as well as chemical and physical stability over common organics and antibiotics compounds. This study provided relevant information about the applications of green synthesis of zinc oxide and Mg-doped zinc oxide nanoparticles. It also provided much information about the importance of zinc oxide nanoparticles that were synthesized using *M. stenopetala* leaf extract and can be used as a baseline for any further studies. Currently, the synthesis of zinc oxide nanoparticles is the most important all over the world, due to its eco-friends and application in many fields of modern science and technology, so its synthesis and use progressively increasing. This study benefits the science community by providing evidence on the ability of *M. stenopetala* leaf extract. *M. stenopetala* was used because it is cost-effective and its diverse health benefits.

2. REVIEW OF RELATED LITERATURE

2.1. Nanotechnology

Nanotechnology may be the next big thing in science, and before long we will probably find ourselves immersed in it. It has attracted considerable attention in the scientific community ever since it emerged as a powerful basic and applied science tool. Nanotechnology provides the tools and technology platform for the investigation and transformation of biological systems, and biology offers inspiration models and bio-assembled components to nanotechnology. Nanobiotechnology is defined as a field that applies the nanoscale principle and techniques to understand and transform biosystems (living and non-living) and which uses biological principles and materials to create new devices and systems integrated from the nanoscale [17]. Key advances have been made in the ability to make measurements at the sub-cellular level and in understanding the cell as highly organized, self-repairing, self-replicating, information-rich molecular machines, classified nanotechnologies into wet and dry nanotechnology, the first one describes the living biosystems and the second one deals with man-made objects at nanoscale structures. The presence of nanoparticles in commercially available products is becoming more common [18- 20].

Nanoparticles, according to the ASTM standard definition, are particles with lengths that range from 1 to 100 nanometers in two or three dimensions. A nanoparticle is defined as the smallest unit that can still behave as a whole entity in terms of properties and transport. Nanoparticles are used in bioapplications such as therapeutics, antimicrobial agents, drug delivery agents, biosensors, imaging contrast agents, transfection vectors, and fluorescent labels. Nanoparticles are of great scientific interest as they bridge the gap between bulk materials and atomic or molecular structures. A bulk material has constant physical properties regardless of its size, but at the nanoscale, this is often not the case. Several well-characterized bulk materials have been found to possess the most interesting properties when studied on the nanoscale. There are many reasons for this including the fact that nanoparticles possess a very high aspect ratio. In the case of silver nanoparticles (Ag NPs), this allows them to easily interact with other particles and increases their antibacterial efficiency. This effect is extremely robust, and as little as 1 g of AgNPs is known to impart antibacterial properties to hundreds of square meters of substrate material [21].

2.2. Classification of nanoparticles

Nanoparticles can be broadly grouped into two: namely organic and inorganic nanoparticles. Organic nanoparticles may include carbon nanoparticles (fullerenes) while some inorganic nanoparticles may include magnetic nanoparticles, noble metal nanoparticles (like gold and silver), and semiconductor nanoparticles (like titanium dioxide and zinc oxide). There is a growing interest in inorganic nanoparticles as they provide superior material properties with functional versatility. Due to their size features and advantages over available chemical imaging drugs agents and drugs, inorganic nanoparticles have been examined as potential tools for medical imaging as well as for treating diseases. Inorganic nanomaterials have been widely used for cellular delivery due to their versatile features like wide availability, rich functionality, good biocompatibility, the capability of targeted drug delivery, and controlled release of drugs [22]. For example, mesoporous silica when combined with molecular machines proves to be excellent imaging and drug-releasing system. Gold nanoparticles have been used extensively in imaging, as drug carriers, and in the thermotherapy of biological targets. Inorganic nanoparticles (such as metallic and semiconductor nanoparticles) exhibit intrinsic optical properties which may enhance the transparency of polymer-particle composites. For such reasons, inorganic nanoparticles have found a special interest in studies devoted to optical properties in composites. For instance, the size-dependent color of gold nanoparticles has been used to color glass for centuries [23].

2.3. Metals and Metal Oxide Nanoparticles

Metallic nanoparticles have possible applications in diverse areas such as electronics, cosmetics, coatings, packaging, and biotechnology. For example, nanoparticles can be induced to merge into a solid at relatively lower temperatures, often without melting, leading to improved and easy-to-create coatings for electronics applications (eg, capacitors). Typically, nanoparticles possess a wavelength below the critical wavelength of light. This renders them transparent, a property that makes them very useful for applications in cosmetics, coatings, and packaging. Metallic nanoparticles can be attached to single strands of DNA nondestructively. This opens up avenues for medical diagnostic applications. Nanoparticles can traverse through the vasculature and localize any target organ. This potentially can lead to novel therapeutic, imaging, and biomedical applications. Based on all of the above, the synthesis of metallic nanoparticles is an active area of academic and, more importantly, “application research” in nanotechnology.

Metal oxides play a very significant role in material science for instance fabrication of microelectronic circuits, sensors, piezoelectric devices, fuel cells, and coatings for the passivation of surfaces against corrosion and as a catalyst. Metal oxides have also been employed as sorbents for environmental pollutants. In the domain of nanotechnology, oxide nanoparticles can exhibit unique chemical properties owing to their limited size and high density of corner or edge surface sites. Many physical and chemical preparative methods for accessing nanostructured oxides are on record. Among the metal oxides, nano ZnO exhibits a wide band gap (~3.4eV) and large exciting binding energy (60meV) and thus is considered the most promising candidate for nano optoelectronics, sensors, transistors, nano piezo electronics, and UV-detection [24].

Various metal-oxide nanoparticles have been also prepared by Pulsed laser ablation in liquid media (PLAL). The concept of fabricating oxide using laser irradiation of metal targets in water was demonstrated in 1987, when iron and tantalum oxides were formed on target surfaces in water using a Q-switched ruby pulsed laser [25]. Although the preparation of various kinds of NPs by ablation of different targets in deionized water has been extensively studied in recent days, papers reporting the fabrication of metal oxide-based nanomaterials from metal targets by PLAL are still scarce compared with reports concerning the production of noble-metal nanoparticles [26].

Of the inorganic materials, metal oxides such as TiO2, ZnO, MgO, and CaO are of particular interest as they are not only stable under harsh process conditions but also generally regarded as safe materials for human beings and animals [27] and [28]. The use of nanoparticles of silver and zinc oxide has been seen as a viable solution to stop infectious diseases due to the antimicrobial properties of these nanoparticles. The intrinsic properties of a metal nanoparticle are mainly determined by size, shape, composition, crystallinity, and morphology [29]. The solution phase synthesis of metal oxide nanoparticles typically involves the reaction of a metal salt with hydroxide ions [30]. The particle size is dependent on the kinetics of nucleation and growth from a supersaturated solution as well as processes such as coarsening, [31, and 32] oriented attachment, and aggregation. Synthesis of crystalline particles with diameters less than 10 nm is often performed in non-aqueous solvents where nucleation and growth are usually completed in a few minutes [33-34]. Processes such as coarsening and oriented attachment occur at longer times and can have a large influence on particle size. The ability to separate nucleation and growth from

supersaturating from processes such as coarsening and oriented attachment is important for controlling the particle size. [35-37]

2.4 Synthesis of nanoparticles

Nanoparticles having one or more dimensions of the order of 100nm or less- have attracted considerable attraction due to their unusual and fascinating properties, with various applications, over their bulk counterparts [38]. Currently, a large number of physical, chemical, biological, and hybrid methods are available to synthesize different types of nanoparticles [39]. Though physical and chemical methods are more popular for nanoparticle synthesis, the use of toxic compounds limits their applications. The development of safe eco-friendly methods for biogenetic production is now of more interest due to the simplicity of the procedures and versatility. Traditionally nanoparticles were produced only by physical and chemical methods. Some of the commonly used physical and chemical methods are ion sputtering, Solvothermal synthesis, reduction, and sol-gel technique. There are two approaches for nanoparticle synthesis namely the Bottom-up approach and the Top-down approach [40].

A. Top-Down Approach

The principle behind the top-down approach is to take a bulk piece of material and then modify it into the wanted nanostructure and subsequent stabilization of the resulting nano-sized metal nanoparticles by the addition of colloidal protecting agents. Cutting, grinding, and etching are typical fabricating techniques, which have been developed to work on the nanoscale. The sizes of the nanostructure which can be produced with top-down techniques are between 10 to 100nm [41].

B. Bottom-Up Self Approach

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. The advantage of this approach is the better possibility to obtain nanostructures with fewer defects and a more homogenous chemical composition [42].

