Synthesis of ZnO NanoParticles Using *Calpurnia Aurea* Leaf Extract and Evaluation of Its Antibacterial Activity

BY:

KUMERA EMIRU ATOMSA

A THESIS SUBMITTED TO THE DEPARTMENT OF CHEMISTRY
SCHOOL OF APPLIED NATURAL SCIENCE
PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

OFFICE OF GRADUATE STUDIES
ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

ADAMA, ETHIOPIA
SEPTEMBER, 2018

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APPROVAL OF BOARD OF EXAMINERS

We, the undersigned, members of the Board of Examiners of the final open defense by Kumera Emiru have read and evaluated his thesis entitled "**Synthesis of ZnONanoParticle Using *Calpurnia Aurea* Leaf Extract and Evaluation of Its Antibacterial Activity** " and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the Degree of Master of Science in Chemistry.

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DECLARATION

I hereby declare that this M.Sc 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.

Name: Kumera Emiru Atomsa

Signature:_____

This MSc thesis has been submitted for examination with our approval as thesis advisors.

Advisor Name: Gemachu Deressa (PhD) Co-Advisor Name: Bedasa Abdisa (PhD)

Signature: _____ Signature: _____

Date of submission: _____

ADVISOR'S APPROVAL SHEET

To: Chemistry Department

Subject: Thesis Submission

This is to certify that the thesis entitled "**Synthesis of ZnO nanoparticle Using *Calpurnia Aurea* Leaf Extract and Evaluation of Its Antibacterial Activity**" submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemistry, the Graduate program of the department of Chemistry, and has been carried out by Kumera Emiru Atomsa under our supervision. Therefore, we recommend that the student has fulfilled the requirements and hence here by he can submit the thesis to the department.

_____	_____	_____
Name of major Advisor	Signature	Date

_____	_____	_____
Name of co- Advisor	Signature	Date

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LIST OF ABREVATION

ATCC	American Type Culture Collection
CIGS	Copper Indium Gallium Selenide
CTAB	Ethyl alcohol, Cetyltrimethyl ammonium bromide
DME	N,N-dimetylformamide
DMF	N,N- dimethylformamide
EDX	Energy Dispersive X-ray spectroscopy
Eg	Energy band gap
E v	Electron volt
FT-IR	Fourier Transform Infrared Spectroscopy
FWHM	Full Width at Half Maximum
GRAS	Generally Recognized As Safe
IR	Infrared
IZ	Inhibition Zone
K.pnmonea	*Klebsiella pneumonia*
KBr	Potasium Bromide
LEDS	Light Emitting Diodes
MW	molecular weight

nD	Refractive Index
NIR	Near Infrared
Nm	Nano meter
NP	Nano particle
PL	Photoluminescence
PVP	Poly (N-vinylpyrrolidine)
SEM	Scanning Electron microscope
S.aureus	Staphylococcus aureus
TGA	Thermal Gravimetric Analysis
UV	Ultera-Violet
XRD	X –Ray Diffraction

ABSTRACT

The progress of green chemistry in the synthesis of nanoparticles using plants exstract has attracting a great attention nowadays due to its low cost, simple to handle, non-toxic and environmentally-friendly in nature. The present study focuses on the green synthesis and characterization of pure zinc oxide nanoparticles using different ratios of calpurnea aurea leaf extract for antibacterial application on the drug resistant bacteria. Zinc acetate dihydrate was used as precursor to synthesize ZnO nanoparticles using the calpurnea aurea leaf extract. Zinc acetate dihydrate was used to synthesize ZnO nanoparticles. The synthesized nanoparticles were characterized by advanced techniques like Thermo Gravimetric Analysis (TGA), X-Ray Diffraction (XRD, Scanning Electron Microscopy (SEM), Energy dispersive X-ray and Fourier Transform Infrared Spectroscopy (FTIR). TGA was performed for ZnO nanoparticles to study the thermal stability of materials. The XRD analysis reveals all the synthesized particles have pure hexagonal phase with particle size in the range of 8.43-27.3nm. The SEM analysis indicates the spherical shape morphology for the samples that calcinated at 400 ^{0}C and grain size increases with the decrease of the concentration of the capping agents. EDX spectrum showed the elemental composition of ZnO NPs. The UV-Visible result showed the absorbance peak at 454nm, 466nm and 431nm corresponding to the characteristic band of zinc oxide nanoparticles (1:1), (3:2),and(9:1)respectively. The FT-IR analysis shows the interaction between the capping agents and the synthesized ZnO nanoparticle. The antibacterial activity studies of the synthesized materials against selected bacteria gram-posative (Staphylococcus aureus,) and gram-negative(Klebsiella pneumonia) have been checked. The synthesized ZnONPs show antibacterial activity against both gram-posative and gram-negative bacteria,the gram-negative show more resistance than the gram-posative bacteria.

Keywords: *Calpurnia aurea, Zincoxide nanoparticles, Strophylococcus aurea, klebsiella pneumonea*

1. INTRODUCTION

1.1 .Background of the study

Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new material at the nanoscale [1]. In nanotechnology, a nanoparticle (NP) is defined as a small object that behaves as a whole unit in terms of its transport and properties. Nanotechnology involves synthesis, characterization and application of nanomaterial's and devices by controlling shape and size. In the field of nanotechnology, metal nanoparticles have attained a great importance due to their unique optical, catalytic, electronic, magnetic and antimicrobial properties owing to their small size [2-4].

The field of nanotechnology is one of the most active researches now days in modern material science and technology. Nanoparticles are particles that have at least one dimension that is1-100 nm in size. Physical and chemical methods are more popular for nanoparticle synthesis but the use of toxic compounds and expensive processing and instruments limits their application. Synthesis of nanoparticles can be performed using a number of routinely used chemical methods such as chemical precipitation sonochemical, solvothermal, sol-gel process, hydrothermal decomposition *etc* [5-7].

An eco- friendly green mediated synthesis of inorganic nanoparticle is a fast growing research in the limb of nanotechnology [8, 9]. Although, physical and chemical methods are prevalent in nanoparticles synthesis, the green synthesis is the best improvement due to the protection of the environment as well as the synthesized nanoparticles with small size and large surface area. The plant phytochemicals with antioxidant properties is responsible for the synthesis of metal and metal oxide nanoparticles. This benign reaction is quite rapid, readily conducted at room temperature and pressure, and easily scaled up. Among the metal oxide nanoparticles, zinc oxide is interesting because of its physical and chemical properties such as stability, high catalytic activity, luminous transmittance, effective antibacterial property, intensive IR and UV absorption [10, 11]. ZnO nanoparticles are reported by several studies as non-toxic to human cells [12, 13]. ZnO have extensive applications in water purification [14]. This aspect necessitated their usage as antibacterial agents, noxious to microorganisms and hold good biocompatibility to human cells [15].

Laterally, synthesis of nanoparticles has been accomplished by bacteria, fungi, actinomycetes [16]. Moreover, the use of the extract of neem, *Camellia sinensis, Corriandrum, Nelumbolicifera, Ocimum sanctum, Aloe Vera, Calpurnea aurea,* and many other plants comply with the principles of green chemistry and is environmentally benign [17].

