GREEN METHODS OF SYNTHESISING ZINC OXIDE NANOPARTICLES USING MORINGA OLEIFERA LEAF EXTRACT FOR THE APPLICATION OF ANTIMICROBIAL ACTIVITIES

BY: ALEMAYEHU YADETE

A THESIS SUBMITTED TO: THE APPLIED PHYSICS PROGRAM

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Approval board of the examiner

We, the undersigned, members of the Board of Examiners of the final open defense by Alemayehu Yadete have read and evaluated his thesis entitled "**GREEN METHODS OF SYNTHESISING ZINC OXIDE NANOPARTICS USING MORINGA OLEIFERA LEAF EXTRACT FOR THE APPLICATION OF ANTIMICROBIAL ACTIVITIES**" and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirements of the Degree of Masters of science in Physics.

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Declaration

I hereby declare that this MSc.thesis is my original work and has not been presented for a degree in any other university, and all sources of material used for this thesis have been duly acknowledged.

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Advisor's approval sheet

To: Applied Physics Program

Subject: Thesis Submission

This is to certify that the thesis entitled "**GREEN METHODS OF SYNTHESISING ZINC OXIDE NANOPARTIClLES USING MORINGA OLEIFERA LEAF EXTRACT FOR THE APPLICATION OF ANTIMICROBIAL ACTIVITIES**" submitted in partial fulfillment of the requirements for the degree of Master of Science in Physics, the Graduate Applied Physics program, and has been carried out by Alemayehu Yadete ID.No GSU/0457/06, under our supervision. Therefore, we recommend that the student has fulfilled the requirements and hence hereby he can submit the thesis to the Applied Physics program.

Name of major Advisor	Signature	Date
Name of Co-Advisor	Signature	Date

Dedication

This thesis manuscript is dedicated to my beloved wife Alganesh Kassa for her concern, love, patience and affection.

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List of Abbreviations

B.subtilis	Bacillus subtilis
C.albicans	Candida albicans
E.coli	Escherichia coli
FTIR	Fourier Transform Infrared
FWHM	Full width at half-maximum
IR	Infra-red
NIR	Near Infra- red
P.aeruginosa	Pseudomonas aeruginosa
ROS	Reactive oxygen species
S.aureus	Staphylococcus aureus
Thyphus	Typhus folia
SEM	Scanning electron microscopy
UV/Vis	Ultraviolet visible
XRD	X-ray diffraction
ZnO NPs	Zinc oxide nanoparticles
ZOI	Zone of inhibition

Abstract

In this study, the ZnO NP_S were synthesised by dissolving 2 g and 4 g of zinc nitrate hexa-hydrate into 20 mL and 50 mL of extracted solution of moringa oleifera leaf powder by using Green synthesis method calcined at 400 ^{0}c and 500 ^{0}c respectively for antimicrobial applications. The average crystallite size of the ZnO Nps synthesized by dissolving 2 g of zinc nitrate hexa-hydrate into 20 mL 0f extracted solution of moringa oleifera leaf powder and 4 g of zinc nitrate hexa-hydrate by dissolving into 50 mL of extracted solution of moringa oleifera leaf powder determined by XRD were about 8.82 nm and 13.724 nm respectively. Additionally the ZnO NP_S synthesized by dissolving each of 1 g and 3 g of zinc nitrate hexa-hydrate into 50 mL of extracted solution of moringa oleifera leaf powder calcined at a temperature of 500 ℃.Their crystal sizes were about 11.57 nm and 12.62 nm respectively. The lattice parameters,a = 0.287 nm and c = 0.497 nm,ratio of c / a =1.731. The UV-Vis absorption peak of the ZnO NPs was observed at 364.2 nm and 370 nm for ZnO Nps synthesized by dissolving 2 g of zinc nitrate hexa-hydrate into 20 mL 0f extracted solution of moringa oleifera leaf powder and 4 g of zinc nitrate hexa-hydrate by dissolving into 50 mL of extracted solution of moringa oleifera leaf powder respectively. The band gap energy of samples M_1 and M_2 are 3.4 eV and 3.35 eV respectively. The vibrational phonon of ZnO NP_S observed in the region of 433 cm^{-1}-510 cm^{-1}confirms the successful production of ZnO nanoparticles. The excitation wavelength of the ZnO NP_S were synthesized by dissolving 2 g of zinc nitrate hexa-hydrate into 20 mL 0f extracted solution of moringa oleifera leaf powder and 4 g of zinc nitrate hexa-hydrate bydissolving into 50 mL of extracted solution of moringa oleifera leaf powder performed at 275 nm, 355 nm and respectively.The ZnO NP_S having particle sizes of 8.82 nm and 13.724 nm were showed a larger inhibition zone on gram positive bacterial such as S.aureus and B.subtilis and a smaller inhibition zone on gram negative bacterial such as E.coli,P.aeurginosa,Typhus and C.albicans fungus. Among the two ZnO NPs, the ZnO NP_s having particle size 8.882 nm showed an excellent antimicrobial activity on gram positive bacterial than gram negative bacterial and C.albicans fungus.

Key words: Nanoscience, zinc oxide nanoparticles, spectroscopy, and well diffusion agar method

1. Introduction

1.1 Background of the Study

Nanoscience is a branch of science which deals about nanomaterials that displays significant properties that are not available in bulk materials and exhibit vast applications due to their small size [1].

Nanoparticles are the class of materials and remarkable scientific tools that has been used for various biotechnological, pharmacological and technological uses. They are cornerstones of nanoscience and nanotechnology. Nanoparticles are different from bulk materials due to their small size, higher surface area to volume ratio, crystallographic surface structure, specific magnetic properties and new quantum effects. The area of Nanotechnology is one of the most dynamic fields in advanced material science [2]. Nanomaterials significantly affect the areas of physics, chemical science and biomedical science [3].

The metal oxide nanoparticles are most commonly used in catalysis, sensors, biomedicine and antimicrobial activity, etc.

Zinc oxide nanoparticles has unique physical and chemical properties, such as high chemical stability, high electrochemical coupling coefficient, broad range of radiation absorption and high photo stability[4].It has tremendous scientific and technological interest due to direct wide band gap energy (3.37eV),large exciton-binding energy (60meV) and high thermal and mechanical stability at room temperature. Due to this property it is attractive for potential use in electronics, optoelectronics and laser technology. The piezoelectric properties of ZnO mean that it can be used as sensor, energy generator and photo catalyst in hydrogen

production [5]. Because of its hardness, rigidity and piezoelectric constant it is an important material in the ceramic industry, while its low toxicity, biocompatibility and biodegradability make it a material of interest for biomedicine and in pro-ecological systems [6].

The discovery of new application areas may further increase the production volumes, and lead to a proliferation of ZnO-NPs in the environment in the near future. Currently, there is no evidence to suggest that humans are adversely affected by ZnO-NPs through their use in consumer products. However, ZnO NPs are known to partially dissolve in water, hence ZnO-NP-containing products are likely to release both dissolved zinc and ZnO-NP into the environment which are likely to persist and bioaccumulation. Zinc oxide (ZnO) among nanosized metal oxides has been extensively used due to its antimicrobial and antitumor activities.

ZnO is generally included in cosmetic lotions as it is known to possess UV absorbing and blocking properties.The fluorescent ZnO nanocrystals have become a biofriendly candidate for biological technology applications. ZnO nanoparticles have considerable attention to their unique antibacterial, antifungal, UV filtering properties, high catalytic and photochemical activity [7]. Most ZnO nanoparticles are produced green synthetically have advantage of low cost and white appearance over the silver nanoparticles [8]

Moringa oleifera belongs to the single genus of family moringaceae. It is small fast – growing ornamental tree widespread over the tropical regions of Africa and Asia [9]. Moringa leaf have been reported to be a rich source of carotene, protein, Vitamin C, calcium and potassium and act as a good source of natural antioxidants; and thus enhance the shelf-life of fact containing foods due to the presence of various types of antioxidant compounds

such as ascorbic acid, flavonoids, phenolics and carotenoids. [10].

The various parts of the world M. oliefera used in medicine because it is rich in amino acids, k, Ca, Fe, ascorbate, and growth regulating hormones kezeatin which will promotes cell division, cell elongation and antioxidant properties [11].

The antibacterial properties of ZnO NPs were investigated and developed as antibacterial agent against wide range of micro organisms to control and prevent the spreading and persistence of bacterial infections [12]. Antibacterial activity of ZnO tested and the effect was more pronounced with the Gram- positive than the Gram-negative bacteria and also of NPs exhibited a preferential ability to kill cancerous HL60 cells as compared with normal peripheral blood mononuclear cells [13]. The most likely mechanism of antibacterial activity of ZnO NPs is attributable to photochemical property of ZnO NPs. The photo induced charge carriers (electrons and holes) interact with oxygen and H2O NPs to produce reactive oxygen species (ROS) like, singlet oxygen, hydroxyl radical, etc [14,15,16]. These reactive oxygen species might trigger membrane lipid peroxidation and cause antibacterial effect [17].