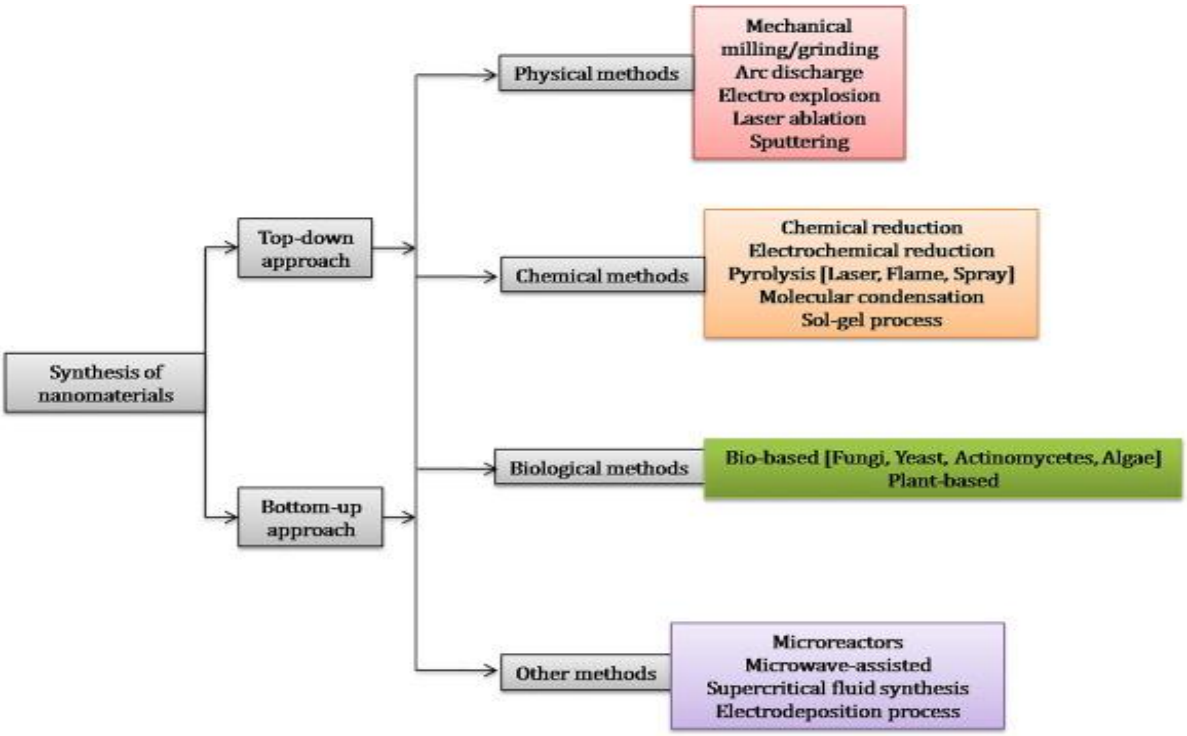


Figure 2. Top-down and Bottom-up Approach of Synthesis of nanoparticles [43].

2.4.1 Physical and chemical methods of nanoparticle synthesis

Some of the commonly used physical and chemical methods include:

- The sol-gel technique is a wet chemical technique used for the fabrication of metal oxides from a chemical solution which acts as a precursor for an integrated network (gel) of discrete particles or polymers. The precursor sol can be either deposited on the substrate to form a film, cast into a suitable container with the desired shape, or used to synthesize powders.
- Solvothermal synthesis is a versatile low-temperature route in which polar solvents under pressure and at temperatures above their boiling points are used. Under Solvothermal conditions, the solubility of reactants increases significantly, enabling reactions to take place at a lower temperature.
- Chemical reduction is the reduction of an ionic salt in an appropriate medium in the presence of surfactant using reducing agents. Some of the commonly used reducing agents are sodium borohydride, hydrazine hydrate, and sodium citrate.
- Laser ablation is the process of removing material from a solid surface by irradiating it with a laser beam. At low laser flux, the material is heated by absorbed laser energy and evaporates or sublimates. At higher flux, the material is converted to plasma. The

depth over which laser energy is absorbed and the amount of material removed by a single laser pulse depends on the material's optical properties and the laser wavelength. Carbon nanotubes can be produced by this method.

- Inert gas condensation, where different metals are evaporated in separate crucibles inside an ultra-high vacuum chamber filled with helium or argon gas at a typical pressure of 100 Pascal's. As a result of inter-atomic collisions with gas atoms in the chamber, the evaporated metal atoms lose their kinetic energy and condense in the form of small crystals which accumulate on liquid nitrogen-filled cold fingers. For E.g. gold nanoparticles have been synthesized from gold wires [43].

2.5 Biosynthesis of nanoparticles

The need for the biosynthesis of nanoparticles rose as the physical and chemical processes were costly. So in the search for cheaper pathways for nanoparticle synthesis, scientists used microorganisms and then plant extracts for synthesis. Nature has devised various processes for the synthesis of nano- and micro-length scaled inorganic materials which have contributed to the development of a relatively new and largely unexplored area of research based on the biosynthesis of nanomaterials.

Biosynthesis of nanoparticles is a kind of bottom-up approach where the main reaction occurring is reduction/oxidation. The microbial enzymes or the plant phytochemicals with anti-oxidant or reducing properties are usually responsible for the reduction of metal compounds into their respective nanoparticles.

The three main steps in the preparation of nanoparticles that should be evaluated from a green chemistry perspective are the choice of the solvent medium used for the synthesis, the choice of an environmentally benign reducing agent, and the choice of nontoxic material for the stabilization of the nanoparticles. Most of the synthetic methods reported to date rely heavily on organic solvents. This is mainly due to the hydrophobicity of the capping agents used. Synthesis using bio-organisms is compatible with the green chemistry principles: the bio organism is (I) eco-friendly as are (ii) the reducing agent employed and (iii) the capping agent in the reaction [43]. Often chemical synthesis methods lead to the presence of some toxic chemical species adsorbed on the surface that may have adverse effects on medical applications [44]. This is not an issue when it comes to biosynthesized nanoparticles as they are eco-friendly and biocompatible for pharmaceutical applications.

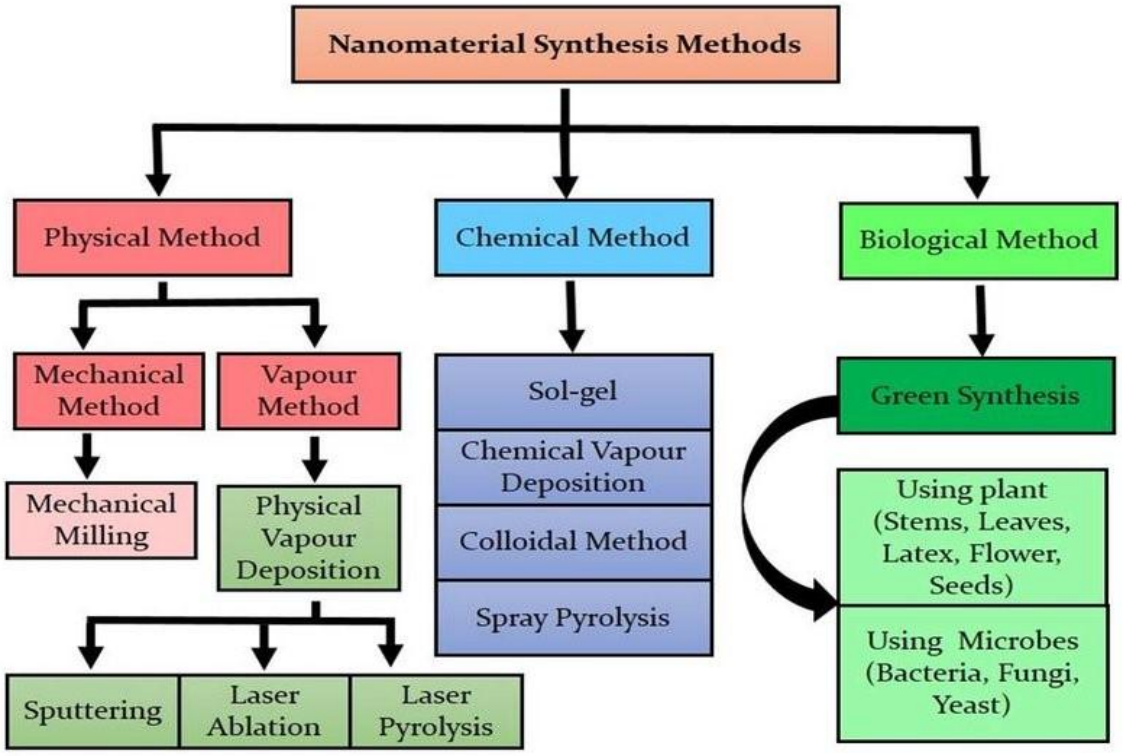


Figure 3. Physical and Chemical Methods of Nanoparticle Synthesis [45].

2.6 About Moringa Stenopetala

M. stenopetala, commonly known as African Moringa or cabbage tree, Shiferaw in Amharic is belongs to Moringaceae family and native to Kenya and Ethiopia [46]. A drought-resistant species, is characterized by its bottle-shaped trunk, long twisted seed pods, and edible leaves likened to cabbage, from which its common name is derived. *M. stenopetala* is extirpated in the wild in Ethiopia, though still grown there as a crop on the terraces of the Ethiopian highlands, mainly in the Konso region. *M. stenopetala* is known under various vernacular names throughout its native regions, including Aleko in the Wolayita, Gamogofa language, Shelagdaor telchada in the Konso languages, and Shiferaw in Amharic. In English, it is the most commonly known as the African Moringa or cabbage tree, though these names may be shared with other Moringa species from Africa. The local communities in the southern part cook the leaves as cabbage and eat it with their traditional food known as "Kurkurfa/Fosses" [45].

M. stenopetala is a perennial tree with a shrubby, rounded habit [46].Growing to a height of 6-12 m in all but the most exceptional cases where it may reach 15 m high. The leaves are light green when mature, up to 55 cm long, and attached alternately to the stem by short petioles.