In Ethiopia, *Calpurnia aurea* (locally known as "ceekaa" in Afan Oromo) is used traditional medicine as an insecticide as well as for the treatment of amoebic dysentery and diarrhea in humans as well as animals. It is better to develop antibacterial from natural product source which are more active as well as effective and tolerate resistance from the bacteria. The extracts of the leaves and steam of Calpurnia aurea contain antioxidants and antibacterial properties [18]. In this research work the extracts of the leaves of *Calpurnia auria* would be used as capping (antioxidant agents) for the preparation of ZnO nanaparticles.

The antibacterial activity of zinc oxide nanoparticles is well known and hence make use of this property to inhibit growth of gram positive bacteria (Staphylococcus aureus) and gram-negative bacteria (*Klebsiella pneumonia)* using disc diffusion method. These two bacterial strains will be selected as they are highly contagious.

Hence, evaluation of the potential antibacterial activity of zinc oxide nanoparticles against these pathogenic bacteria was evaluated in this work. These two different types of pathogenic bacteria have been chosen for the study because of the following reasons:

Staphylococcus aureus: is a gram-positive, round-shaped bacterium that is a member of the Firmicutes, and is frequently found in the nose, respiratory tract, and on the skin [19].

***Klebsiella pneumonia*:** Gram- negative bacteria, virulent strain causes pneumonie, bloodstream infection, wound infections, surgical site infections, meningitis, urinary tract infections [20].

1.2. Statements of the Problem.

The rapidly increment in the development of new antibacterial materials has been observed as consequence to the spread of antibiotic resistant infections, which has become a major issue in health care. The emerging infectious diseases and the development of drug resistance bacteria at an alarming rate is a matter of serious concern and increasing public health problem. New strate-

gies for controlling bacterial activity are urgently needed and nanomaterials can be a very promising approach. A possible alternative is the synthesis of new inorganic compounds with antimicrobial properties. Zinc oxide nanoparticle has the advantage of durability as well as chemical and physical stability over common organics and antibiotics compounds. Many research works had been concerned with green synthesis of nanoparticles for the antibacterial applications which is the recent research focus. Hence this research is intended to fill the gap in the area of green synthesis of ZnO nanoparticles from the leaves of *Calpurnia auria* and its application for drug resistant bacteria. Therefore, the work is planned to synthesize zinc oxide nanoparticles using *Calpurnia auria* leaf extract and investigate its effect against gram positive bacteria (*Staphylococcus aureus*) and gram negative bacteria (*Klebsiella pneumonia*).

1.3. Objectives of the study

1.3.1. General Objective

The general objective of this study is green synthesis of zinc oxide nanoparticle (ZnO) using aqueous extract of *Calpurinia aurea* leaves and evaluation of its antibacterial activity.

1.3.2. Specific Objectives

The specific objectives of this work are:

- To synthesis ZnO nanoparticles using aqueous extract of *Calpurinia auria* leaves and the precursor salt zinc acetate dihydrate (Zn $(CH_3COO)_2.2H_2O$).
- To characterize the synthesized ZnO nanoparticles using TGA, XRD, FT-IR, SEM, EDX, Uv.
- To evaluate effect of proportions of $Zn(CH_3COO)_2 2H_2O$ and the plant extract on size and antibacterial activity.
- To evaluate the antibacterial activity of the synthesized ZnO nanopartecles against gram- positive (*staphylococcus aureus*) and gram- negative bacteria (*klebsiella pneumonia*).

1.4. Significance of the Study

This research provides important information about the green synthesis of ZnO nanoparticles by using *Calpurinia auria* leaves extract and its antibacterial activity.

1.5. The Scope of the Study

The scope of this study is limited to green synthesis of pure ZnO nanoparticles using plant extracts of *calpurinia aurea* leaves, characterization of the synthesized NPs using, XRD, FT-IR, SEM, EDX, Uv and TGA techniques and its application for antibacterial activities on selected gram-positive (*staphylococcus aureus*) and gram-negative bacteria (*klebsiella pneumonia).*

2.LITERATURE REVIEW

2.1.Definition of NanoParticles

Nanoparticles are defined as small particles sized between 1 to 100 nanometers in at least one dimension. Currently there are more than 1,317 nanotechnology-based consumer products according to an analysis by Nanotechproject.com [21]. Compared to their counterparts in bulk states, manufactured nanomaterials have the merits of better adjustable electronic properties, better tunable optical properties, and higher reactivity [22].

2.2 Synthesis of ZnO NanoParticles

Synthesis of dimensionally controlled particles in large quantities and study of their properties to investigate novel applications is a preferred activity for researchers in nanomaterials. There are several ways of preparation of ZnO nanoparticles. These methods are: physical method, chemical method and biological method [23].

2.2.1.Physical method

Physical methods developed for synthesis of ZnO nanoparticles often require special equipment's or operational control. The physical method often called top-down approach which includes methods like diffusion, irradiation, thermal decomposition, and arc discharge. The thermal decomposition method is one of the significant top-down approaches. It is used for the creation of mono disperse nanoparticles. These approaches use larger initial structures or macroscopic units which can be externally controlled in the processing of nanostructures. Etching through the mask, ball milling and application of severe plastic deformation are common examples of physical top-down method of synthesis of nanoparticles. The top-down method of synthesis is a model which generates on a larger scale for further reduction of nanoscale. Mostly the top-down methods are not cheap and quick to manufacture. This method is slow and not suitable for large scale production [24, 25].

2.2.2.Chemical method

Moderate reaction conditions and convenient synthetic manipulation have rendered chemical methods an attractive option for accessing ZnO nanoparticles. Traditional chemical routes that are frequently used in recent years include sol-gel approach, chemical precipitation and colloidal Synthesis. Surfactants or polymer based preparative method for ZnO nanoparticles are also quite popular to avoid agglomeration and achieve better control of ZnO nanoparticles. Removal of surfactants or polymers, however, poses serious limitations to such methods. Hence, delicate and precise control of experimental factors for the synthesis of nanoparticles with desired morphologies is a cherished goal for future device applications. Homogeneous chemical precipitation method is often considered economically viable for preparation of mono disperse metal oxide particles of different shapes and sizes. The method also provides better control of chemical and morphological characteristics.

Chemical methods, including precipitation from inorganic or organic solutions and sol-gel techniques can conveniently provide control of nucleation, growth and ageing of particles in the solution. The methods rely on advanced solution and coordination chemistry theories, enabling the synthesis of the required precursor particles utilizing a variety of parameters to enable control of the solid formation process. A major contribution to the growth of particles is through Ostwald ripening, where small particles with lower solubility product dissolve and re-precipitate on the surface of larger particles in solution. Agglomeration takes place in solution as the particles clog together to minimize surface energy [26].

Mostly the chemical methods of synthesis of nanoparticles are the bottom up approaches. These approaches include the miniaturization of material components (up to atomic level) with further self-assembly processes leading to the formation of nanostructures. During self-assembly the physical forces operating at nano scale are used to combine basic units into larger stable structures. Typical examples are quantum dot formation during epitaxial growth and formation of nanoparticles from colloidal dispersion. In these methods different metal particles are reduced to form nanoparticles. Various types of metal particles used like Sodium borohydrate and Sodium citrate etc [27]. Other chemical reagents used are N,N-dimethylformamide (DMF), Poly (N-vinyl pyrrolidine (PVP), ethyl alcohol, Cetyltrimethyl ammonium bromide (CTAB),*etc* [28].This

method initiates with a pattern which is generate larger scale, then reduced to nano scale. This method of synthesis of nanoparticles is cheap and quick to manufacture.