In addition, dissolution of zinc ions from ZnO NPs as Zn^{2+} or as complex hydroxide anions of Zn in the culture medium has been attributed to enhanced antibacterial activity of ZnO NPs [18, 19].

Many researchers have been synthesised and characterized zinc oxide nanoparticles, and studied antibacterial activities depend on size, concentration, dimensions and morphology of nanoparticles [20].

The antimicrobial activity of zinc oxide nanoparticles have been studied against Gram-negative bacteria such as *Pseudomonas aeruginosa, campylobacter jejuni, Escherichia coli* and Gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus.* They have been reported that Gram-positive bacteria have higher susceptibility to zinc oxide nanoparticles than Gram-negative bacteria due to their polarity difference of cell membrane, structural difference in bacterial membrane and the difference in intracellular antioxidant content [18].

On the other hand, as far as our knowledge concerns there is no research reports on synthesis of ZnO NPs using moringa leaf extracted solution for the antimicrobial application of the specific microbial such as *S.aureus, E.coli, P.aeruginosa, B.subtilis, Thyphus and C. albican*. Therefore, the general objective of this study is green methods of synthesizing ZnO nanoparticles moringa leaf extracted solution for the application of antimicrobial activities.

The specific objectives of this research were:

1. Synthesis ZnO nanoparticles using moringa oleifera leaf,
2. Characterize ZnO nanoparticles by XRD, SEM., FTIR, UV-Vis spectroscopy and fluorescence spectroscopy.
3.Apply ZnO nanoparticles for antimicrobial activities such as *S.aureus, E.coli, Bacillus, Pseudo and S.thuyphus* and *Candida*.

The thesis is organized as follows: In chapter1, introduction to nanoscience and nanotechnology, nanoparticles, metal oxide NPs and zinc oxide, general synthesis routes of ZnO nanoparticles, the applications of ZnO NPs and objectives of the study are presented.

In chapter 2, literature reviews related to ZnO NPs are presented. The ZnO NPs, the crystal structures, chemical and optoelectronic properties of ZnO and the synthesis routes of ZnO NPs are discussed. Furthermore, the characterization techniques (XRD, SEM, UV-Vis Spectroscopy, Fluorescence Spectroscopy and FTIR Spectroscopy) of ZnO nanoparticles, application of ZnO NPs for antimicrobial activities and antifungal activities are discussed.

In chapter 3, the materials and methods of the study are presented. This chapter has six sections; in the first and the second sections of this chapter, the various chemicals, solvents and apparatus used to carry out this research work are described respectively. In the third, fourth and fifth sections, the methods of synthesis and characterization are presented respectively. In section six, the antimicrobial and antifungal activity procedures are presented.

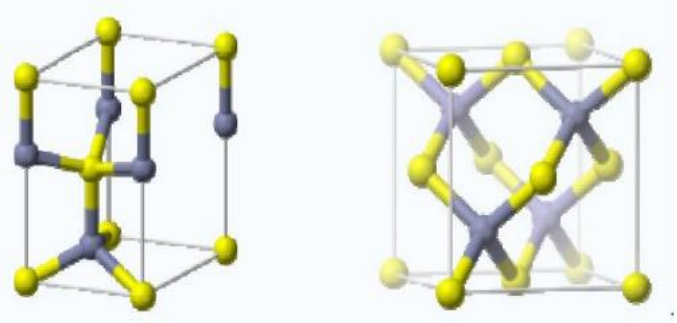
In chapter 4, the results and discussions of the thesis are presented. The average crystallite size, SEM images of ZnO NPs is reported. Moreover, the UV-Vis absorption peak, emission spectra and FTIR spectra of ZnO NPs are reported. In the final section of this chapter, antimicrobial activities of ZnO NPs are reported. Finally, the conclusion of the thesis is drawn in chapter 5.

Chapter 2: Literature review

2.1 Background of Zinc Oxide Nanoparticles

ZnO NPs have more toxic effects on bacteria and fungi due to some factors like light Intensity, surface chemistry and particle morphology. However, they have minimal toxic effect on human cells [24].

Zinc oxide has three crystal structures such as hexagonal wurtzite, cubic zinc blend cubic rock salt. Among these, wurtzite crystalline structure is the most stable phase at ambient conditions. In this structure every zinc atom is tetrahedrally coordinated with four oxygen atoms. It has lattice parameters, a = 0.32495 nm and c = 0.52069 nm, ratio of c / a =1.6023, which is similar to the ideal value of hexagonal cell *c/a* = 1.633. It belongs to the space group of $P6_3mc$ and is characterized by two interconnecting sub lattices of Zn^{2+} andO^{2-}. The cubic rock salt is observed only at relatively high pressures ~10 GPa. The tetrahedral symmetry of wurtzite structure makes an imperative role for the polarity of ZnO that arises along the hexagonal axis. The polar symmetry of ZnO along the hexagonal axis results piezoelectricity, pyroelectricity and spontaneous polarization. Since ZnO has sensitive lattice constants to the presence of structural point defects (oxygen vacancies and interstitial Zn ions act as donors in its lattice) and extended defects (threading/planar dislocations), it has an n-type nature. These structural point and extended defects are commonly found in ZnO resulting in a non-stoichiometric compound $Zn_{1+d}O$ with excess zinc. They are responsible for visible photoluminescence [25, 26, and 27].



Hexagonal Wurtz structure Cubic zinc blend structure

Figure 2.1: crystal structures of ZnO.

Pure ZnO is occurs as a white powder usually called as white zincite or mineral zincite. Because crystalline ZnO is thermo-chromic, its color is changing from white to yellow when it is heated in air. It also reverts to white on cooling due to a very small loss of oxygen at high temperature to form the non-stoichiometric $Zn_{1+x}O$. Since zinc oxide is an amphotericOxide, it is insoluble in water and alcohol, but in most acids like hydrochloric acid and in alkalis. ZnO forms cement like material which are used in dentistry when reacted with phosphoric acid and a strong aqueous solution of zinc chloride. It produce carboxylates like asoleate or stearate when react with fatty acids in oils [28, 29]

ZnO shows a better optoelectronic property because of its large exciton binding energy (60 meV) which could be attended at and above room temperature due to excitonic recombination. The optical properties of ZnO are heavily influenced by the energy band structure and lattice dynamics. The processes of optical absorption and emission have been influenced by an extrinsic transition called bound exciton. This bound exciton is related to defects which are responsible for creating discrete electronic states in the band gap. Exciton can be bound with neutral or charged donors and acceptors and it depends on the band

Structure of semiconductor material. Zinc oxide films which made from single crystal can be applied for modulation of UV radiation due to its directionally dependent optical property. It is also used for different electronic application due to its high breakdown stress and high saturation velocity. Due to its higher radiation, chemical, and thermal resistance,ZnO is used to form transparent contacts of solar cells. Fluorescence spectra of ZnO nanowire shows increase of green emission intensity witha decrease of nanowire diameter and continuous reduction of diameter of ZnO nanowire gives quantum size effect and due to this size confinement exciton binding energy is enhanced.The performance of optoelectronic devices have been influenced by transport characteristics and interaction of phonon with free carrier [30].

2.2 Synthesis routes of ZnO nanoparticles

Top-down method is a physical method in which a microcrystalline or bulk material has been broken down again and again in to small pieces with various lithographic techniques like grinding, milling etc. The microcrystalline or bulk material becomes like a powder, which is in the form of nanomaterials. All the solid state routes fall in to this category including, Mechanical alloying, equal channel angular pressing (ECAP), high-pressure torsion (HPT), accumulative roll bonding (ARB), nanolithography, etc [31].

Bottom- up method is a chemical method where nanomaterials are building up from bottom to upwards. This means that adding (self-assembling) a very small atoms or molecules up to require size. The two vital processes in the bottom-up method are nucleation and growth. Nucleation is the first step of phase transformation where an aggregate of atoms are formed.

All techniques that start with liquid and gas as the starting material fall in to this category including, physical vapour deposition (PVD), chemical vapour deposition, spray conversion processing, sol-gel process, wet chemical method, self-assembly and green synthesis method [32]. Among these, green synthesis method is the most simple and popular method due to its low cost, reliability and environmental friendly synthetic routes. It also provides rigorous control of the size and shape of the nanoparticles [33].

2.3 Characterization Techniques of ZnO Nanoparticles

In order to apply zinc oxide nanoparticles for any of applications characterization should be hold after synthesis of the material. Several physical techniques have been developed to characterize the size, morphology and other remarkable properties of zinc oxide nanoparticles. The most commonly used characterization techniques are X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), UV-visible Spectroscopy, Fluorescence Spectroscopy and FTIR Spectroscopy. The detail information about these techniques are explained as follows.