They are bi- or tri pinnate in composition, with about five pairs of pinnate and three in nine leaflets on each pinna. Each leaflet is 3.5-6.5 cm in size and elliptical to ovate. *M. Stenopetala* features a busy, aromatic inflorescence, organized as dense panicles up to 60 cm long. The individual flowers are bisexual, radially symmetrical, and pentamerous. The calyx is polysepalous and cream-colored, sometimes flushed pink, with 4–7 mm long sepals. The corolla is polypetalous and variably white, pale-yellow, or yellow-green; its petals are roughly oblong and 8–10 mm in length. Each flower features five stamens with white 4–6.5 mm long filaments and yellow 2 mm long anthers, as well as an indeterminate number of shorter staminodes. The ovary is densely haired and superior, 2 mm long, and ovoid, transitioning to a smooth cylindrical style sans stigmatic lobes [47].

Figure 4. Images of *M. stenopetala.*

2.6.1. Taxonomy of Moringa Stenopetala

The species was first described as Donaldsonia stenopetala by botanist Edmund G. Baker in 1896, based on the type specimen collected by Donaldson Smith along the northeastern shore of Lake Turkana. Along with at least twelve other species, *M. Stenopetala* belongs to the monotypic genus Moringa, which is the sole representative of the family Moringaceace. Researchers have divided these species into three distinct groups: Moringa with eight, Dysmoringa with one, and Donaldsonia with four. *M. Stenopetala* belongs to the latter groups, along with *M. drouhardii* and *M. Hildebrandtii*, characterized by winged seeds and regular flowers with short receptacles and superior ovaries [48].

The different parts of this medicinal plant have locally been utilized by the Ethiopian communities to manage diseases like malaria, hypertension, asthma, diabetes, and stomach pain [49].

Table 1. Scientific Classifications of Moringa Stenopetala

Scientific classification	
Kingdome	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Clade	Rosids
Order	Brassicales
Family	Moringaceae
Genus	Moringa
Species	*Moringa stenopetala*

2.7 Zinc Oxide Nanoparticles (ZnO NPs)

Semiconductor nanoparticles are very important materials that have attracted the attention of researchers because of their wide applications in material science, and chemical and electrical engineering during the past years. Nanoparticles have mainly two different excitations. These are excitonic transitions and deep-trap transitions. Whilst excitonic transitions come from the band-edge (from the conduction band to the valance band), deep-trap transitions are caused by deep trap energy levels and the surface defects that appear especially at the higher surface-to-volume ratios. This is a result of the physical properties of nanoparticles becoming size-dependent when their radius becomes comparable to the Bohr radius of the bulk material. It is observed that the emission spectra from the excitonic level do not show any shift but if the emission spectra come from the deep trap level they show some considerable shifts upon changing the excitation wavelength. The emission spectra area direct function on the size of the nanoparticles and the optical properties of semiconductor nanoparticles are strongly dependent on the effective particle size. The surface-to-volume ratio is the most important point for the optical properties of the nanoparticles. As this ratio increases the effects of the deep-trap transitions, become inefficient [50].

ZnO is a kind of wide band gap (3.37 eV) semiconductor with large exciton binding energy (60 meV) [51]. ZnO is a bio-friendly oxide semiconductor and an inexpensive luminescent material. It has attracted intensive research efforts for its unique properties and versatile applications in antireflection coatings, transparent electrodes in solar cells, ultraviolet (UV) light emitters, diode lasers, varistors, piezoelectric devices, spin-electronics, surface acoustic wave propagators [52, 53], antibacterial agent [54], photonic material [55] and for gas sensing [56].

Among all the inorganic semiconducting nanoparticles, zinc oxide nanoparticles have attracted increasing attention because ZnO nanoparticles can be easily synthesized and ZnO is a green material that is biocompatible, biodegradable, and nontoxic for medical applications and environmental science [57]. Recently, there are several physical or chemical synthetic methods of preparing ZnO, such as thermal evaporation, pulsed laser deposition (PLD), ion implantation, reactive electron beam evaporation, thermal decomposition, and sol-gel technique. Recently, great effort has been made into the synthesis of size-controlled ZnO nanoparticles to explore their potential [58-60].

2.8. Electronic properties

ZnO has a relatively large direct band gap of ~3.3 eV at room temperature; therefore, pure ZnO is colorless and transparent. Advantages associated with a large band gap include higher breakdown voltages, ability to sustain large electric fields, lower electronic noise, and high temperature and high-power operation. ZnO has n-type character, even in the absence of intentional doping. Native defects such as oxygen vacancies or zinc interstitials are often assumed to be the origin of this, but the subject remains controversial. An alternative explanation has been proposed, based on theoretical calculations, that unintentional substitution of hydrogen impurities is responsible. Controllable n-type doping is easily achieved by substituting Zn with group-III elements Al, Ga, In, or by substituting oxygen with group-VII elements chlorine or iodine [61-62].

Reliable p-type doping of ZnO remains difficult. This problem originates from the low solubility of p-type dopants and their compensation by abundant n-type impurities, and it is pertinent not only to ZnO but also to similar compounds GaN and ZnSe. Measurement of p-type in "intrinsically" n-type material is also not easy because inhomogeneity results in spurious signals. The current absence of p-type ZnO does limit its electronic and optoelectronic applications which usually require junctions of n-type and p-type material. Known p-type dopants include group-I elements Li, Na, and K; group-V elements N, P, and As; as well as copper and silver. However, many of these forms deep acceptors and do not produce significant p-type conduction at room temperature. Electron mobility of ZnO strongly varies with temperature and has a maximum of ~2000 cm2/ (V·s) at ~80 Kelvin [63-64].

2.9 Optical properties

ZnO is a wide band gap semiconductor that displays luminescent properties in the near ultraviolet and visible regions. The emission properties of ZnO nanoparticles in the visible region widely depend on their synthetic method as they are attributable to surface defects. Recently, we have developed a novel organometallic synthetic method for the preparation at room temperature of crystalline ZnO nanoparticles of controlled size and shape. The studies on the emission properties of nanocrystalline ZnO nanoparticles and nanorods were prepared following this organometallic synthetic method. They observed a clear influence of the shape of the particles and the ligands on the luminescence properties in the visible domain. They observed two different emissions at 440 nm and at 580 nm that are associated with the presence of surface defects on the particles. The first one corresponds to the well-known

yellow emission located at 580 nm with a lifetime of 1850 ns for 4.0 nm size particles. The second emission at 440 nm is observed when amine ligands are present. Based on the optical measurements, they have proposed a mechanism at the origin of the two emissions. They also observed that the modification of the relative intensity between the two emissions is related to a specific location of the amine ligands on the surface of the particles [65].

2.10. Lattice parameters

The lattice parameters of a semiconductor usually depend on the following factors:

i. Free electron concentration acting via deformation potential of a conduction-band minimum occupied by these electrons,
ii. the concentration of foreign atoms and defects and their difference in ionic radii for the substituted matrix ion,
iii. External strains, for example, those induced by substrate, and
iv. Temperature. The lattice parameters of any crystalline material are common and most accurately measured by high-resolution x-ray diffraction XRD by using the Bond method for a set of symmetrical and asymmetrical reflections [60]. For the wurtzite ZnO, lattice constants at room temperature determined by various experimental measurements and theoretical calculations are in good agreement. The lattice constants mostly range from 3.2475 to 3.2501 Å for the parameter and from 5.2042 to 5.2075 Å for the c parameter. The c/a ratio and u parameter vary in a slightly wider range, from 1.593 to 1.6035 and from 0.383 to 0.3856, respectively. The deviation from that of the ideal wurtzite crystal is probably due to lattice stability and ionicity. It has been reported that free charge is the dominant factor responsible for expanding the lattice proportional to the deformation potential of the conduction-band minimum and inversely proportional to the carrier density and bulk modulus. The point defects such as zinc antisites, oxygen vacancies, and extended defects, such as threading dislocations, also increase the lattice constant, albeit to a lesser extent in the heteroepitaxial layers. For the zinc-blende polytype of ZnO, the calculated lattice constants based on a modern abi nitio technique are predicted to be 4.60 and 4.619 Å. A high-pressure phase transition from the wurtzite to the rocksalt structure decreases the lattice constant down to the range of 4.271–4.294 Å [61].

2.11. Applications of ZnO NPs

At present, the most widely revealed application for ZnO is an ITO replacement for displays and photovoltaic panels, where ZnO could lower the costs of transparent conductors. In addition to its conductive nature, it also can be used as a semiconductor for making inexpensive transistors for disposable electronics or even low-cost LEDs. ZnO is also finding applications in thin-film batteries, and ZnO's ability to be engineered into interesting nanostructures hints at new applications down the road. ZnO already is being tapped in spintronics. Due to their excellent optical and electrical properties, ZnO nanoparticles have become predominant semiconductor materials for nanoscale devices, such as nano-generators, gas sensors, highly efficient solar cells, field-emission transistors, ultraviolet photodetectors, and biomedical systems ZnO is attracting considerable attention for its possible application to UV light emitters, spin functional devices, gas sensors, transparent electronics and surface acoustic wave devices [67].

3. MATERIALS AND METHODS

3.1 Chemicals

The following chemicals and solvents were used in this research work. Zinc acetate dihydrate ($Zn(CH_3COO)_2 . 2H_2O$, MW = 219.5 (Made in China)), Magnesium nitrate hexahydrate ($Mg(NO_3)_2 . 6H_2O$, MW= 256.41 g/mol Sodium hydroxide (NaOH, M.W = 39.99971 g/mol (Ranchem Industry & Trading)), Ethanol absolute (99.9%) (Ranchem Industry & Trading), distilled water and plant leaf extract were used. All chemicals were analytical grade and used without further purification.