2.2.3.Biological method

Green synthesis method is proved as beneficial over other methods which are implemented for the synthesis of nanoparticles. Green synthesis methods are eco-friendly approach and compatible for pharmaceutical and other biomedical applications, as the toxic chemicals are not used in these methods. Besides, this method does not require high pressure, temperature. Synthesis of various nanoparticles by microorganisms is used more than the other techniques due to its eco-friendly nature. The use of environmentally beneficial materials like plant leaf extract, bacteria, fungi and enzymes for the synthesis of zinc nanoparticles proposes abundant benefits of eco-friendliness and compatibility for pharmaceutical and many other biomedical applications [29]. Lot of attention has been diverted to the green synthesis of metal nanoparticles using biological material as the reducing and stabilizing agents and due to the usage of eco-friendly, non-toxic and safe reagents during the biosynthesis process, green synthesis has been considered in the field of toxic chemical and physical methods [30].In the biological method, plant extracts are used for controlled and precise synthesis of several metallic nanoparticles. High surface and a large fraction of surface atoms are responsible for the nanoparticles' atom-like behavior [31]. Despite the fact that conventional methods use less time for synthesizing nanoparticles, they contribute to environmental toxicity because they require toxic chemicals as capping agents. Green nanotechnology is an eco-friendly alternative and is cost effective [32] and utilizes proteins as natural capping agents. Synthesis of metal nanoparticles by plants utilizes various secondary metabolites, enzymes, proteins and or other reducing agents.

2.3.ZnO Nanoparticles

Zinc oxide (ZnO) is a class of inorganic metal oxides available and exhibits a wide range of nanostructures. Photo catalytic and photo oxidizing ability against chemical and biological species are used to characterize these metal oxides. U.S.Food and Drug Administration have recognized ZnO is considered "generally recognized as safe" (GRAS) [33].Lower cost, UV blocking properties, high catalytic activity, large surface area, white appearance and their remarkable applications in the field of medicine and agriculture are the advantages of ZnONPs particles. Re-

cently, ZnO have been used extensively in environmental remediation and antibacterial activity [34].

ZnO nanoparticles exhibit strong antibacterial activity against high temperature and pressure resistant spores. It is postulated that the generation of hydrogen peroxide or due to the electrostatic binding of the particles on the microbial surface contribute to the antimicrobial activity of ZnO nanoparticles [35]. Antibacterial activity of ZnO nanoparticles is of remarkable applications in designing microbial resistant articles for preserving food and wood products, cosmetics, novel nanomedicines, wound dressing and disinfecting agents. Photo catalytic activity of ZnO nanoparticles offers a promising method for waste water treatment [36] .Toxic water pollutants released from textile and dying industries by utilizing natural source of energy, sunlight are degraded by ZnO and exhibit photochemical reactivity. This could be because of the presence of many active sites and fabrication of hydroxyl radicals on ZnO surface [37].

Zinc oxide has vast applications in optical, piezoelectric, magnetic, and gas sensing. They exhibits high catalytic efficiency, strong adsorption ability and used in sunscreens manufacture, ceramics and rubber processing, waste water treatment, and fungicide. ZnO nanoparticles can absorb UV radiation and therefore offers better protection and improved opaqueness [38].

2.4 Properties of Zinc Oxide Nanoparticles

The semiconductors with the dimensions in the nanometer are important because of their electrical, optical and chemical properties which can be tuned by the changing the size of particles.

2.4.1 Electrical Properties

The fundamental study of the electrical properties of ZnO nanostructures is crucial for developing their future applications in nanoelectronics. ZnO has a moderately large band gap of 3.3 eV at room temperature. The benefits of a large band gap comprise higher values of breakdown voltages, sustaining large electric fields, high-temperature and high-power operations. ZnO has n-type character, in the absence of doping. The origin of n-type character is typically the non-stoichiometry analysis. Due to the imperfections like the oxygen vacancies, zinc interstitials and ZnO nanowires are reportedly show n-type semiconductor behavior. The major hindrance of ZnO

is the broad series of applications in electronics and photonics with the complexity of p-type doping. The favorable and advantageous p-type doping for ZnO nanostructures will enormously enhance their upcoming advance applications in nano scale electronics and optoelectronics. The p-type and n-type ZnO nanowires can serve as p-n junction diodes and the light emitting diodes (LED) [39].

2.4.2 Optical Properties

Zinc oxide is generally transparent to visible light but strongly absorbs ultra violet light below 365.5 nm. The absorption is typically stronger than other white pigments. In the region of visible wavelengths, regular zinc oxide appears white, but, rutile and anatase titanium dioxide have a higher reactive index and thus has a superior opacity. ZnO is a wide band gap of 3.4 eV semiconductor with a large exciton binding energy of 60 meV and also considered as one of the most promising semiconductor material for electronic, photonic, optical and biological applications [40]. The band gap energy occurs between the valence and conducting bands corresponds to the energy of 3655 photons. Zinc oxide is considered as the photoconductive under the analytical study of ultra violet light.

The arrangement of optical and semiconductor properties construct a doped zinc oxide which is a contend or for new generations of devices. Solar cells require intrinsic optical properties of ZnO nanostructures are being intensively deliberated for the implementing photonic devices. Photoluminescence (PL) spectra of ZnO nanostructures have been extensively reported. Excitonic emissions have been examined from the photoluminescence spectra of the ZnO nanorods. It is shown that quantum size confinement can significantly enhance the exciton binding energy. Strong emission peak at 380 nm due to band-to-band transition and green-yellow emission band related to oxygen vacancy are observed. Photoluminescence spectra show that the ZnO nanowire is a capable and promising material for the UV emission. Its UV lasing property is additionally more significant and interesting. Due to its near-cylindrical geometry and large refractive index of nearly about 2.0, ZnO nanowire or nanorod is a natural candidate for the optical waveguide.

The additional advantages of ZnO nanowire lasers are that the excitonic recombination lowers the threshold of lasing, and quantum confinement yields a substantial density of the band edges and enhances radiative efficiency. Optical wave guiding using dielectric nanowire also achieved con-

siderable progress. In recent times, ZnO nanowires were stated as sub-wavelength optical waveguide. Optically forced light emission was guided by ZnO nanowire and coupled into SnO_2 nanoribbon. These discoveries show that the ZnO nanostructures can be potential building blocks for integrated optoelectronic circuits. Optical properties have a great interest towards the vast application of optoelectronics, photovoltaics and biological sensing [41].

2.4.3 Chemical Properties

Zinc oxide occurs as the mineral zincite or as white powder known as zinc white. It is usually orange or red in color due to manganese impurity. Crystalline zinc oxide is thermochromic in nature which changes from white to yellow color when heated and turns back to white color on cooling. This change in color is caused by a very small loss of oxygen at high temperatures. Zinc oxide is amphoteric in nature which reacts with both the acids and alkalis. When ZnO reacts with acid, it forms zinc sulfate. Similarly when it reacts with alkali, it forms zincates. ZnO decomposes to form zinc vapor and oxygen indicating its considerable stability. ZnO decomposes to form zinc vapor and oxygen indicating its considerable stability. Exposure of zinc oxide to air absorbs both water vapor and carbon dioxide and forms zinc carbonate [42].