X-ray is an energetic electromagnetic radiation appearing at the region between gamma-rays and ultraviolet, which is comparable with the size of an atom. When X-rays interact with a crystalline substance (phase), one gets a diffraction pattern. The reflection of incident monochromatic X-ray from successive planes of crystal lattices when the difference between the planes of complete number n of wavelengths leads to famous Bragg's law [34]. The Bragg's law can be described according to equation (2.1).

$$n\lambda = d\sin\theta \qquad 2.1$$

Where **n** is an integer 1, 2, 3..., **λ** is wavelength in nanometer, d is interatomic spacing and θ is the diffraction angle in degrees.

XRD is a non destructive analytical technique which does not require intricate sample preparation. Mostly it is used for the fingerprint characterization of crystalline materials and the efficient determination of their structure, chemical composition and physical properties.
It is a very functional and powerful tool to structural characterization including how the atoms pack together in the crystalline state and what the interatomic distance and angle are etc [35].

XRD can also used to characterize nanoparticles such as ZnO NPs. It determines the average crystallite size of ZnO NPs. A great advantage of X-ray powder diffraction is that it requires virtually no sample preparation, in comparison to other characterization techniques. The sample can be either in the form of smear or compact flat pack or a capillary and is exposed to monochromatic beam of x-ray and the diffracted beam intensity is collected in a range of angles (2θ) with respect to the incident beam. The intensity corresponding to a constructive interference of the diffracted beam from a crystallographic plane is observed as peak, corresponding to the Bragg angle (θ).

A powder diffraction pattern contains information on phases present (peak positions), phase concentrations (peak heights), structure, degree of crystallanity or amorphous content (background hump) and crystallite size/strain (peak widths/broadening).The width of the peaks in a particular phase pattern provides an indication of the average crystallite size. Large crystallites give rise to sharp peaks, while the peak width increases as crystallite size decreases. Crystallite size does not equal the size of the particle, as one particle can be a conglomerate of several crystallites. Peak broadening also occurs as a result of variation in d-spacing caused by micro strain. The positions of the peaks provide an accurate fingerprint of the crystal structure of the nanocrystals.

The average crystallite size of the ZnO nanoparticles can be obtained from the line broadening using Debye Scherer formula:

$$D = \frac{0.9\lambda}{\beta cos\theta} \quad 2.2$$

Where D is averaged crystallite size, **λ** is wavelength of incident beam (1.5406Å), β is full width at half-maximum (FWHM) in radians and θ is scattering angle in degree. The lattice constants a' and 'c' and the spacing 'd_{hkl}' for the structures of ZnO can be calculated according to equation (2.3 and 2.4) and (2.5).

$$a = \sqrt{\frac{1}{3}}\ \frac{\lambda}{Sin\theta} \quad 2.3$$

$$c = \frac{\lambda}{Sin\theta} \quad 2.4$$

$$d_{hkl} = \frac{ac}{2}\sqrt{\frac{12}{4c^2(h^2 + hk + 3(al)^2}} \quad 2.5$$

Scanning electron microscopy (SEM) is a microscopic instrument which gives detail information about size, external morphology (texture), microstructure, composition, topography, and crystal orientation of ZnO nanoparticles. Electrons are thermoionically emitted from a tungsten or lanthanum hexa-boride cathode and are accelerated towards an anode; alternatively electrons can be emitted via field emission. The electron beam, which has an energy ranging from a few hundred eV to 100 KeV, is focused by one or two condenser lenses in to a beam. Characteristic X-rays are emitted when the primary beam causes the ejection of inner shell electrons from the sample and are used to determine the elemental composition of the sample.

As most biological specimens are made up of non-dense material the amount of secondary electrons produced is too low to be of much use in creating an image and therefore they are usually coated with a very fine layer of a metal which readily produces secondary electrons. The back scattered electrons emitted from the sample can be used .These signals are monitored by photomultiplier tubes and magnified. An image of the investigated microscopic region of the specimen is observed in cathode ray tube and is photographed [38].

UV-visible Spectroscopy is the type of absorption spectrophotometer which involves the excitation of electrons present in the molecule from ground state to excited state. It is the most reliable and accurate analytical method which used to measure scanning or transition of electron and optical properties of materials. Absorption spectrophotometer is the analytical technique in which compounds absorb light of radiation of a specific wavelength.

UV-Vis spectroscopy is almost entirely used for quantitative analysis in which the estimation of the amount of a compound known to be present in the sample solution. The components of UV-Vis spectroscopy are light source that generates a broad band of electromagnetic radiation, monochromater (wavelength selector), sample holder, detector to measure the intensity of radiation and computer (read signal). The absorption spectra of atoms, molecules, or ions are generated when a beam of electromagnetic energy or light is passed through a sample, and absorbs a portion of the photons of electromagnetic energy passing through the sample [39].

UV-Vis spectroscopy can also used to characterize the nanoparticles such ZnO nanoparticles. It measures the absorption peak of ZnO nanoparticles, which occurred due to transition or scanning of electron from valence band to conduction band.

When an electron gets some amount of photon energy from UV or visible light, it is jumping from valence band (lower energy) to conduction band (higher energy).

It is the peculiar property for ZnO. The absorption peak is depend on some factors like selection of wavelength, nature of the solvent, band gap, oxygen deficiency, size and structure of the nanoparticles, surface roughness, impurity centers, PH of the solution and temperature. Generally, as it was reported in several literatures, the UV-Vis absorption peaks of ZnO NPs are found between 350 nm- 380 nm. The band gap energy, the energy required for the transition of electron from a state of lower energy (E_1) to state of higher energy (E_2) is equivalent to the energy of electromagnetic radiation (photon energy) that causes transition [39]. It is given by equation:

$$E_g = E_2 - E_1 = \frac{\square c}{\lambda} \qquad 2.6$$

Where Eg is band gap energy, h is Planck's constant, C is speed of light and λ is related wavelength of the sample. From the UV-Vis graph, energy band gap is calculated using the equation:

$$E_g = \frac{1240}{\lambda max.}\ eV \qquad 2.7$$

FTIR Spectroscopy is a non-destructive and highly flexible instrument which able to measure bond vibrational motions of atoms in molecules. It is a powerful technique for chemical characterization, identification of functional groups and used to achieve information regarding the attachment of the capping agents. In recent time,it has been replaced dispersive instruments for most applications due to its superior speed and sensitivity[40].

The three basic spectrometer components of FTIR spectroscopy are radiation source, interferometer and detector. It uses a beam of infrared light to analyze the structure of organic compounds, identify unknown materials, and determine the quality or consistency of a sample and the amount of components in a mixture [41]. FTIR analyze a wide range of materials in bulk or thin films, liquids, solids, pastes, powders, fibers, and other forms. Its spectra of pure compounds are generally so unique that they are like amolecular "fingerprint" [42].

Vibrational motion of atoms in a molecule is influenced by the masses of atoms, their geometrical arrangement and strength of their chemical bonds. There are IR and Raman spectra, which are the results of transition between quantized vibrational states and provide a complementary image of molecular vibrations. Both IR and Raman spectra involves IR radiation [43].

Specific types of chemical bonds or functional groups can be identified by infrared spectroscopy based on their unique absorption signatures. Identification of functional groups is the cornerstone of IR spectroscopy, which produces broad absorptions. Molecules absorb resonant frequencies that are characteristic of their structure. This means the frequency of the absorbed radiation matches the frequency of the bond or group that vibrates and the detection of energy is done on the basis of shape of the molecular potential energy surfaces, the masses of the atoms, and the associated vibronic coupling.

IR encompasses a spectral region from red end of visible spectrum (12500 cm^{-1}) to 10 cm^{-1} region in the electromagnetic spectrum.

It is the most influential tool to identify compounds by matching spectrum of unknown compound with reference spectrum (finger printing) and functional groups in unknown substances. IR is commonly divided into near IR (12500 to 4000 cm^{-1}), mid IR (4000 to 400 cm^{-1}) and far IR (400 to 10 cm^{-1}). Among these, mid IR (4000 to 400 cm-1) spectra region is the most significance and richest region in chemical information due to the occurrence of the most fundamental molecular vibrations [43, 44].

FTIR spectroscopy also used to characterize ZnO NPs to investigate their vibrational motions. It gives detail information about the surface chemistry of ZnO nanoparticles and functional groups attached to the zinc oxide nanoparticles surface. These functional group shows various FTIR pattern than those of free groups. Zinc oxide generally gives vibrational phonon in fingerprint region from 400 cm^{-1}- 600 cm^{-1} arising from inter-atomic vibrations. The IR spectra of ZnO NPs greater than 600 cm^{-1} is due to some other carboxylic and hydroxyl groups like O-H, C=O, C-H, C-O, which are attached to zinc oxide nanoparticles during synthesis [45].