3.1.2 Apparatuses and Instruments

Whatman filter paper No 1, analytical balance with an accuracy of 0.01, Centrifuge, Magnetic stirrer, Cuvettes, pH meter, hot plate, drying oven, beakers, round bottom flask, mortar and pestle, and measuring cylinder, Ultraviolet-Visible Spectrometer (JENWAY 6705 UV-Vis Spectroscopy), FTIR (Simadzu IR-470 Spectrometer), XRD (XRD-7000) and Cyclic voltammetry (CV) were used for the experiment.

3.2. Methods

3.2.1 Collection of Plant Material

Fresh *M. stenopetala* leaves were collected from South West region of Bench Sheko zone Gurafarda district. The leaves were washed thoroughly with distilled water and allowed to dry in air at room temperature for 5 days.

3.2.2. Preparation of Moringa Stenopetala Leaf Extract

Leaves of *M. stenopetala* were washed thoroughly with distilled water and allowed to dry for five days. Then the dried leaves were crushed using mortar and pestle. Then 10 g of leaves powder was mixed with 100 mL of distilled water and heated for 40 min at 50 ^{O}C. The extract was filtered with Whatman filter paper. The filtrate was then stored in a refrigerator at around 4 ^{O}C for further use.

Procedures for the preparation of *Moringa stenopetala* leaf extract

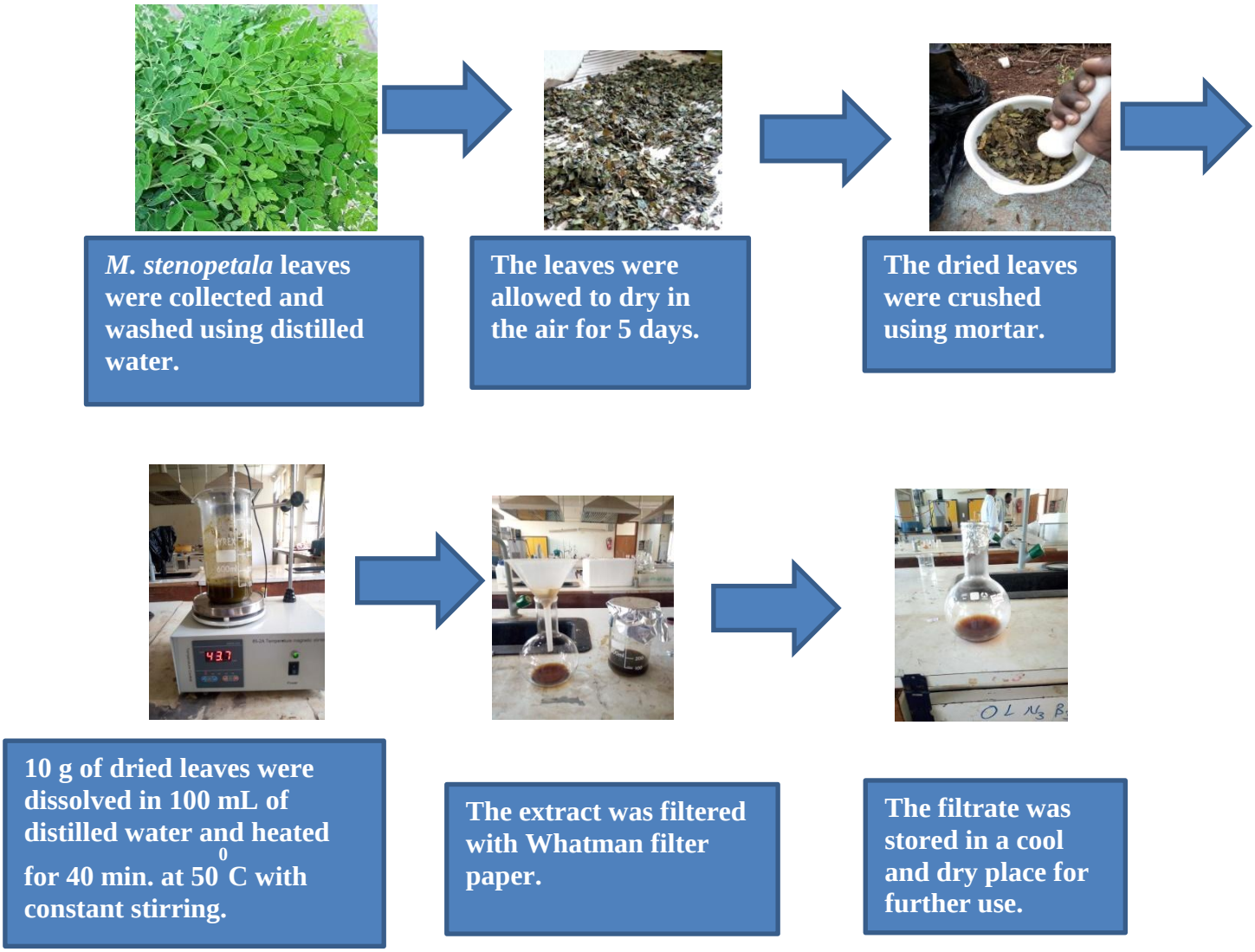


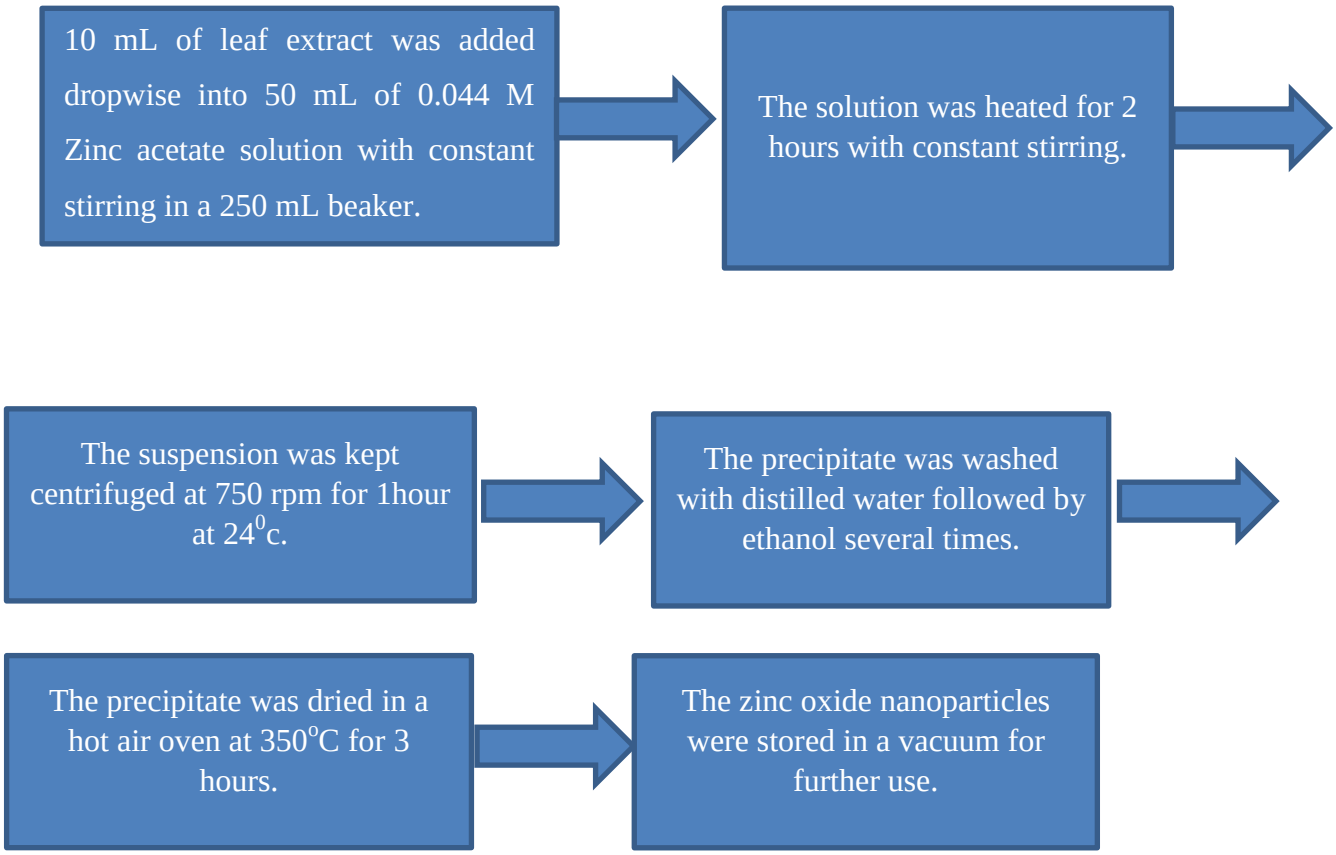
Figure 5. Diagrammatic steps of preparations of *M. stenopetala* leaf extract.

3.2.3. Synthesis of Zinc Oxide Nanoparticles

For the synthesis of zinc oxide nanoparticles different optimizations were conducted using different parameters. 10 mL of *M. stenopetala* leaf extract was mixed with 50 mL of 0.044 M zinc acetate solutions in a beaker. The solution was heated for 2 hours with constant stirring. After the completion of the reaction, the white precipitate was kept centrifuged at 750 rpm for 1hour at 24 ^{o}C temperature.