2.5 Antibacterial Activity of ZnO NanoParticles

The biological method of the ZnO NPs synthesis is gaining importance due to its simplicity, eco-friendliness and extensive antimicrobial activity [43]. The main factor for the increase of the resistance pathogenic bacteria is over use of antibiotics and this has led to the emergence and spread of resistant pathogens and resistant genes in them [44]. The studies about toxicity have shown that zinc ions do not cause any damage to the DNA of human cells. The antibacterial activity of ZnO nanoparticles is due in part to their electrostatic interaction with cell surfaces which shows that on contact with bacteria, the cytotoxic actions of ZnO nanoparticles ruptures the lipid bilayer of bacterium, resulting in leakage of the cytoplasmic substances [45]. Nanoparticles as antimicrobial agents are their better efficiency on resistant bacteria, less toxicity and heat resistance. Among metal oxide nanoparticles, ZnO have many significant features such as chemical and physical stability, high catalysis and effective antibacterial activity [45].

The possible mechanism for the bactericidal activity, explained as follows, it involves the factors including mainly reactive oxygen species (ROS) and the release of Zn ion. It has been found that the activity depends up on crystallite size, morphology, composition, specific surface and phase of crystalline material. Because of larger surface area than the bulk and distinguishable porosity as the particle size reduces, greater number of reactive oxygen species (ROS), namely O_2^-, hydrogen peroxide (H_2O_2), OH^-, and organic hydroperoxides are generated due to small crystallites, which in turn are considered responsible for the damage of cellular composition such as lipids, phosphorus-containing elements (DNA) and disabling of proteins by oxidative stress. It should be noted that oxidative stress results when reactive oxygen species production surpasses the capability of antioxidant defense of the cell. Moreover, that the ZnO nanoparticles have positive zeta potential. Owing to this, nanoparticles provide enhanced particle surface reactivity and adherence with the microbial pathogens, and so surface properties also affect the interactions with the cell walls of the bacteria by allowing easy penetration into the bacteria. The exposure of nanoparticles to the surface of bacteria or accumulation of nanoparticles in the cell or even in the cytoplasm inhibit several functions in the cell thereby inducing structural abnormalities such as distraction of cellular function or deformation or rupture or blebs in membranes, leading eventually to death of bacterial cells [46, 47].

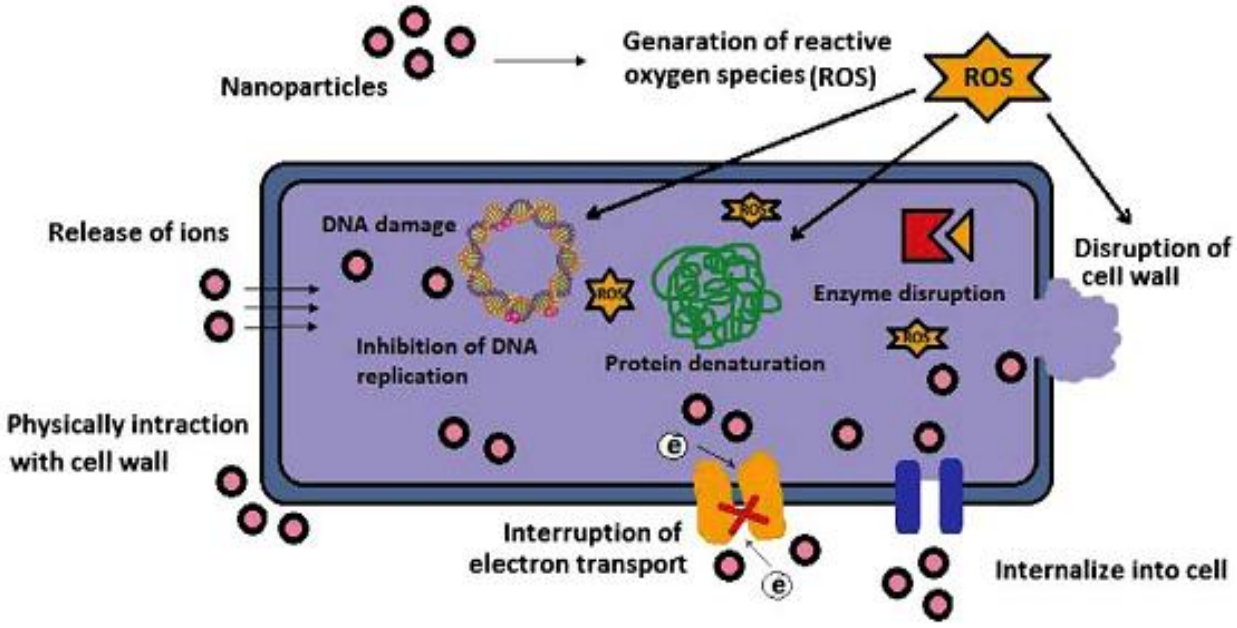


Figure- 1 Various mechanisms of antimicrobial activity of the metal nanoparticles [47].

Figure-1 shown the mechanism by which ZnO NPs deactivate (inhibit) or kill bacteria by generating the reactive oxygen species (ROS) and the ion of Zn.

2.6 Medicinal properties of Calpurnia aurea

The genus Calpurnia is represented by shrubs or small trees, and has about 7 species all of which except one are confined in South Africa. *C. aurea* is a yellow flowered fabaceae small tree or shrub (1 to 10 m tall) widely distributed in Africa from Cape Province in South Africa to Ethiopia and which also occurs in Southern India [48]. In Ethiopia, *C. aurea* (locally known as "ceekaa" in Afan Oromo) is used in indigenous medicine as an insecticide as well as for the treatment of amoebic dysentery and diarrhea in humans as well as animals. The extracts of the leaves in particular are used for killing head lice in humans and ticks in cattle/calves. The head is washed with the juice from the leaves to treat Tinea capitis. Powdered seeds are pasted with honey and swallowed to treat syphilis and the ground fruits are mixed with butter and applied topically for treating scabies [49, 50]

In addition, the leaves are used for the treatment of diarrhea, leishmaniasis, Tinea capitis, and wound and as an insecticide, seeds for eczema, toothache, root for lung TB, swelling, cough and snake bite. Chemical investigations of *C. aurea* have resulted in the isolation of alkaloid. The genus Chemical investigations of *C. aurea* have resulted in the isolation of quinolizidine alkaloids. The leaves and twigs of Ethiopian *C. aurea* yielded 13-hydroxylupanine. The South African species yielded the well known alkaloids: hydroxylupanine, calpurnine, virgiline and its pyrrolylcarboxylic acid ester as found in Ethiopian sample. In addition, the alkaloid 10, 13-dihydroxylupanine was found in CH_2Cl_2 extract of the pods. This compound having a MW of 280 and also occurring in *Cadia purpurea* was absent from the Ethiopian species. Two alkaloids (calpurmenine and 13-2'-pyrrolylcarboxyl) calpurmenine), specifically present in the South African material were isolated from the extracts of leaves and pods [51, 52]. Natural products from microorganisms have been the primary source of antibiotics, but with the increasing acceptance of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very important because these may serve as promising sources of novel antibiotic prototypes [53, 54].