Fluorescence spectroscopy is a rapid, sensitive and selective characterization method. It is a contactless and non-destructive method to investigate the electronic structure of materials. It is also a type of electromagnetic spectroscopy that analyzes fluorescence from a sample using a beam of ultraviolet light. This beam of light excites the electrons in molecules of certain compounds and causes them to emit light. In the single molecule fluorescence spectroscopy, intensity fluctuations from the emitted light are measured from either single fluorophores or pairs of fluorophores [46].

Bulk metal does not usually possess photo luminescent properties due to the absence of band gaps in the atomic solid-state structure. Fluorescence spectroscopy measures the intensity of photons emitted from a sample, an excitation spectrum and emission spectrum. The three basic parts of fluorescence spectroscopy are source of light, sample holder and detector. Photons impinge on the excitation monochromater, which selectively transmits light in a narrow range centered about the specified excitation wavelength. The transmitted light passes through adjustable slits that control magnitude and resolution by further limiting the range of transmitted light. The filtered light passes into the sample cell causing fluorescent emission by fluorophores within the sample.Emitted light enters the emission monochromater, which is positioned at a 90º angle from the excitation light path to eliminate background signal and minimize noise due to stray light. Again, emitted light is transmitted in a narrow range centered about the specified emission wavelength and exits through adjustable slits. Spectral resolution and signal to noise is directly related to the selected slit widths. The particle cannot emit energy at the place where it was absorbed [47, 48].

Fluorescence spectroscopy also used to characterize nanoparticles such ZnO NPs. This technique involves measuring the energy distribution of emitted photons after optical excitation of ZnO nanoparticles. Since ZnO is a semiconductor, free electrons are there in the conduction band and holes are in the valence band. When electrons make a transition from ground state to excited state because of some photon energy which is emitted from the source, energy is absorbed. While this electron came back to valence band, emission is occurred i.e. energy is emitted. The emission spectra of ZnO nanoparticles have been exceed their absorption spectra due to their semiconducting properties.

This is implies when some amount of radiation (photon) is supply, electrons are climb from valence band (lower energy) to conduction band (higher energy), and when it came backs to valence band (lower energy) from conduction band (higher energy), the wavelength is greater since photon energy is inversely proportional to wavelength. Generally, ZnO exhibits two kinds of emissions: one is at ultraviolet near band-edge emission and the other a visible deep level emission with a peak

in the range from 450 to 700 nm. UV emission is related to the transition from conduction band edge to valence band. The Visible emission spectra is caused by the transition from deep donor level to valence band due to oxygen vacancies and by the transition from conduction band to deep acceptor level due to impurities and defect states [49]

2.4 Applications of ZnO Nanoparticles

2.4.1 Antimicrobial Applications of Zinc Oxide Nanoparticles

The real progress towards the use of ZnO NPs as an antimicrobial agent was started in 1995, when Sawai and his colleagues found that MgO, CaO and ZnO nanoparticles powders had antimicrobial activities against some bacteria strains [50]. Now a days, Zinc oxide is listed as a generally recognized as safe (GRAS) material by the Food and Drug Administration and is used as food additive. Zinc is an essential micronutrient which used for intensification, improvement and health in humans and animals [51].

Invitro antibacterial activity of ZnO nanoparticles have been performed by different methods, but the most one is broth dilution method which is followed by colony count.

This method consists of determining the number of doable bacteria by plating, in appropriate agar medium, serial dilutions of intention microorganisms incubated in culture broths containing ZnO nanoparticles at different concentrations and appropriate time/temperature conditions.

The antibacterial activity of ZnO nanoparticles have been tested against some gram-positive *Bacillus subtilis* and *Staphylococcus aureus*; and gram-negative bacteria *Pseudomonas aeruginosa, Campylobacter jejuni* and *Escherichia coli* [52]. *E.coli* and *pseudo* have higher susceptibility to ZnO nanoparticles than *S. aureus* and *bacillus*. The higher resistance of S.aureus and bacillus to ZnO nanoparticles than *E.coli and pseudo* is due to the fact that *S. aureus* and bacillus contain carotenoid pigments, which promote a greater oxidant resistance and due to the presence of potent detoxification agents such as antioxidant enzymes, particularly catalase [53].

Gram-positive bacteria have more sensitivity to reactive oxygen species (ROS) than Gram-negative bacteria due to two main reasons. The first is structural difference in bacterial membrane, i.e. Gram-positive bacteria have a membrane, which surrounds the cell, and a cell Wall primarily made up of peptidoglycan layer, teichoic and lipoteichoic acids. Gram-negative bacteria have more complex cell wall due to the presence of an outer membrane, which is composed mainly of lip polysaccharide (LPS), in addition to a thin peptidoglycan layer. This outer membrane acts as a permeability barrier; this result the absorption of ROS into the cell is reduced [54].

The second one is polarity difference of cell membrane, since the membrane of *S.aureus* and *Bacillus* have more positive charge than *E.coli*, *P.seudo* and *thyphus* which allow a greater penetration level of negatively charged free radicals such as hydroxyl radicals, superoxide and peroxide ions causing damage and cell death in *S.aureus* and *pseudo* at concentrations below that required to cause the same effect in *E.coli and bacillus*. The antimicrobial activity of ZnO nanoparticle is affected by some factors like size of nanoparticle, concentration and surface area, but strongly influenced by their size. This implies that the antimicrobial activity of ZnO on *E.coli*, *Bacillus*, *pseudo*,*Thyphus* and *S.aureus* has been improved with diminution of particle size due to an increase in the surface area to volume ratio, which results in the increased reactivity of ZnO nanoparticle surface. This is the fact that a larger surface area will result in more ROS on the surface of ZnO and, therefore, in a greater antibacterial activity of smaller nanoparticles. The toxicity of ZnO nanoparticles could be depend on their shape due to the increase proportionality of interaction forces of lengthwise-oriented nanoparticles to their lengths [55]. The three mechanisms for the antimicrobial activity of ZnO nanoparticles are the release of antimicrobial ions, interaction of nanoparticles with microorganisms and the formation of ROS by the effect of light radiation [56].

Many researchers have been reported the occurrence of ROS is the main mechanism [17, 37, and 57]. When ZnO nanoparticles activate by visible and UV light, ROS is generating on their surface. Electrons move from the valence band (Vb) to the conduction band (Cb) of the ZnO nanoparticles due to the higher incident radiation with photon energy of ZnO compared to its band gap energy (~3.37eV). As a result a positive area, an electron hole (h^+) in the valence band and a free electron (e^-) in the conduction band is formed [58].

The electron hole (h+) reacts with H_2O molecules (from the suspension of ZnO) separating them into •OH and H^+; and O_2 molecules dissolved in the medium are transformed into superoxide anion radicals O_2^-, which in turn react with H^+ and generate (HO_2). Consequently, this species collides with electrons producing hydrogen peroxide anions (HO_2). Thus,the hydrogen peroxide anion reacts with hydrogen ions to produce H_2O_2 molecules [59].

Chapter 3: Materials and Methods

3.1 Chemicals and Solvents

The chemicals and solvents required for synthesis of ZnO nanoparticles were double distilled water(DIH_2O) , zinc nitrate hexa-hydrate $Zn(NO_3)_2.6H_2O$ (Himedia, Maximum Assay 90 % , High Media Laboratory Pvt .Ltd. India), and moringa oleifera leaf.

The chemicals and solvents used for culturing of microorganisms and antimicrobial activities were double distilled water, Dimethyl sulfoxide (DMSO), Sabourdad dextrose agar (Himedia,TITAN BIOTECH LTD,India), Mueller Hinton Agar (Himedia,TITAN BIOTECH LTD, India) and Gentamacin.

3.2 Apparatus

The apparatus used for green synthesis of ZnO nanoparticles were:Digital electrical analytical balance (FA.Model 2104) : measures the mass of moringa oleifera leaf powder and zinc nitrate hexa-hydrate.

Magnetic stirrer (MH 2It.): boils double distilled water and stires the stock solutions of moringa leaf extracted water and zinc nitrate hexa-hydrate salt.

Furnace (Model-BK-5-12GJ , BIOBASE BIODUSTRY (SHANGDONG)Co.Ltd.,China) : anneals the powder.

Ceramic crucible cups: hold the stock solution in the furnace and as well as in the drying oven.

Thermometer: measures the temperature of the solution of zinc nitrate and moringa leaf extracted water during stirrer.

Funnels: extract moringa leaf water after moringa powder dissolved in the boiled double distilled water.

Different sizes of beakers: were used for heating double distilled water as well as the stock solution of zinc nitrate hexa hydrate and moringa leaf extracted water.

Filter paper (Watt Mann. 1): filters moringa leaf extracted water after moringa leaf powder mixed with boiled double distilled water.