Finally, the precipitate was washed with distilled water followed by ethanol several times to remove impurities. Then the precipitates were dried in a hot air oven at 350 ^{o}C for 3 hours. Then zinc oxide nanoparticles were collected and stored in a vacuum for further use [68].

Procedures for the Synthesis of undoped Zinc Oxide Nanoparticles



3.2.4. Synthesis of Mg-doped Zinc Oxide Nanoparticles

For the synthesis of Mg-doped ZnO nanoparticles, 1 %, 2 %, and 3 % (Mg $(NO_3)_2$. $6H_2O$ were dissolved in 250 mL distilled water individually. Then 40 mL of magnesium nitrate solution was mixed with 50 mL of 0.044 M zinc acetate solution and 10 mL of leaf extract. The solutions were heated for 2 hours with constant stirring. After the completion of the reaction, the white precipitate was kept centrifuged at 750 rpm for 1hour at 24^{o}C temperature. Finally, the precipitate was washed with distilled water followed by ethanol several times to remove impurities. Then the precipitates were dried in a hot air oven at 350 ^{o}C for 3 hours. Then Mg-doped zinc oxide nanoparticles were collected and stored in a vacuum for further use.

3.3. Optimization of Sample

3.3.1. Optimization of pH

Table 2. Optimization of pH

Volume of precursor	Plant extract	pH	Absorbance
50 mL	10 mL	6	0.169
50 mL	10 mL	8	0.344
50 mL	10 mL	10	0.369

Optimization of pH shown in table 2 was conducted by making the volume of precursor and plant extract constant and having variable values of pH. As a result, a different value of absorbance was obtained. There for, the one that has the best absorbance was a pH of 10.

3.3.2. Optimization of Plant Extract

Table 3. Optimization of plant extract

Precursor solution	Plant extract	pH	Absorbance
50 mL	10 mL	10	0.442
50 mL	15 mL	10	0.404
50 mL	20 mL	10	0.329

Optimization of plant extract shown in table 3 was conducted by making the volume of precursor and pH constant and having a variable volume of plant extract. To experiment 10 mL, 15 mL, and 20 mL of the volume of plant extract were used. At the end of the experiment absorbance of 0.442, 0.404, and 0.329 were obtained for each volume respectively. From the experiment, using 10 mL of plant extract obtained was the best result.

3.3.3. Optimization of Precursor (zinc acetate)

Table 4. Optimization of precursor

Volume of precursor (zinc acetate) solution	Plant extract	pH	Absorbance
30 mL	10 mL	10	0.321
40 mL	10 mL	10	0.333
50 mL	10 mL	10	0.384

Optimization of precursor (zinc acetate solution) shown in table 4 was conducted by making the volume of plant extract and pH constant and having a variable volume of precursor (zinc acetate solution). To conduct the experiment, 30 mL, 40 mL, and 50 mL of volumes of zinc acetate solution were used. At the end of the experiment absorbance of 0.321, 0.333, and 0.384 were obtained for each volume respectively. From the experiment, using 50 mL of plant extract obtained the best result.

3.4. Phytochemical Analysis

Phytochemical analysis of distilled water extracts for the qualitative detection of flavonoids, steroids, phenolics, and tannins was carried out as follows.

3.4.1. Flavonoids test

Base test- for the flavonoids test of the leaf extract, 3 mL of each extract was added to 10 mL of distilled water and the solution was shaken. 1 mL of 10 % NaOH solution was added to the mixture. Yellow color formation indicates the presence of flavonoids.

3.4.2. Alkaloid test

Wagner's test for the alkaloid test of the leaf extract, 1 g of iodine was used with 2 g of potassium iodide solution. The formation of reddish brown color indicates the presence of alkaloids.

3.4.3. Steroids test

Salkowski Test- 5 drops of concentrated H_2SO_4 were added to 1 mL of leaf extract in a test tube. The reddish brown color was formed.

3.4.4. Tannins test

2 mL of each extract in a separate test tube were boiled gently for 2 min and allowed to cool. 3 drop of ferric chloride solution was added to each extract. Finally, a blue-black color was formed. That was taken as evidence of the presence of tannins

3.4.5. Phenolic compound test

Ferric chloride test- for the test of phenolic compounds, a few drops of $FeCl_3$ were added into 5 mL leaf extract and dark brown color was formed which indicates the presence of phenolic compounds in the leaf extract.

3.5. Characterization

ZnO and Mg-doped ZnO NPs formation and characterization were studied by Ultraviolet-Visible Spectrometer, FTIR, XRD (X-ray diffraction), and cyclic voltammetry (CV).

3.5.1. UV-Vis Spectroscopy Analysis

The optical characterization of the synthesized ZnO nanoparticles and Mg-doped zinc oxide nanoparticles was carried out using UV-Vis Spectroscopy in the wavelength region of 300–600 nm for Zinc oxide nanoparticles and 300-500 nm for Mg-doped zinc oxide nanoparticles. This spectrophotometer was used to analyze the unique optical properties and optical band gap which depends on the size and the shape of the nanoparticles.

3.5.2. FTIR Spectroscopy Analysis

The functional groups present in the synthesized ZnO and Mg-doped zinc oxide nanoparticles were studied by using Fourier Transform Infrared spectroscopy. FTIR gives information on the vibrational and rotational modes of motion of a molecule and is hence an important technique for the identification and characterization of a substance. The vibrational data of ZnO NPs were characterized by FTIR spectroscopy in the wave number range of 500-4000 cm^{-1}.

3.5.3. X-Ray Diffraction (XRD) Analysis

The crystalline structures of the undoped and Mg-doped ZnO NPs were investigated by X-ray diffraction. The XRD spectrum was recorded from 10^0 to 80^0 with 2θ angles using CuKα (λ =1.54 Å) radiation operated at 40KV and 30mA.

Debye Scherrer's equation was used in this study.

$$CS = \frac{0.94\lambda}{\beta\cos\theta}$$

Where CS is the crystalline size constant [K] =0.94.

β is the full width at half maximum [FWHM].

λ= X-ray wavelength, =1.540x10-10m.

Cosθ= Bragg angle.

3.5.4. Cyclic Voltammetry (CV) Analysis

Cyclic voltammetry is one of the most versatile electrochemical techniques for the study of the redox behavior of electroactive species. Electrochemical analysis of ZnO nanoparticles using cyclic voltammetry was conducted over a potential range of -1.5 volt to +1.5 volt.

3.5.5. Scanning Electron Microscope (SEM) Analysis

For the study of crystal morphology size observation, SEM was used. The morphology of zinc oxide and 3% magnesium-doped ZnO nanoparticles was studied using a scanning electron microscope.

3.5.6. Evaluation of Antimicrobial Activities

The antibacterial and antifungal activity of undoped and Mg-doped ZnO NPs were tested against gram-positive bacteria (*S. aureus B. cereus,)*, and gram-negative bacteria (*E. coli, S. typhus,)* and fungus(C. albicas) using agar plate disc diffusion method. The bacterial and fungi strains were obtained from the Microbiology laboratory, Biology Department at Jimma University. The test organisms were first grown on agar plates at 37^0C for 24 h before inoculation onto the nutrient agar. Gentamicin and Clotromazole were used as a positive control and DMSO was used as a negative control. Then the antibacterial and antifungal tests were performed using plant extract, ZnO nanoparticle, 1% Mg-doped ZnO nanoparticle, 2 % Mg-doped ZnO nanoparticle, and 3% Mg-doped ZnO nanoparticle, and placed on the plate and incubated at 37 ^{0}C for 24 h. At the end of the incubation period, the plates were examined for evidence of a zone of inhibition which appeared as a clear area around the wells and was measured in mm units.

4. RESULTS AND DISCUSSION

4.1. Phytochemical Analysis

Phytochemical tests conducted using distilled water as a solvent for the qualitative detection of flavonoids, steroids, phenolics, and tannins were discussed using the table below.

Table 5. Phytochemical analysis

Solvent used	Flavonoid	steroid	Alkaloid	Tannins	Phenolic compound
Distilled water	+	-	+	+	+

The presence and absence of useful bioactive substances such as flavonoids, steroids tannins, and phenolic compounds in leaf extracts of *M. stenopetala were* revealed by the confirmatory test, involving color changes. Based on the results described in the above table, the naturally existing phytochemicals in the leaf extract were used as a reducing and capping agent. Therefore the study confirmed the presence of secondary metabolites such as flavonoids, tannins, and phenolic compounds and the absence of steroids.

4.2. UV-Vis Spectroscopic Analysis

The UV-Vis spectra analysis of undoped zinc oxide nanoparticles synthesized at pH of 6, 8, and 10 were studied. Absorption spectra of ZnO nanoparticles at pH 6, 8, and 10 were 371 nm, 377 nm, and 381 nm respectively. The best peak at a wavelength of 381 nm indicates that ZnO NPs display excitation absorption (at 381 nm) due to their large excitation binding energy at room temperature. The sharp bands of zinc colloids were observed at 381 nm, which proves that the zinc ion is efficiently reduced by the Moringa Stenopetala leaf extract. The absorption peak confirms the occurrence of blue-shifted absorption spectrum for the bulk value (388 nm) of the ZnO NPs, due to the quantum confinement effect, which is good agreement with the previous report [68].