3 MATERIALS AND METHOD

3.1 Chemicals and solvents

3.1.1 Chemicals

The following chemicals and solvents were used in this research work. Zincacetate dihydrate ($Zn(CH_3COO)_2.2H_2O$,MW=219.5g/mol,98%,made in China), Sodium hydroxide (NaOH, MW=39.99971g/mol, 99.8%,ALPHA CHEMICAL Made in *Indea*), Gentamycine, dimethyl sulfixode DMSO, MW=78.147g/mol,99.5%) Muller-Hinton agar, Ethanol absolute(99.9%,Form Ranchem industry and Trading), and distilled water. All chemicals were analytical grade and used without further purification.

3.1.2 Apparatus

The laboratory apparatus used for this work were the following: Beakers, Conical flasks, funnel, volumetric flasks, volumetric cylinder, Borosilicate glass, mortar and pestle, filter paper (whatman no.1), crucible, cuvette, spatula, analytical balance, centrifuge, centrifuge tube, Magnetic stirrer with hot plate, drying oven, tongs, ruler and Muffle furnace.

3.2 Preparation of *Calpurnea aurea* Leaf Extract

The young, healthy *Calpurnea aurea* leaves were collected from Dembi Dolo town and taken to Jahan Sayo Secondary and Preparatory school. Fresh leaves of *Calpurnia aurea* were thoroughly cleaned with running tap water to remove dust particles and other unwanted impurities, followed by distilled water and air dried at room temperature under shadow for 12days. Then, the dried leaves were taken to Adama Science and Technology University. The Leaves were finely crushed into small pieces and cut it into fine pieces.10g of the leaves were mixed with 200 mL of distilled water in 250mLConical flasks and heated at 60 °C for 20 minutes, until the color of the aqueous solution changes from watery to light brown. The resulting extract was cooled at room temperature and filtered by whatman filter paper no.1 [19].The extract was stored in a refrigerator at 4°C in order to be used for further experiments [56, 57]. Figure 2 shows the plant extract that was used for synthesis of ZnONPs extracted from *Calpurnea aurea* and figure-3 shows the plant leaf of *Calpurnea aurea*.

Figure 2. Calpurnea aurea leaf extract.

Figure3 Picture of leaves of “Ceeka” (Calpurnia aurea) taken from Dembi-Dolo Town (By Kumera Emiru), April 13/2018.

3.3 Synthesis of Zinc Oxide Nanoparticles.

Zinc acetate dihydrate [$Zn(CH_3COO)_2.2H_2O$] was used as precursor to synthesize ZnO nanoparticles using aqueous extract of *Calpurnea aurea* leaf. 0.1M Zinc acetatedihydrate solution was prepared by dissolving 2.195g of Zinc acetate dihydrate in 100 mL destilled water using 250 mL volumetric flask and stored for further use. 1M NaOH was prepared using distilled water to be used as precipitating agent. Then four different samples were prepared by taking different ratios

by volume of precursor salt [Zn $(CH_3COO)_2.2H_2O$] solution and *Calpurnea aurea* leaf extract Sample by volume.

1: Zn $(CH_3COO)_2.2H_2O$: *Calpurnea aurea* Leaf Extract(9:1) ratio (90mL:10mL) labeled as Sample A

2: Zn $(CH_3COO)_2 2H_2O$:*Calpurnea aurea* Leaf Extract (7:3) ratio (70 mL:30mL) labeled as Sample B

3: Zn $(CH_3COO)_2.2H_2O$:*Calpurnea aurea* Leaf Extract (3:2) ratio (60 mL:40mL) labeled as Sample C

4: Zn $(CH3COO)_2.2H_2O$:*Calpurnea aurea* Leaf Extract (1:1) ratio(50 mL:50mL) labeled as sample D

$Zn(CH_3COO)_2.2H_2O$ + Plant extract + 2NaOH $\longrightarrow$ ZnO + $2NaOOCCH_3$ + $3H_2O$ + by products

For sample A (9:1 Ratio)

10 mL of *Calpurnea aurea* leaf extract was added to 90 mL of 0.1M $Zn(CH_3COO)_2.2H_2O$ and 20 mL of 1M NaOH was added to the mixture drop wise. Then, the mixture was stirred continuously for 2 hours at room temperature, while adding drop wise 1M NaOH (0.8g dissolved in 20mL) with magnetic stirrer and resulted in solution with white precipitate. This white precipitate indicate that the formation of ZnO NPs. The precipitate was centrifuged using centrifuge tube in centrifuge with 1000 r.p.m for 15 minutes and washed repeatedly with distilled water for 5 minutes followed by ethanol in order to remove the impurities for 5 minute in a centrifuge. Finally, the precipitate was dried in an oven at 60℃ for 2 hours and grinded to fine powder using agate mortar. The yellowish white powder obtained from the above method was calcineted at 400 ℃ for 1 hour. After calcination, a white powder is obtained.

For sample B (7:3 Ratio)

30mL of *Calpurnea aurea* leaf extract was added to 70mL of 0.1M $Zn(CH_3COO)_2.2H_2O$ and 20mL of 1M NaOH was added to the mixture drop wise.Then the mixture was stirred continuous-

ly for 2 hours at room temperature, while adding drop wise 1M NaOH (20mL of 1MNaOH) ,with magnetic stirrer and resulted in solution with yellowish white precipitate. The precipitate was filtered using centrifuge tube in centrifuge with 1000 r.p.m for 15 minutes and washed repeatedly with distilled water for 5 minutes followed by ethanol in order to remove the impurities for 5 minute in a centrifuge. Finally, the precipitate was dried in an oven at 60℃ for 2 hours and grinded to fine powder using agate mortar. The yellow powder obtained from the above method was calcineted at 400 ℃ for 1 hour.

For Sample: C (3:2 Ratio)

40 mL of *Calpurnea aurea* leaf extract was added to 60 mL of 0.1M $Zn(CH_3COO)_2.2H_2O$ and 20 mL of 1M NaOH was added to the mixture drop wise, while the mixture was stirred continuously for 2 hours with magnetic stirrer at room temperature and resulted in solution with light yellow precipitate. The precipitate was filtered using centrifuge tube in centrifuge with 1000 r.p.m for 15 minutes and washed repeatedly with distilled water for 5 minutes followed by ethanol in order to remove the impurities for 5 minute in a centrifuge. Finally, the precipitate was dried in an oven at 60℃ for 2 hours and grinded to fine powder using agate mortar. The yellow powder obtained from the above method was calcineted at 400 ℃ for 1 hour.

For Sample D (1:1 Ratio)

50 mL of *Calpurnea aurea* leaf extract was added to 50 mL of 0.1M $Zn(CH_3COO)_2.2H_2O$ and 20 mL of 1M NaOH was added to the mixture drop wise, while the mixture was stirred continuously for 2 hours with magnetic stirrer at room temperature and resulted in solution with yellow precipitate. The precipitate was filtered using centrifuge tube in centrifuge with 1000 r.p.m for 15 minutes and washed repeatedly with distilled water for 5 minutes followed by ethanol in order to remove the impurities for 5 minute in a centrifuge. Finally, the precipitate was dried in an oven at 60℃ for 2hrs and grinded to fine powder using agate mortar. The yellow powder obtained from the above method was calcinated at 600 ℃ for 1 hour.