Electric constant temperature blast drying oven (Model-DGH-9055,JANGSU GANGDU MECHANIC ELECTRONIC EQUIPMENT Co.Ltd., China) : dries the stock solutions of zinc nitrate and moringa leaf extracted water. Grinder: used for powdering of moringa dried leaf.

The apparatus used for culturing of bacterial and fungus were Petri plates (dish), Micro petite, laminar air flow, Test tubes , Aluminum foil , Gloves, Sterilizer, Incubator ,force puncher , Cotton swab , Beakers , Refrigerator (LG) and Rulers.

3.3 Instruments

3.3.1 X-ray Diffraction spectroscopy

The X-ray powder diffraction patterns of the ZnO NPs were recorded on X-ray Diffractometer (PANalytical XPERT-PRO Diffractometer) equipped with nickel filtered Cu-$K\alpha_1$ radiation (λ= 1.5406 A^0) operating at 45 KV and 30 mA. The data have been collected in the scan range (2θ) from 10^0 -100^0. X-ray Diffraction spectroscopy is used to characterized the particle size of ZnO NP_S.

3.3.2 UV- Vis spectroscopy Characterization

The UV-Vis absorption peaks of ZnO NPs were recorded with UV-Vis spectroscopy (PerkinElmer, Lambda 950 UV/VIS/NIR Spectrophotometer) in the wavelength region of 240 nm - 600 nm at room temperature. The ZnO nanoparticles were dissolved in DMSO and its absorbance measured using 1cm quartz cuvette. The ASCII files of the measured spectra were collected by computer which interfaced with the instrument. Finally, the absorption spectra of ZnO NPs were analyzed using origin 8 software. It is used to measure the absorption peak of ZnO nanoparticles.

3.3.3 Fluorescence spectroscopy

The emission spectra of ZnO NPs were measured with fluorescence spectroscopy (Fluoromax-4 Spectrofluorometer) in the wavelength region of 300 nm- 500 nm at room temperature. The samples was dissolved in di-methyl sulphoxide (DMSO) and stirred until it dissolved. The emission spectra of the sample were measured by 1 cm quartz cuvette. The excitation wavelengths of the samples were performed at 275 nm and 355 nm for M_1 and M_2 respectively. The slit width of the instrument was adjusted to 5 nm. Fluorescence spectroscopy measures the intensity of photons emitted from a sample, an excitation spectrum and emission spectrum.

3.3.4 FTIR spectroscopy

The vibrational phonon and the quality of ZnO NPs were also characterized by Fourier transform infrared spectroscopy (PerkinElmer, Spectrum 65 FTIR) in the wave number region of 4000-400 cm^{-1} according to the procedures reported by [62]. The ZnO NPs spectra measurements were performed by putting the powder ZnO NPs on KBr pellet. It uses a beam of infrared light to analyze the structure of organic compounds, identify unknown materials, and determine the quality or consistency of a sample and the amount of components in a mixture . FTIR analyze a wide range of materials in bulk or thin films, liquids, solids, pastes, powders, fibers, and other forms

3.5 Moringa oleifera plant leaf collection

Fresh and healthy Moringa oleifera leaves were collected from Adama city, Lethenal Coronel Dejene Sime elementary School (Ethiopia).

3.6 preparation of Moringa oleifera plant leaves powder

The leaf of Moringa oleifera were washed twice with distilled water and air-dried at room temperature.These dried leaves were grinded using a grinder in Adama Science and Technology University, Chemistry laboratory.

3.7 Synthesis Methods of the Targeted Zinc Oxide Nanoparticles

3.7.1 First Synthesizing Method of ZnO NP_S (M_1)

About 20 g powder of Moringa oleifera leaves, 200 mL of double distilled water was added. Then, the solution was stirred under magnetic stirrer for 20 min in the temperature of 60℃. The Moringa oleifera leaf water extract was used for synthesis of ZnO nanoparticles. Also, 20 mL extract was added to 2 g of zinc nitrate hexa-hydrate ($Zn(NO_3)_2 . 6H_2O$) .

The solution was stirred for 30 min maintaining the temperature at 80℃. After this time the solution was cooled at room temperature. The color of the solution was yellow, and it transferred to a ceramic crucible cup. Then, the solution in a crucible cup was carried out in furnace at 400^0c for 2 hrs. Finally white powder was obtained. The overall chemical reaction of the first green synthesis method (M_1) of ZnO NP_S is summarized in the following box:

20mL of Moringa Oleifera leaf extracted solution + 2g of zinc nitrate hexa-hydrate

$(Zn(NO_3)2.\ 6H_2O \xrightarrow{400℃}$ **white powder of ZnO NP_S**

3.7.2 The second synthesizing method (M_2)

50mL of filtered Moringa oleifera water extract was added to 4 g of zinc nitrate hexa-hydrate in a beaker. The wall of a beaker was covered with aluminum foil to avoid any photo induced phenomenon and kept to room temperature. Yet there was an obvious color change in to yellow after 18hrs, and it transferred to crucible cup. Accordingly the concentration that is the mixture of zinc nitrate hexa- hydrate and the water extracted Moringa leaf was dried at 100^0C in the standard oven and the powder was collected. Finally the powdered annealing of remains was carried out in a muffle furnace at 500^oC for 1 h.

3.7.3 The third synthesizing method (M_3)

50mL of filtered Moringa oleifera water extract was added to 1 g of zinc nitrate hexa-hydrate in a beaker. The wall of a beaker was covered with aluminum foil to avoid any photo induced phenomenon and kept to room temperature. Yet there was an obvious color change in to yellow after 18hrs, and it transferred to crucible cup. Accordingly the concentration that is the mixture of zinc nitrate hexa- hydrate and the water extracted Moringa leaf was dried at 100^0C in the standard oven and the powder was collected. Finally the powdered annealing of remains was carried out in a muffle furnace at 500^oC for 1 h.

The overall chemical reaction of the second green synthesis method of ZnO NP_S (M_3) is summarized as follows:

50mL of Moringa oleifera leaf extracted solution + 1 g of zinc nitrate hexa-hydrate $(Zn(NO_3)2.\ 6H_2O \xrightarrow{500℃}$ white powder of ZnO NP_S

3.7.4 The second synthesizing method (M_4)

50mL of filtered Moringa oleifera water extract was added to 2 g of zinc nitrate hexa-hydrate in a beaker. The wall of a beaker was covered with aluminum foil to avoid any photo induced phenomenon and kept to room temperature. Yet there was an obvious color change in to yellow after 18hrs, and it transferred to crucible cup. Accordingly the concentration that is the mixture of zinc nitrate hexa- hydrate and the water extracted Moringa leaf was dried at 100^0C in the standard oven and the powder was collected.

Finally the powdered annealing of remains was carried out in a muffle furnace at 500^{o}C for 1 h.

The overall chemical reaction of the second green synthesis method of ZnO NP_S (M_4) is summarized as follows:

50mL of Moringa oleifera leaf extracted solution + 2 g of zinc nitrate hexa-hydrate ($Zn(NO_3)2.\ 6H_2O$ $\xrightarrow{500℃}$ **white powder *of* ZnO NP_S**

3.7 Antimicrobial activity study

The antimicrobial activity of ZnO was evaluated against Gram-positive bacteria such as *B. subtilis*, *S.aures* and Gram-negative bacteria such as *E.coli*, *P.aeruginosa and Thyphus*, and also evaluated against *C.albicans* fungus by agar well diffusion method.

The wells of 8mm diameter were punched Muller-Hinton Agar having the above six mentioned test microorganisms. The wells were filled with 100 μL of ZnO at the concentration of 1028, 516, 256 and 125 $\frac{\mu g}{mL}$. Gentamicin was used as a positive control. The plates were incubated for 24 h and 72 h for bacterial and fungus respectively. Antimicrobial activity was evaluated by measuring the inhibition zone of the test microorganisms.

Chapter 4: Results and discussions

4.1 XRD Patterns of ZnO NP_S

XRD used to characterize nanoparticles such as ZnO NPs. It determines the average crystallite size of ZnO NPs. A great advantage of X-ray powder diffraction is that it requires virtually no sample preparation, in comparison to other characterization techniques.

Figure 4.1 a-d: shows the XRD pattern of ZnO nanoparticles of samples $M_{1,}$ M_2, M_3 and M_4 respectively. The diffraction peaks of the two ZnO NPs correspond to (100), (002), (101), (102), (110), (103), (112) and (201). Among these, the (101) and (001) planes are the most prominent for samples $M_{1,}$ M_2, M_3 and M_4.The variation of diffraction peaks of the ZnO nanoparticles is due to the difference in the volume of extracted solution of Moringa leaf and calcinated temperature. The average crystallite size of the ZnO NPs calculated using Eq (was about 8.882nm and 13.742nm for M_1 and M_2 respectively. The average crystallite size variation among the ZnO NPs is due to a difference in the diffraction peaks results in the variation of calcinations temperature. The results are quite similar with the previous research reported by [64]. The diffraction peaks scattered at an angle (2Ɵ), and the corresponding crystal plane (hkl), full width at half-maximum (FWHM) and crystallite size of the ZnO NPs of M_1 and M_2 respectively is given in table 4.1a-b respectively.