The UV-Vis spectra analysis of Mg-doped zinc oxide nanoparticles was shown in figure 6. The peak absorption spectrum of 1 %, 2 %, and 3 % Mg-doped ZnO NPs for the sample was 385 nm, 388 nm, and 389 nm respectively. It indicates that the 3 % Mg-doped zinc oxide nanoparticle has a sharp absorption peak at 389 nm and decreased the band gap.

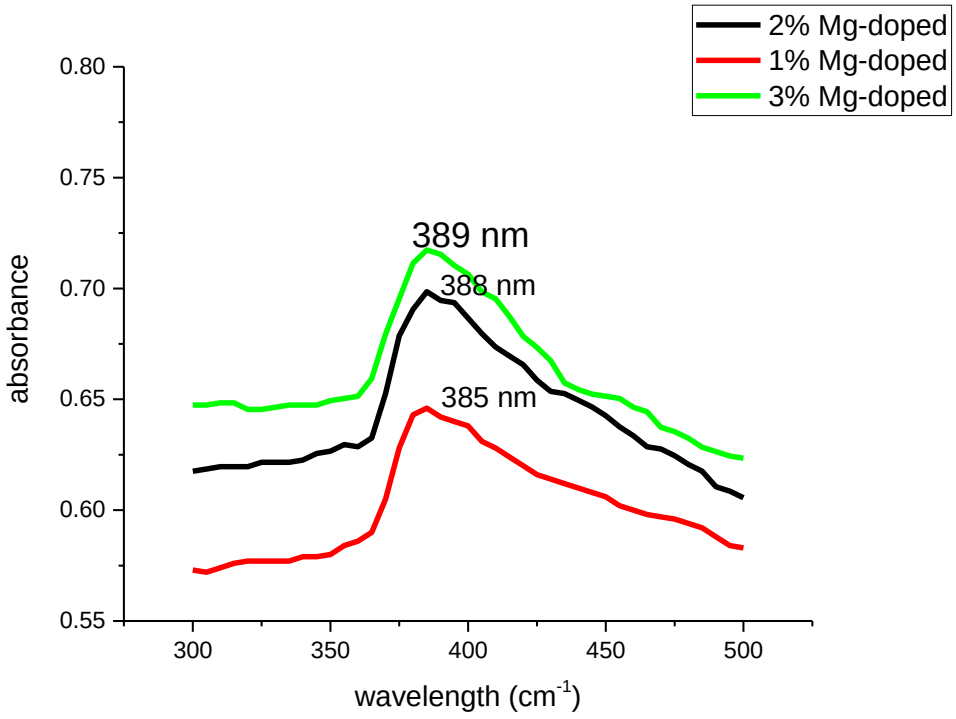


Figure 6. UV-Vis analysis of 1 %, 2 %, and 3 % Mg-doped ZnO nanoparticles

4.3. Fourier Transform Infrared Spectroscopic Analysis

The functional groups present in ZnO and Mg-doped zinc oxide nanoparticles were studied by using Fourier Transform Infrared (FTIR) spectroscopy in the wave number range of 500-4000 cm^{-1}.

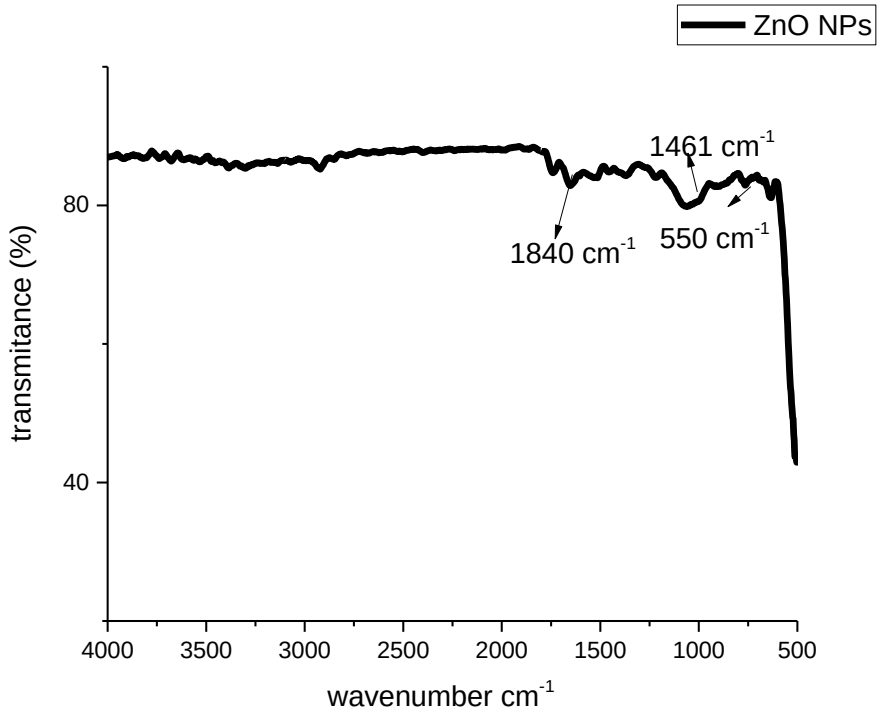


Figure 7. The FTIR spectrum analysis for ZnO NPs.

The FTIR spectrum for ZnO NPs studied in figure 7 confirmed that the peak at 1461 cm-1 results from the stretching bands of C=O functional groups. The peak at 1840 cm^{-1} indicates C=C groups and the region between 550 and 600 cm^{-1} is assigned for the metal-oxygen bond (Zn-O).

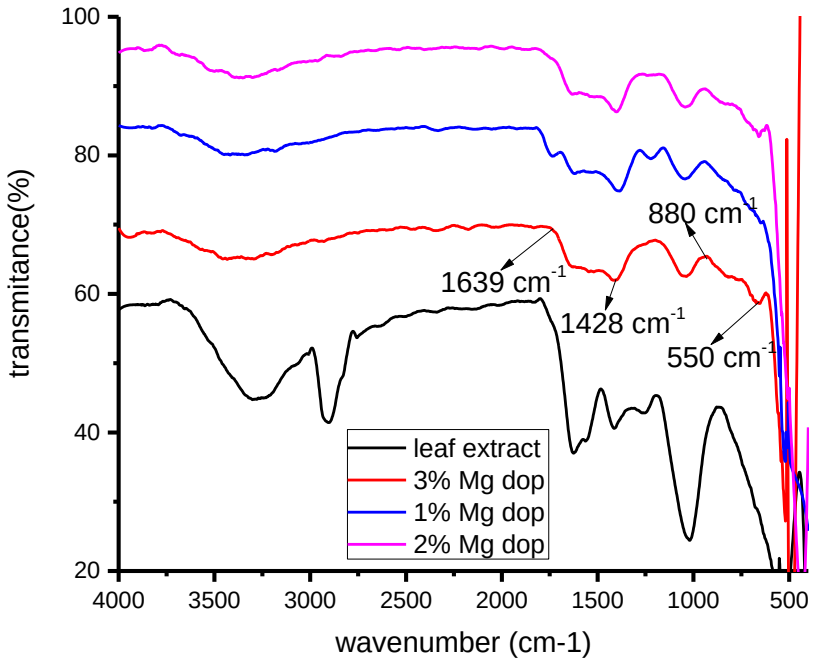


Figure 8. The FTIR spectrum for 1 %, 2 % and 3 % Mg-doped ZnO NPs and leaf extract.

The FTIR spectrum for Mg-doped ZnO nanoparticles was shown in figure 8. From the spectrum of 3% Mg-doped ZnO nanoparticles, the broad absorption in the frequency band around 550 cm^{-1} is due to Zn-O stretching and weak metal-oxygen bond at 880 cm^{-1}. The peak at 1428 cm^{-1} indicates that C=O stretching. There is C=C stretching at 1639 cm^{-1} [69].

4.4. X-Ray Diffraction (XRD) Analysis

Figure 10 and figure 11 show the XRD patterns of ZnO NPs and 1%, 2%, and 3% Mg-doped ZnO NPs synthesized from zinc acetate and magnesium nitrate precursors using *M. stenopetala* leaf extract.

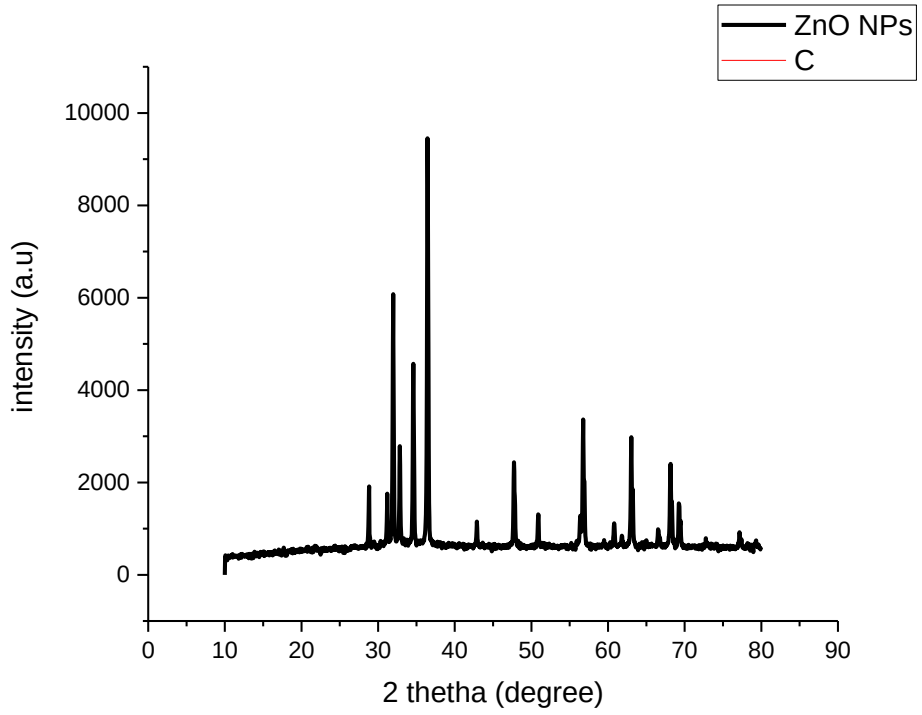


Figure 9. The XRD study of ZnO NPs.