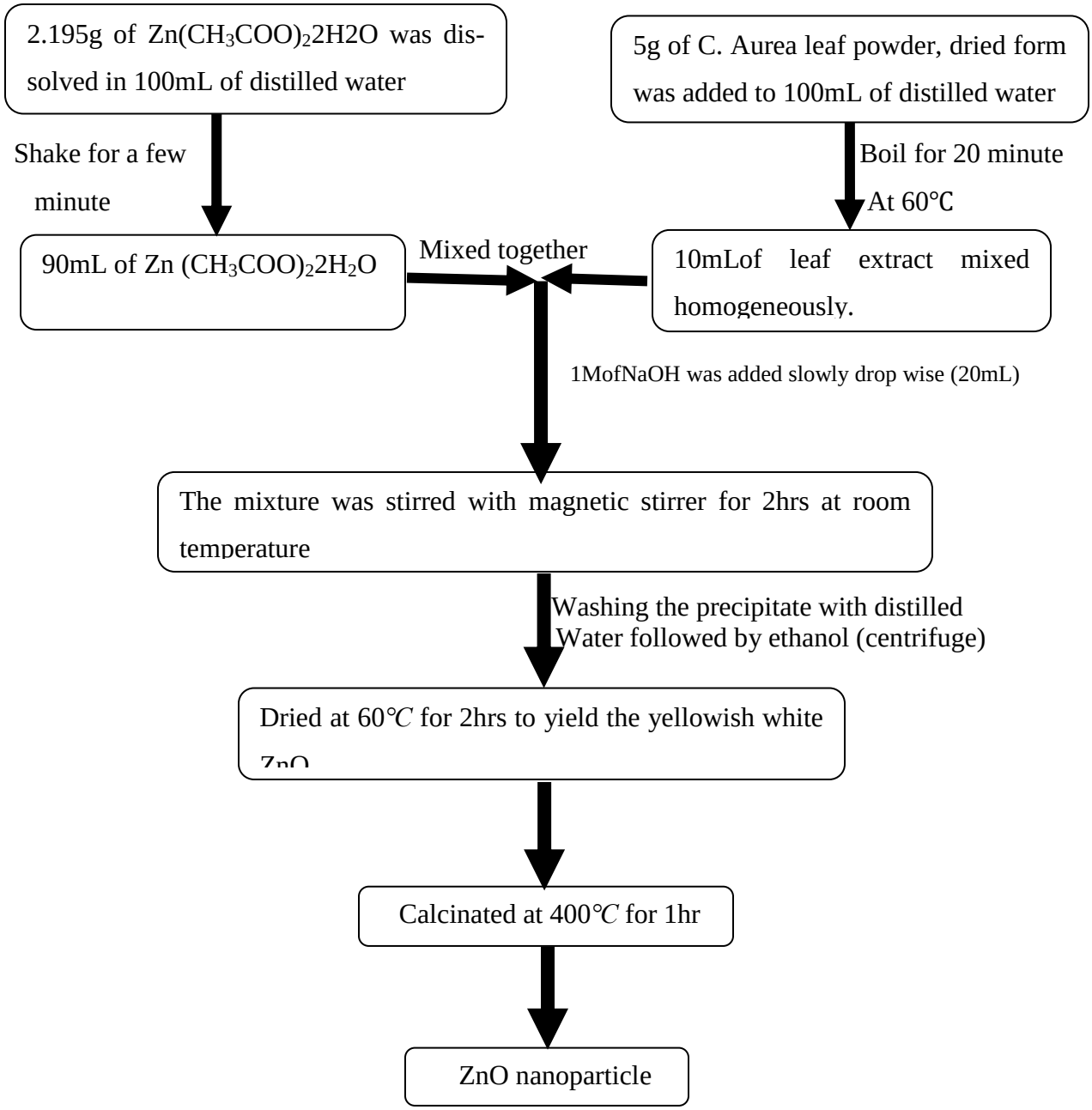


Figure4 A flow diagram of preparation of ZnO nanoparticles from zincacetate dehydrate and

C.aurea leaf extract (for 9:1) ratio. The flow diagram given on fig.4 shows the procedure of preparation of ZnONPs usig zinc acetatedihydrate and C.aurea leaf extract. This works for all the rest ratios by changing the volum of the salt and the plant extract.

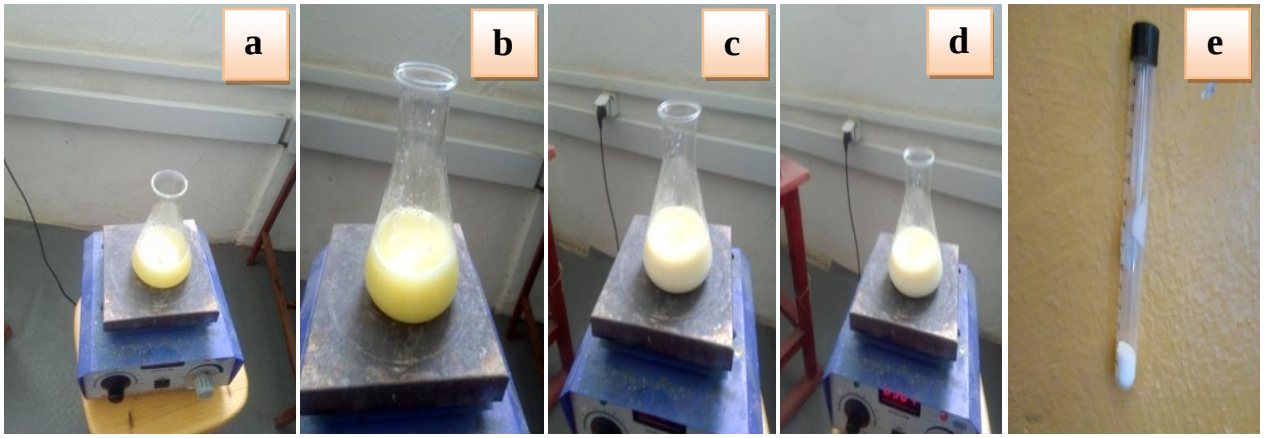


Figure5 (9:1 ratio) color change progress during the synthesis of ZnO nanoparticle from zinc acetate dehydrate and Calpurnea aurea plant leaf extract