Table 4.1(a): The X-ray diffraction peaks and the corresponding crystal plane, FWHM and crystallite size of the ZnO nanoparticles of M1.

hkl	FWHM (Deg.)	2θ	θ	β(rad)	Cos(θ)	Kλ	Crystal Size(D)	Average Cry.size($D_{av.}$)
100	0.866	31.671	15.835	0.0151	0.962	0.1386	9.534	8.882 nm
002	1.048	34.390	17.195	0.0183	0.955	0.1386	7.937	
101	0.930	36.139	18.069	0.0162	0.950	0.1386	8.983	
102	1.065	47.442	23.721	0.0185	0.915	0.1386	9.220	
110	0.942	56.464	28.232	0.0164	0.881	0.1386	9.579	
103	1.135	62.746	31.373	0.0198	0.853	0.1386	8.203	
200	1.064	67.842	33.921	0.0196	0.829	0.1386	8.999	
112	1.120	68.842	34.421	0.0190	0.825	0.1390	8.603	

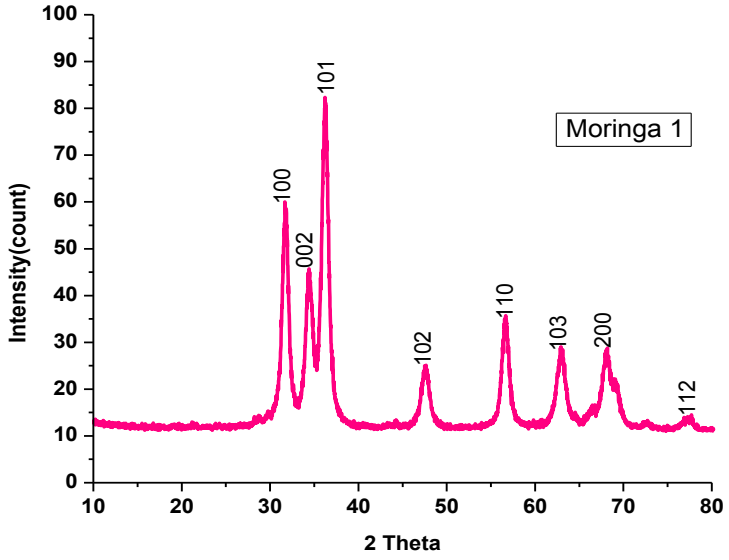


Figure 4.1(a):- XRD patterns of ZnO NP_S of sample M_1

Table 4.1 (b): The X-ray diffraction peaks and the corresponding crystal plane,FWHM and crystallite size of the ZnO nanoparticles of M_2.

Hlk	FWHM (degree)	2θ	θ	β(rad)	cos(θ)	kλ	crystal size(D)	D Average
100	0.5612	31.7104	15.8552	0.00978	0.962	0.1386	14.732	13.724nm
002	0.567	34.4349	17.2175	0.00989	0.955	0.1386	14.675	
101	0.5931	36.2053	18.1026	0.01035	0.951	0.1386	14.081	
102	0.6557	47.5272	23.7636	0.01145	0.915	0.1386	13.231	
110	0.6165	56.5095	28.2548	0.01075	0.881	0.1386	14.634	
103	0.7094	62.8667	31.4334	0.01238	0.853	0.1386	13.407	
200	0.7256	67.9091	33.9546	0.01266	0.829	0.1386	12.477	
201	0.76806	68.9616	34.4808	0.0134	0.824	0.1386	12.553	

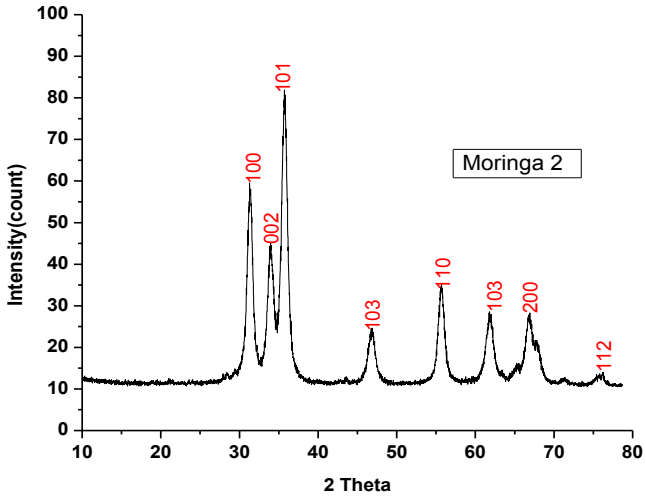


Figure 4.1 (b): XRD patterns of ZnO nanoparticles of sample M_2

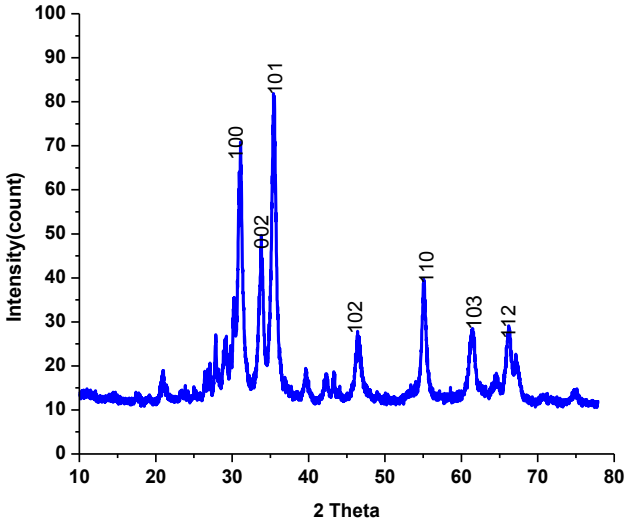


Figure 4.1 (c): XRD patterns of ZnO nanoparticles of sample M_3

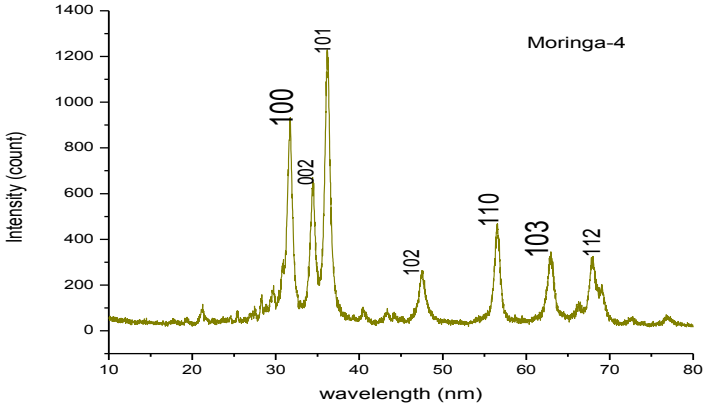
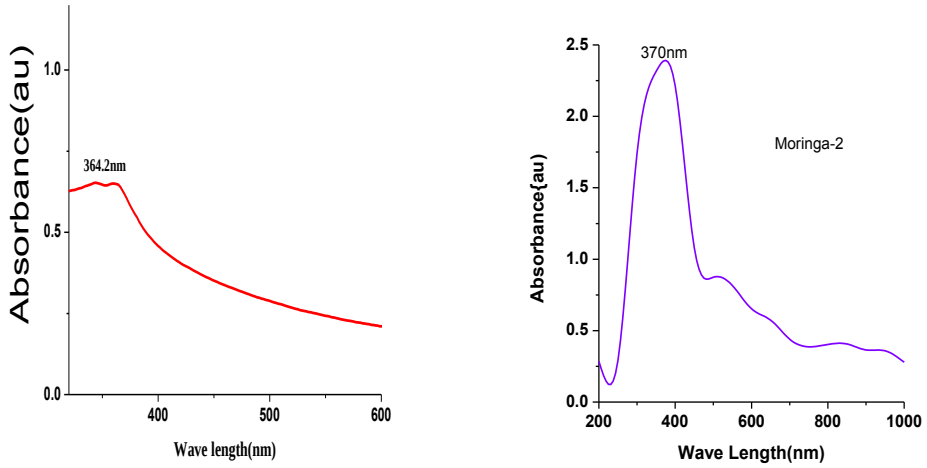


Figure 4.1 (d): XRD patterns of ZnO nanoparticles of sample M_4

4.2 UV-Vis absorption peak of ZnO nanoparticles

Figure 4.3:a-b shows the UV-Vis absorption peak of ZnO NPs of M_1 and M_2 samples were dispersed in DMSO. The absorption peak of ZnO nanoparticles were observed at 364.2nm and 370nm for M_1 and M_2 samples respectively. These peaks are due to electronic transition from deep level of valence band to conduction band.