The XRD peaks are located at angles (2θ) of 31.82, 34.81, 34.74, and 36.18 corresponding to (100), (002), (102), and (101) planes of the ZnO NPs. There is no impurity phase observed for ZnO NPs. The standard diffraction peaks show the hexagonal wurtzite structure of ZnO NPs. The calculated particle size for ZnO was 27.72 according to Debye-Scherrer's formula. The mean crystallite size was calculated by using the Scherrer formula

$$CS = \frac{0.94\lambda}{\beta\cos\theta}$$

Where λ is the wavelength of the radiation (1.54056 Å), β is the full width at half maximum intensity, and θ is the diffraction angle. From the calculated values, it is observed that the mean crystallite size increases with an increase in Mg doping concentration [70].

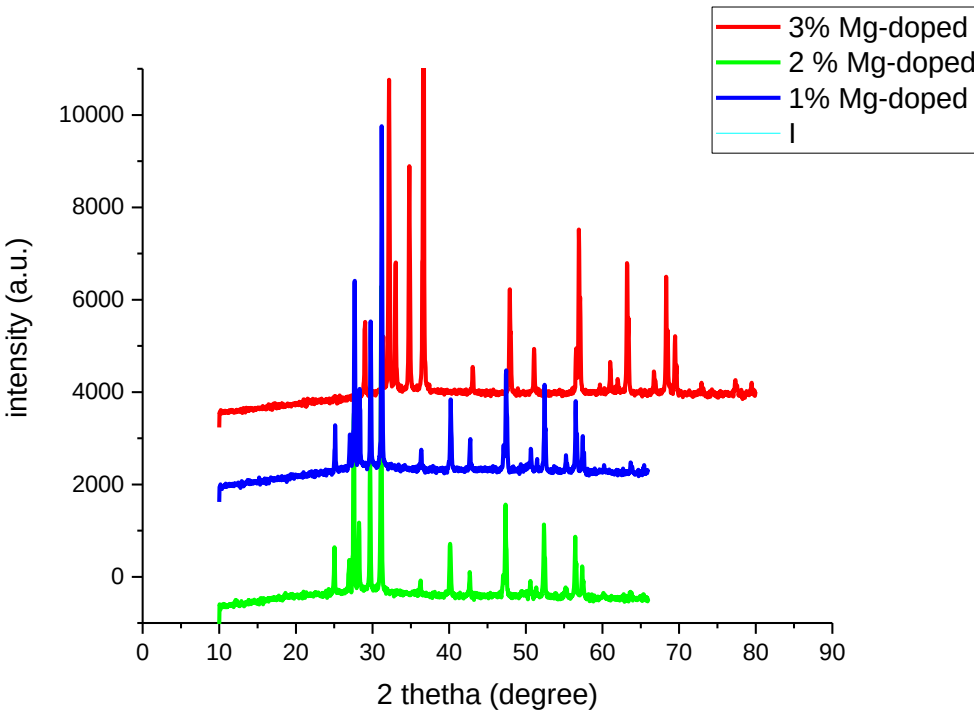


Figure 10. The XRD study of 1%, 2 %, and 3 % Mg-doped ZnO NPs.

The XRD peaks located at angles (2θ) of (31.47, 31.69, 32.78 and 34.54 are corresponding to (101), (102), (103), and (100) planes of Mg-doped ZnO NPs. There is an impurity phase observed for 3 % Mg-doped ZnO NPs. Due to Mg ions doping inside the periodic crystal lattice of ZnO, a small amount of strain is persuaded. This results in the swap of the lattice which consecutively leads to a change in the regularity of the crystal. However, very careful inferences indicate that the peak position shifts towards the lower angle values which may be due to the higher doping concentration of Mg. It is well reported in the literature that the lattice characteristics of the host materials get changed due to the incorporation of dopant materials. This happens due to their variance in the atomic radii. Furthermore, the dopant ions may replace Zn^{2+}. Thus the basic structure of ZnO NPs is unaltered and they retain their original wurtzite structure [71].

4.5. Cyclic Voltammetry (CV) Analysis

Cyclic voltammetry study of redox behavior of ZnO nanoparticles was indicated in figure 11. Electrochemical analysis of ZnO nanoparticles using cyclic voltammetry was conducted over a potential range of -1.5 volt to +1.5 volt. A silver/silver chloride (Ag/AgCl) electrode was used as the reference electrode and platinum wire as the counter electrode. Ferrocyanide was the analyte used. The CV analysis shown in figure 12 indicates that ZnO nanoparticles show an oxidation peak at +0.4 volt and a reduction peak at -0.1 volt. The result indicates that ZnO nanoparticles exhibited good electrolytic activity and can be used as a potential electrode material for further electronic applications.

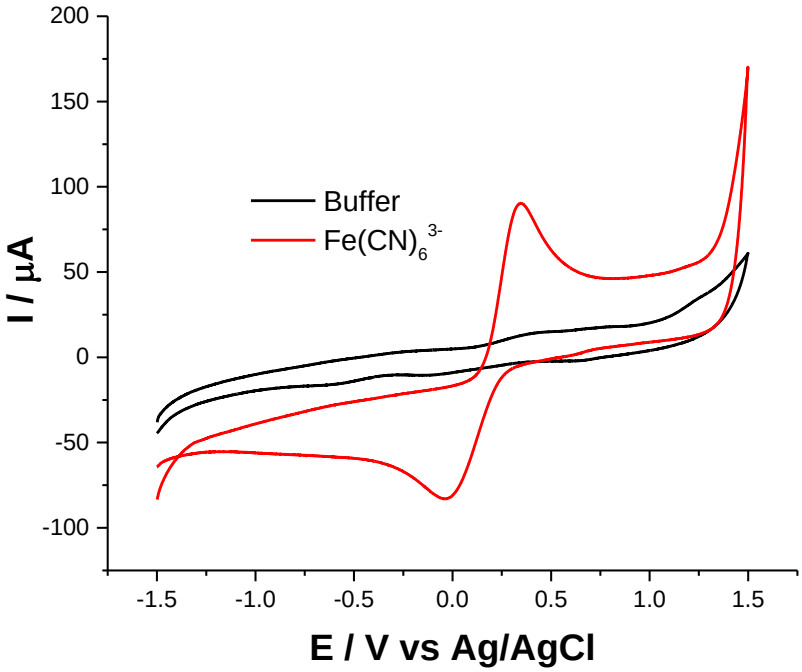


Figure 11. The Cyclic Voltammetry analysis of ZnO NPs.

4.6. Scanning Electron Microscopic (SEM) Analysis

For crystal morphology size observation for all the synthesized samples, SEM was used. The SEM images of zinc oxide and 3% magnesium-doped zinc oxide nanoparticles are shown in Fig. 12 below.