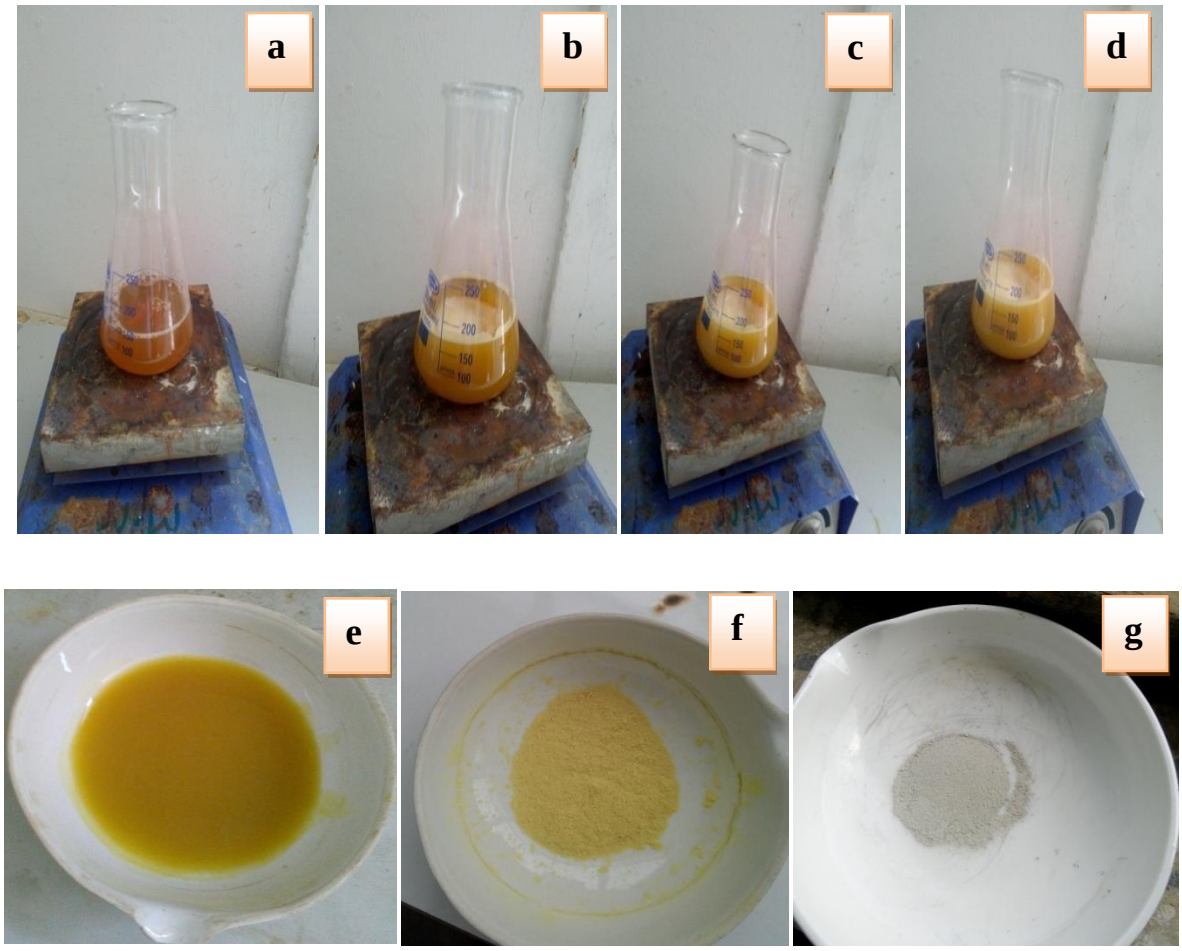


Figure 6 (1:1 ratio) color change progress during the synthesis of ZnO nanoparticle from zinc acetate dehydrate and Calpurnia aurea plant leaf extract and color of the product obtaind

In typical synthesis, 100mL of 0.1 M zinc acetate dehydrate (2.195g) was added first to leaf extract in all ratios with continuous stirring at room temperature for complete dissolution. Subsequently 20 mL of 1.0 M NaOH solution was slowly added under stirring to increase the pH value and in turn to form the precipitation [19]. A progressive change in the color of solution was observed during the stirring process and the change from sturdy brown to yellowish white confirms the nanoparticles formation. But the yellowish white color is not observed for all ratios, for some of them, only a yellow color is observed.

$Zn(CH_3COOH)_2.2H_2O$ + Plant extract + NaOH ⟶ ZnO +2$NaCH_3COO$ + $3H_2O$ + byproducts

The obtained precipitate was centrifuged for 15 minutes, washed with distilled water and centrifuged for 5 minutes & also washed with ethanol for 5 minutes to remove the impurity of all the ions produced during the reaction.And, then dried in an oven for 2 hours at 60℃. After the calcinations of precipitation at 400 °C in furnace, yellowish white powder was obtained but not for all ratios. The above figure 5 a ,b, c, e and 6 a, b, c, d, e, f, and g shows the comparison of the color changes observed during the synthesis of ZnONPs starting from the beginning of stirring with magnetic stirrer up to the end of 2hs for 9:1 and 1:1 ratios of salt and plant leaf extract respectively. And also shows the color of their product formed.

3.4 Characterization of ZnO nanoparticles

3.4.1 Thermal and Gravimetric Analysis (TGA)

The thermal Gravimetric analysis (TGA) was carried out using a simultaneous DTA-TG apparatus (DTG-60H, Shimadzu Co., Japan), to determine the calcinations temperature of the synthesized material. Approximately 13.987 mg of the uncalcinated ZnO nanoparticle using zinc acetate dihydrate precursor sample was placed in a platinum crucible on the pan of the microbalance and heated from room temperature to 1000℃, using Al_2O_3 as inert material.

3.4.2 X-Ray Diffraction (XRD)

The crystalline size of the green synthesized zinc oxide nanoparticles were investigated by the X-ray diffraction using X-ray diffractometer (Bruker D8 Advance Diffractometer). XRD spectrum was recorded from 10° to 80° with 2θ angles using CuKα (λ =1.54 Å) radiation operated at 40kV

and 30 mA. Debye Scherrer's equation indicated in equation 1, was used to estimate the crystallite size [58].

$$\mathbf{D = \frac{0.94\,\lambda}{\beta cos\theta}} \quad \textbf{---------- (1)}$$

Where, D is the Crystallite size, λ is the X – ray source wavelength, β is the Full Width at Half Maximum (FWHM) of a peak, and θ is the Bragg diffraction angle.

The lattice parameter such as "a" and "c" are calculated by the formula given below:

$$a = \frac{\lambda}{\sqrt{3}sin\theta}, \quad \text{---------- (2)}$$

$$c = \frac{\lambda}{sin\theta} \quad \text{---------- (3)}$$

3.4.3 Scanning Electron Microscopy (SEM)

In this research work JSM-6480 LV SEM machine was employed to study the morphology of synthesized nanoparticles. The experiment was performed at an accelerating voltage of 30 kV. The slide was coated with platinum and after the platinum coating, the SEM image was taken. SEM reveals information about the surface morphology of materials making up the sample. SEM provides detailed high resolution images of the sample by rastering a focused electron beam across the surface and detecting secondary or back scattered electron signal. In SEM characterization, nanoparticles powder is mounted on a sample holder followed by coating with a conductive metal. The sample is then scanned with focused fine beam of electrons. The surface characteristics of the sample were obtained from the secondary electrons emitted from the sample surface.

3.3.4 Energy Dispersive X- Ray Spectroscopy.

The chemical composition of the green synthesized zinc oxide nanoparticles was estimated by energy-dispersive X-ray spectroscopy (EDX). Energy dispersive X-ray spectroscopy (EDX) is an analytical technique used to determine the elemental analysis or chemical characterization of a sample through interaction in between sample and source of X-ray excitation. At rest, every atom has definite arrangement of electron in discrete energy levels around the nucleus. When high energy beam of radiation hit the sample (matter), it may excite an electron from inner shell and cre-

ate a hole over there. To compensate this vacancy an electrons from outer shell migrate to inner shell, while the difference in energy between outer and inner shell may be generated in the form of X-ray. As, every element has a definite electronic arrangement, when the sample is being hit with high energy electromagnetic radiations it will generate characteristic X-ray this is like fingerprint for every element. The number and energy of X-rays emitted from sample can be measured by a detector.