The UV-Vis absorption peak variation among the ZnO nanoparticles are due to the difference in their size and shape, which results due to different usage of volumes of extracted solutions moringa leaf and different masses of zinc nitrate hexa-hydrate during synthesis ,calcinations temperature and diffraction peaks.



(a) (b)

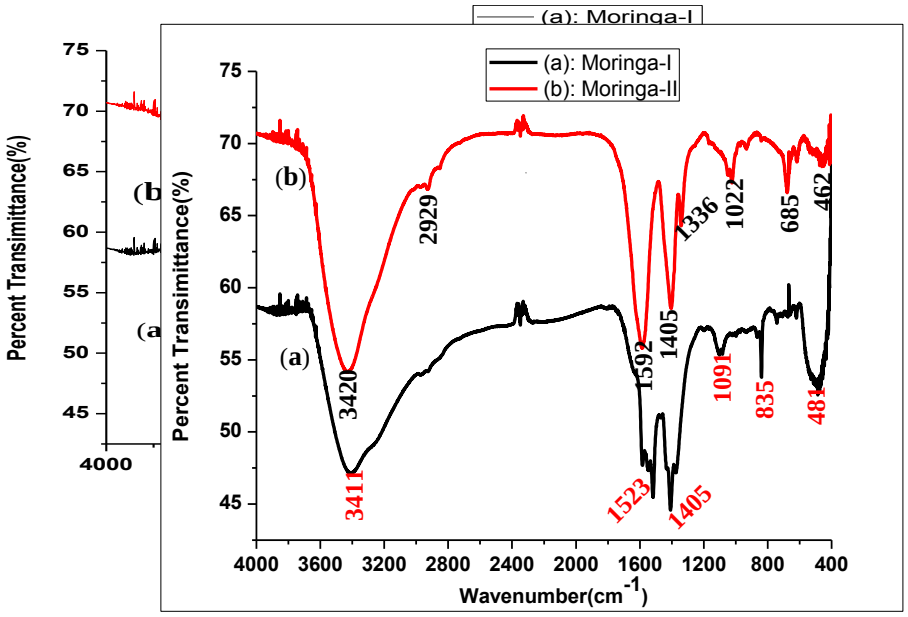
Figure 4.2: a-b: UV-Vis absorption peak of ZnO nanoparticles of M_1 and M_2

4.3 IR spectra of ZnO nanoparticles

Figure 4.3 a-b shows the IR spectra of ZnO nanoparticles of M_1 and M_2 respectively. The broad band observed in the wavenumber region 3437- 3466 cm^{-1} was due to O-H stretching, which represents the presence of water molecule on the surface of ZnO nanoparticles. The band observed at the vibrational spectra of 2422 cm^{-1} was due to the alkyl group C-H, which attached to the ZnO nanoparticles during synthesis. Similarly, the band observed between 1370-1598 cm^{-1} were due to C=O stretching. The sharp peak observed in the range 462 cm^{1}-481 cm^{-1} was attributed to the vibrational phonon of ZnO. This result indicates the successful

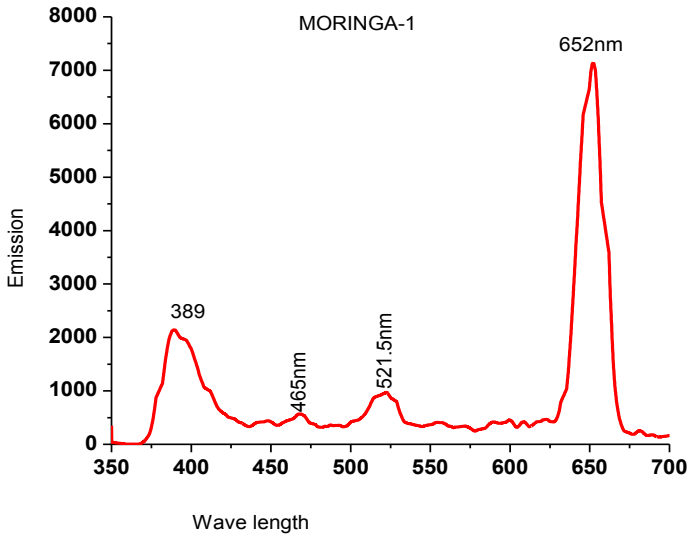
production of ZnO nanoparticles.

This result is also quite similar with previous research reported by[64].

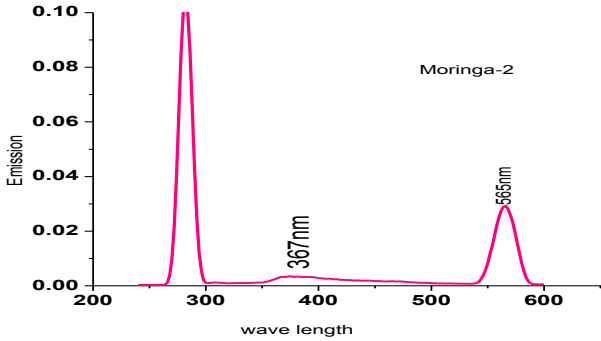


4.4 Emission spectra of ZnO nanoparticles

Figure 4.4 a-b shows the emission spectra of ZnO nanoparticles of sample M_1 and M_2 in DMSO. The UV emission spectra peak of M_1 and M_2 observed at 389 nm and 367 nm respectively are due to transition of free electrons from conduction band edge to valence band. On the other hand, the visible emission spectra were peaked in the wavelength region 465 nm- 652 nm and 565 nm and precursors of sample M_1 and M_2 respectively. The cause of these visible emission spectra are due to the transition of electron from deep donor level to valence band due to oxygen vacancies and by the transition from conduction band to deep acceptor level due to impurities and defect states.



(a)



(b)

Figure4.4a-b: Theemission spectra of ZnO nanoparticles of sample M_1 and M_2

4.5 Antimicrobial activity of the ZnO nanoparticles

Figure 4.5 a-c shows the antibacterial activity of ZnO nanoparticles of M_1 and M_2 on *E.coli* , *S.aureus, and* S.*thyphus* respectively. Antibacterial activity results shown that the ZnO nanoparticle of M_1 has been excellent antibacterial activity against *S.aureus E.coli* ,Pseudo,*S. aureus* ,*Bacillus* and S.thyphus bacteria compared to M_2. However, all the two ZnO nanoparticles were showed admirable antimicrobial activity than the standard antibiotic drugs on *S.aureus* and Bacillus bacteria. This is due to the simple cell wall and less negative charge membrane of *S.aureus and Bacillus.* As shown in the fig below, the growth inhibition *of Pseudo,S.aureus ,Bacillus ,E.coli S.thyphus* and *candida* has been increased by increasing the concentration of ZnO nanoparticles in wells. Furthermore, the antimicrobial activities of the ZnO nanoparticles have been increased with decreasing of particle size. Generally, the result was indicates that ZnO NPs are more effective against Gram-positive bacterial strains compared to Gram-negative bacterial strains. The zone of inhibition produced by the ZnO NPsofM_1andM_2against*E.coli,Pseudo,S.aureus,Bacillus,S.thyphus* and *candida* is given in tabl e 4.5(a-b)

Table 4.5 (a): The antibiotic activity of ZnO NP_S (Moringa-1)

Concentration of ZnO NP_S (Moringa-1) in μg/mL	Zone of inhibition in agar well diffusion of each microorganisms (mm)					
	$C.albicans$	$S.thyphus$	$p.aeruginosa$	$E.coli$	$B.subtilis$	$S.aures$
125	0	0	8.5	9	9.5	10
256	8	8.5	9	10	10	11
516	9	9	10	11	11	13
1208	9.5	10	11	11.5	12	15
Gemanticin	12	12	13	14	15	17

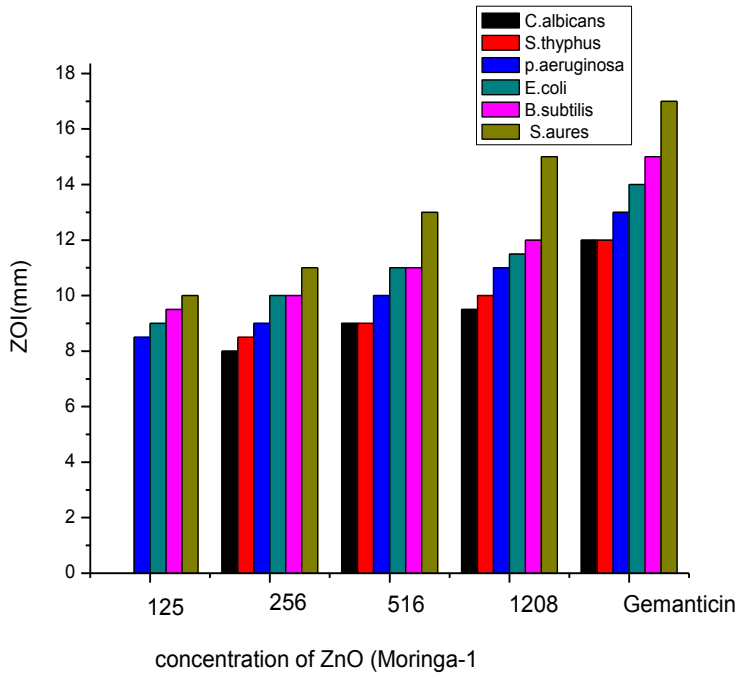


Figure 4.5 (a): The antibiotic activity of ZnO NP_S (Moringa-1)