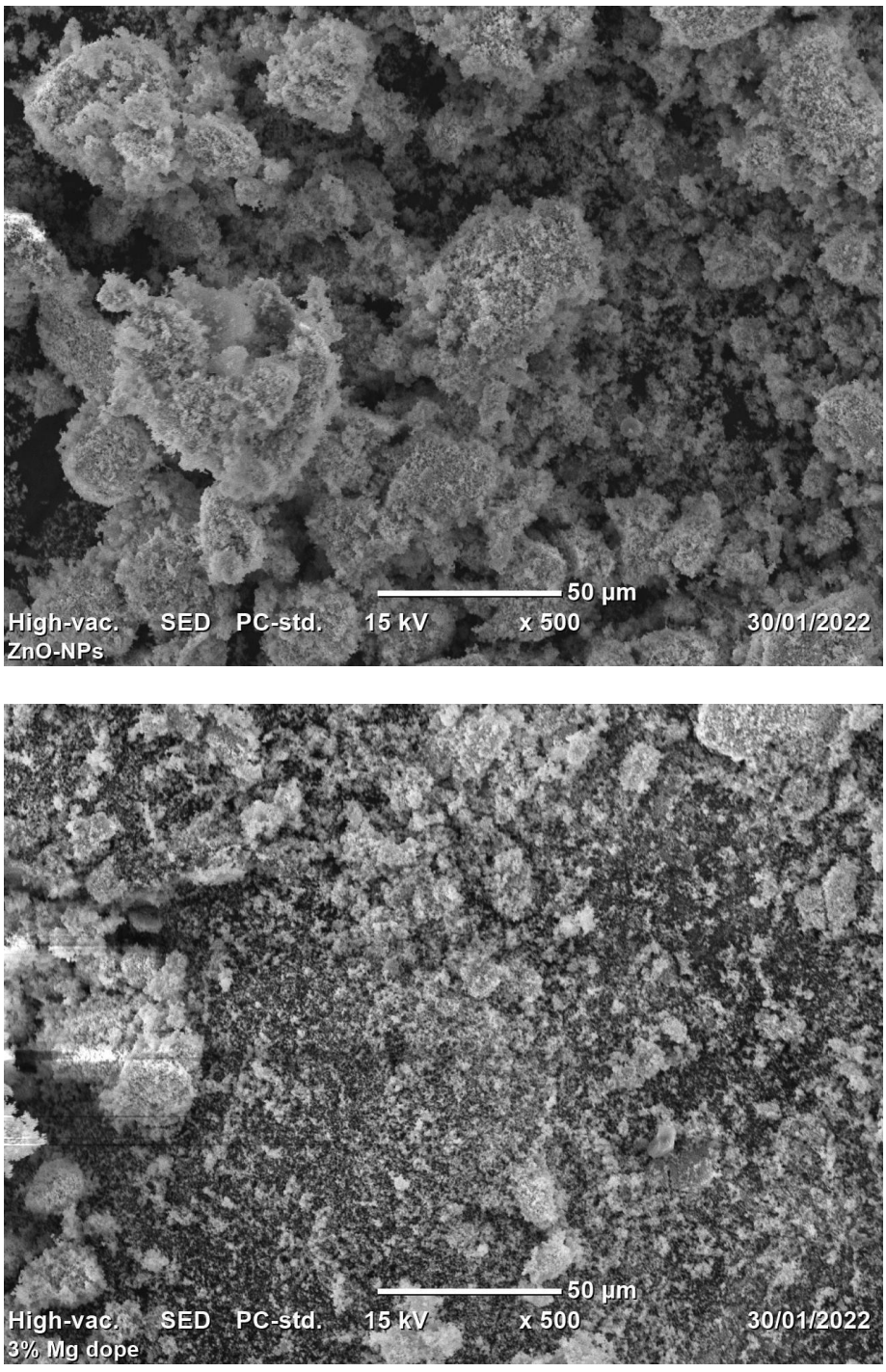


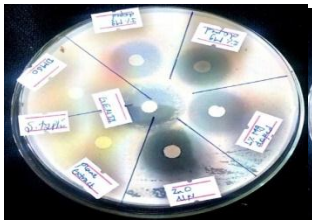
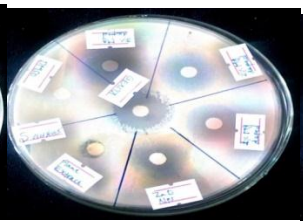
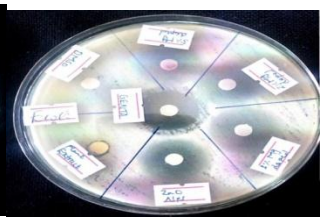
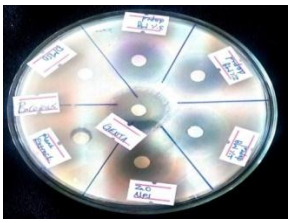
Figure 12. The SEM image of ZnO and Mg-doped ZnO nanoparticles

These images indicate that the shape and morphology of zinc oxide and 3 % magnesium-doped zinc oxide nanoparticles changes with increasing Mg concentration. The formed ZnO nanoparticles by using extract have dominantly spherical and circularly defined surface morphology as seen in the figure above. The influence of Mg-doping on the ZnO particle's homogeneity and morphology is very much in-line with the findings of the previous report assigned by speculating that this could be due to the increasing substitution of different Mg ions with Zn sites [72]. The image for ZnO shows the grown nanoparticles with relatively uniform size distribution with somehow larger size (~50 nm). This could be an indication of the formation of secondary particles through the aggregation of the primary particles. In the 3% Mg-doped ZnO samples, the particles seem to be more and more agglomerated with an increase in Mg content.

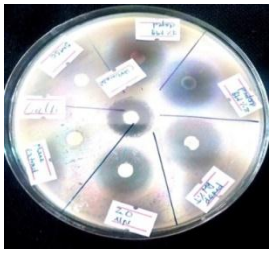
The result obtained from SEM also indicates that ZnO and Mg-doped ZnO NPs have been successfully prepared using zinc acetate and magnesium nitrate precursors within a nano range. The SEM images clearly show microstructural homogeneities and remarkably different morphology for ZnO powder [72].

4.7. Evaluation of Antimicrobial Activity

The antibacterial and antifungal activity of undoped and Mg-doped ZnO NPs and leaf extract were tested against gram-positive bacteria (*S. aureus B. cereus,)*, and gram-negative bacteria (*E. coli, S. typhus,)* and fungus (C. albicas) using agar plate disc diffusion method**.** The results are shown in Table 4, clearly representing the excellent anti-bacterial and antifungal activity of undoped and Mg-doped ZnO nanoparticles against gram-positive and gram-negative bacteria and fungus. The experimental outcomes undeniably suggest an effective growth inhibitory activity of the nanoparticles upon both the microorganisms and strong activity on gram-positive bacteria (*S. aureus B. cereus,)*, gram-negative bacteria (*E. coli, S. typhus,)* and fungus(C. albicas). To conduct antibacterial and antifungal activities of leaf extract, ZnO and 1 %, 2 %, and 3 % Mg-doped ZnO NPs 250 mg/L of concentration were used.

S. typhus *S. aureus* *E. coli* *B. cereus*



C. albicas

Figure 13. Antimicrobial Activities of leaf extract, ZnO NPs. 1 %, 2 % and 3 % Mg-doped ZnO NPs.

Table 6: Diameters of inhibition zone in mm of leaf extract, ZnO NPs, Mg-doped ZnO NPs against bacterial and fungi species.

Organisms	Zone of inhibition in mm							
	Gentamycin	Clotromazole	DMSO	Leaf extract	ZnO NPs	1% Mg-doped	2% Mg-doped	3% Mg-doped
S. aureus	26 mm	-	-	10 mm	21	24 mm	25 mm	26 mm
B. cereus	23 mm	-	-	15 mm	22	24 mm	24 mm	25mm
E. coli	27 mm	-	-	12 mm	23	26 mm	28 mm	29 mm
S. typhus	27 mm	-	-	13 mm	23	26 mm	26 mm	30 mm
C. albicas	-	26 mm	-	10 mm	22	26 mm	26 mm	29 mm

From the above table, Mg-doped ZnO nanoparticles seemed to be more efficient for gram-negative bacteria. It was found that the antibacterial activity of Mg-doped ZnO nanoparticles increased with increasing powder concentration. Furthermore, due to the various surface-

interface characteristics may have different chemical-physical, adsorption-desorption abilities in the direction of bacteria, making sure of different antimicrobial performances.

The interaction between the NPs and the cell wall of bacteria was changed due to the doping of Mg. The growth of Gram-negative bacteria was more commendably affected by Mg^{2+}-doped ZnO nanostructures compared with ZnO NPs. From the Table above, it is noted that Gram-negative and Gram-positive have different inhibition zones. This difference in the antimicrobial activity of Mg-doped ZnO nanostructures against Gram-negative and Gram-positive bacterial strains may be due to the difference in the cell wall structure of those respective bacteria. It was also reported earlier that various bacterial strains had considerably different infectivity and tolerance levels towards the different agents including antibiotics. Also, differences in the antibacterial activity might be due to different particle dissolution [73].

5. CONCLUSION

In this research ZnO and Mg-doped zinc oxide nanoparticles were synthesized by using *M. stenopetala* leaf extract. The XRD study confirms the formation of hexagonal wurtzite structure of the synthesized ZnO and Mg-doped ZnO nanoparticles. The Mg-doped ZnO NPs particle sizes were decreased as compared to pure ZnO NPs. This size reduction was mainly due to the distortion in the host ZnO lattice by foreign impurities like Mg^{2+} ions. From the UV-Vis study, the sharp bands of zinc colloids were observed at 381 nm, which proves that the zinc ion is efficiently reduced by the *Moringa stenopetala* leaf extract. The absorption peak confirms the occurrence of the blue shift. This blue shift may occur from different origins, such as an electron, lattice distortion, localization of charge carriers due to interface effects, and point defects. The vibrational stretching around 550 cm^{-1} (Zn-O) also supports the formation of ZnO nanoparticles. The cyclic voltammetry study confirmed that ZnO nanoparticles exhibited excellent oxidation-reduction behavior and can be used as a potential electrode material for further electronic applications. The result obtained from SEM also indicates that ZnO and Mg-doped ZnO NPs have been successfully prepared using zinc acetate and magnesium nitrate precursors within a nanorange. The synthesized ZnO nanoparticles and Mg-doped ZnO nanoparticles have a significantly efficient antimicrobial effect on gram-positive bacteria (*S. aureus B. cereus,)*, gram-negative bacteria (*E. coli, S. typhus,)* and fungus (C. albicas). The highest zone of inhibition was observed for 3% Mg-doped ZnO NPs for gram-negative bacteria and a medium zone of inhibition was observed for 2 % Mg-doped ZnO NPs. The difference in the zone of inhibition of the antimicrobial activity of Mg-doped ZnO nanostructures against Gram-negative and Gram-positive bacterial strains may be due to the difference in the cell wall structure of those bacteria.

6. RECOMMENDATION

- Attention should be given by the government, NGOs, researchers, etc. to the systemic evaluation of medicinal plants to develop safe, effective, affordable, and accessible products since the majority of the population in Ethiopia depends on medicinal plants.
- Further study on the characterization of zinc oxide nanoparticles and Mg-doped zinc oxide nanoparticles using other medicinal extracts and investigation of antimicrobial activities should be done.

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