Table 4.5 (b): The antibiotic activity of ZnO NP_S (Moringa-2)

Concentration of ZnO NP_S (Moringa-2) in μg/mL	Zone of inhibition in agar well diffusion of each microorganisms (mm)					
	C.albicans	S.thyphus	p.aeruginosa	E.coli	B.subtilis	S.aures
125	0	0	7.5	8	8.5	9
256	8	8	8	9	9.5	10
516	8	8.5	9.5	10	10	11
1208	9	9.5	10	11	11.5	13
Gemanticin	12	12	13	14	15	17

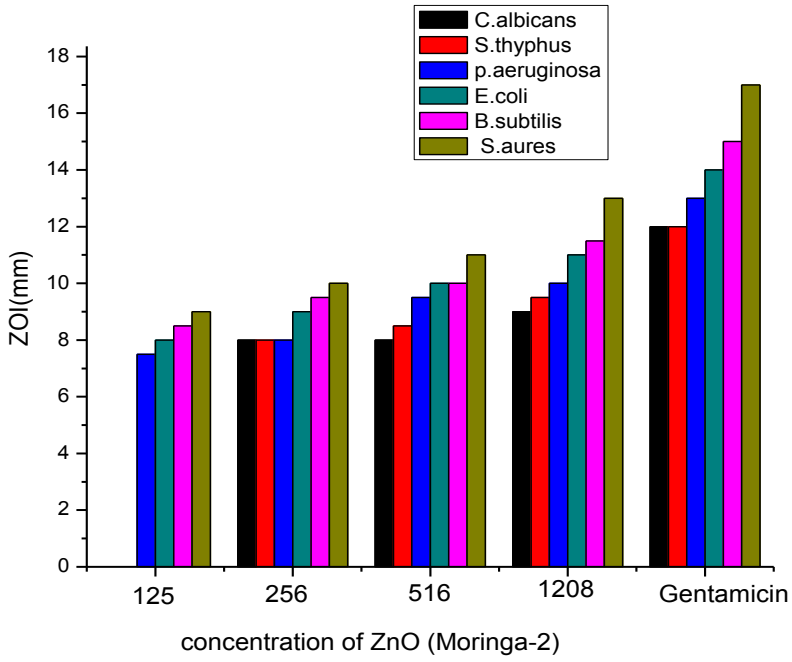
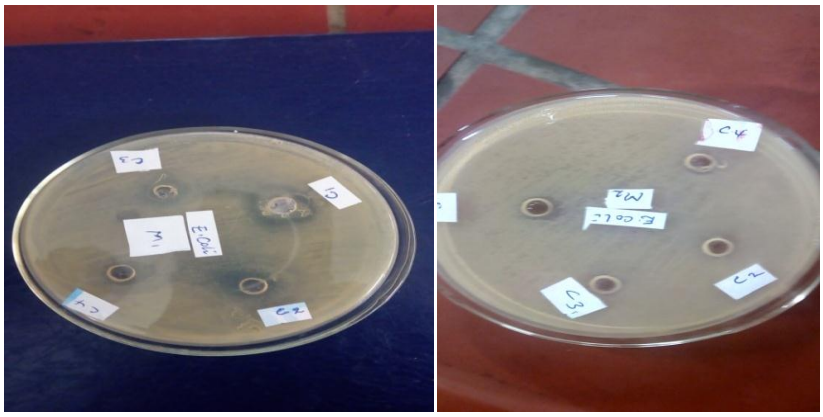
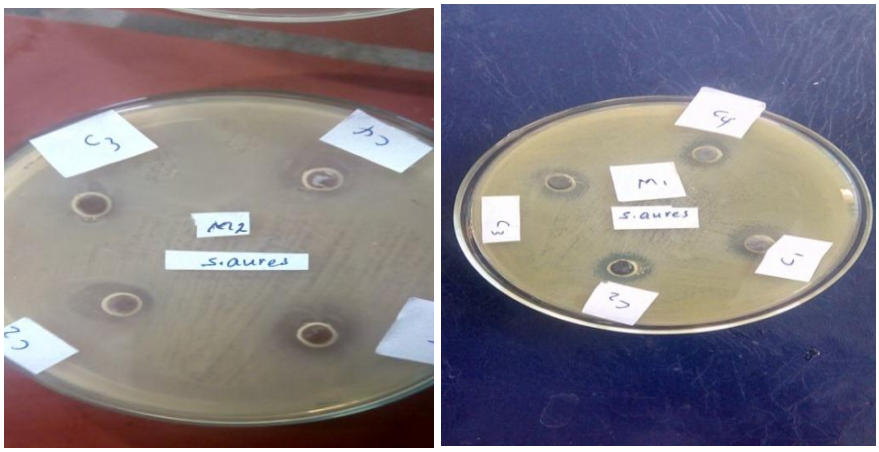


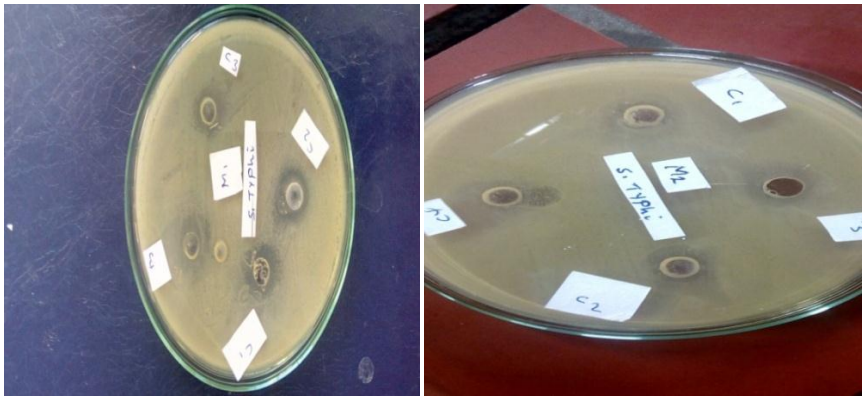
Figure 4.5 (b): The antibiotic activity of ZnO NP_S (Moringa- 2)



(a) ZOI of E.Coli on M_1 ZOI of E.coli on M_2



(b) ZOI of S.aures on M_1 ZOI of S.aures on M_2



(c) ZOI of Typhus on M_1 ZOI of Typhus on M_2

Figure 4.5 a-c: Antibacterial activity of ZnO nanoparticles of M_1 and M_2

4.6 Minimuminhibitoryconcentrations(MIC)

The Minimum inhibitory concentrations of micro organisms are shown in the following table 4.6 (a)

Table 4.6(a): The Minimum inhibitory concentrations of micro organisms

ZnO NP_S	Minimum inhibitory concentrations (mM)					
	C.albicans	S.thyphus	p.aeruginosa	E.coli	B.subtilis	S.aures
M_1	1.8	1.8	1.2	1	0.8	0.6
M_2	2	2	1.6	1.4	1	0.8

5. Conclusions and Recommendations

5.1 Conclusions

It is known that the green synthesis of ZnO nanoparticles is much safer and environmentally friendly as compared to chemical synthesis. In response to this assumption, this study demonstrates the green synthesis of ZnO nanoparticles using Moringa Oleifera leaf water extract. The synthesised ZnO nanoparticles were characterised by UV-Vis absorption spectroscopy, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR) and Fluorescence Spectroscopy. The averaged crystallite size of the ZnO nanoparticles of samples M_1, M_2, M_3 and M_4 were 8.882 nm, 13.724 nm, 11.57 nm and 12.62 nm respectively. The larger nanoparticles of ZnO resulted from the agglomeration of smaller nanoparticles. Moreover, the synthesized ZnO nanoparticles exhibited high activity against *S.aureus*, *E.coli*, *P.aeruginosa*, *B.subtilis*, *Thyphus* and *C.albican*. Generally, the results in this study indicate that ZnO nanoparticles are more effective against Gram-positive bacterial strains compared to Gram-negative bacterial strains and fungus.

5.2 Recommendation

Further study is needed on the application of ZnO nanoparticles for different Antimicrobial and antifungal activity.

6. References

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