3.3.5 UV-Visible Spectroscopy

The optical characterization of ZnO NPs was carried out using UV-Vis Spectroscopy in the wave length region of 200 nm-800 nm. Ultraviolet spectra were collected in the range of 200–800 nm at room temperature in 1 cm path quartz cell, and the absorbance at 376 nm, which is the characteristic absorption peak for wurzite hexagonal ZnO, was registered. The band gap value was obtained from the plot of the Kubelka–Munk function versus the energy of the absorbed light.The energy band gap was calculated by the following formula.

$$\mathrm{Eg} = \frac{hV}{\lambda} \quad \text{----------(4)}$$

h=Plank's constant=6.626×10^{-34}Js, V=3.0×10^{8}m/s(speed of light)

λ= wave length in nm. Eg =Energy band gap

3.4.5 Fourier transform infrared (FT-IR) spectroscopy

The synthesized ZnO nanoparticles was characterized by FT-IR Spectrum (Perkin Elmer, 65) in order to analyze and detect surface functional groups at the scanning range of 4000- 400 cm^{-1}.For the FT-IR characterization, the sample will be mixed with solid KBr uniformly and properly, then, compressed to settle down on a thin transparent film and this thin transparent film was used for FT-IR analysis which will be kept in the chamber of the instrument for scanning.

3.4.6 Chemicals used for antibacterial activitys

Materials used for antibacterial activities were: zinc oxide nanoparticles, Nutrient broth 1.3g, Nutrient agar 5.6g, Agar-agar 0.5g, petriplates, antibiotic discs, cotton swabs, zinc oxide nanopar-

ticle sample, Staphylococcusaureus, and k.pneumoniae. Disc diffusion method was used for antimicrobial activity of zinc oxide nanoparticles [55].

3.5.Preparation of Inoculum

Nutrient broth (1.3 g in 100 mL distilled water) was prepared in 2 conical flasks and sterilized. In the first conical flask clinically isolated strain of gram-positive Staphylococcus aureus was inoculated. In the second conical flask clinically isolated strain of gram-negative k.pneumnia was added.These bacterial cultures inoculated in nutrient broth and was kept on rotary shaker for 24 hours at 100 r.p.m.

3.5.1 Antibacterial Activity

The antibacterial activity of calcinated ZnONPs, uncalcinated ZnONPs and also that of calpurnia aurea leaf extract were checked. The technique was performed using agar well diffusion method. The suspension of bacterial cultivation for 24 h from each isolates of *k.pneumnia* and *S. aureus* adjusted to 0.5 Mc Farland turbidity standard described by Padmavathy [59]. About 100 µL of each bacterial suspension was spreading by cotton swabs onto surface of Mueller-Hinton agar plates and left for (10-15 min) until the surface of agar has dried. Then the wells was made into agar at 6mm diameter using cork borer with the distance between well and another more than 22 mm. Then the plates were incubated at 37°C for 24 h, the diameters of inhibition was recorded in millimeter using metric ruler [59].

3.5.2 Disc diffusion method for antibacterial activity

The antibacterial activity of uncalcinated, calcinated ZnO NPs and that of *calpurnea aurea* leaf extra was checked. Antibacterial tests were carried out by the disc diffusion method using the suspension of bacteria spread on nutrient agar. Antibacterial activity of synthesized ZnO nanoparticles was tested against gram positive (*Staphylococcus aureus*) and gram negative (*k.pneumnia*) bacteria by disc diffusion method. Bacterial cultures was maintained on nutrient Muller-Hinton agar at 37℃ and the cultures were kept in appropriate media slants and stored at 4℃ until used. The antibacterial activities was tested by the disc diffusion agar method, which is a test of the antibiotic sensitivity of the bacteria and it uses the antibiotic discs to test the extent to which bacteria

are affected by those antibiotics. Discs used for antibacterial activity were ciprofloxacin. Discs were applied to the surface of an agar plate that was previously dried. The plates were, then turned upside down and incubated at 37℃ for 24 h in an incubator. The plates were shaken gently to allow evenly mixing of bacteria cells and agar. A 60 mm plate accommodated two discs and ZnO nanoparticles without unacceptable overlapping of zones. Agar plate was divided into 3 sections antibiotic disc, zinc oxide nanoparticles sample, and both antibiotic disc and zinc oxide nanoparticle sample. Then 200 mg of ZnO nanoparticles using zinc acetate precursor was dissolved in 10 mL of distilled water, to obtain 20 mg/mL. From the sample, 200 µL of concentration saturated with discs (6 mm diameter disc) was placed on plate and incubated at 37℃ for 24 hours. The diameters of the inhibition zones were calculated. Clear inhibition zones formed around the discs indicate the presence of antibacterial activity. The results were taken by considering the zone of growth and inhibition of the bacteria by the test fractions. Antibacterial activity was evaluated by measuring the diameter (mm) of the inhibition zone (IZ) around the disc against the test organisms by using a ruler [60, 61].

4. RESULTS AND DISCUSSION

4.1 Thermal Gravimetric Analysis (TGA)

The uncalcinated synthsesized ZnO nanoparticles using zinc acetate precursor was analyzed by using TGA/DTA (Differential Thermal Analysis). The weight loss of the investigated materials was analyzed. Figure-7 shows the thermo gravimetric analysis (TGA) and differential thermal analysis (DTA) of the uncalcined ZnO nanoparticles using zinc acetate dihydrated precursor. TGA curve shows mass loss of the sample whereas DTA curve indicates the energy gain or loss during the process. TGA/DTA transition shows a loss of 14.167 % up to 400℃. No considerable loss is observed after this up to 1000℃ . TGA-DTA data for $Zn(OH)_2$ precursor registered strong endothermic peak at 400℃. This indicates that after 400℃ there was no weight loss for the synthesized ZnO nanoparticles indicating the NPs is thermally stable. This temperature was used for calcinations of the synthesized ZnO nanoparticles using zinc acetate precursor

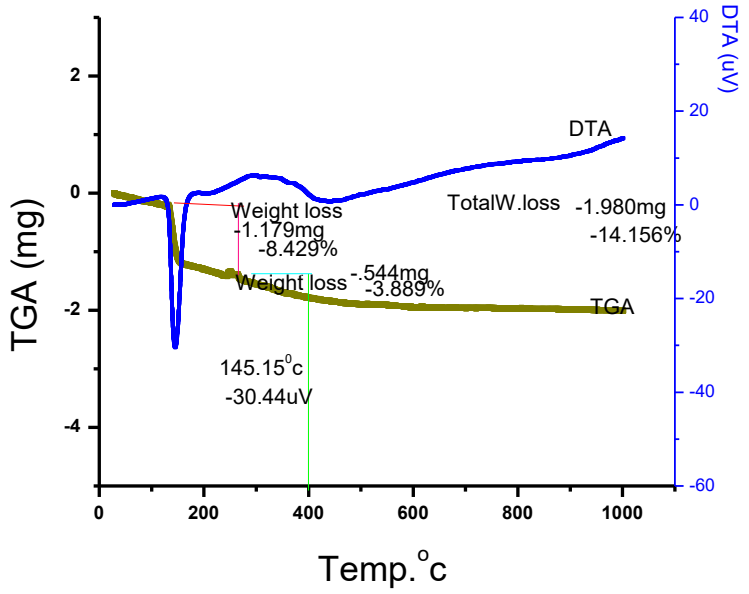


Figure 7 Thermal Analysis result for uncalcinated ZnO NPs using zinc acetate

4.2. X-Ray Diffraction (XRD)

Figure (A, B, C, & D) below shows the XRD patterns of ZnO NPs synthesized from zinc acetate dihydrated ($Zn(CH_3COOH)_2.2H_2O$) precursors, which were synthesized using stoichiometric

amounts of zinc acetate salt at different ratios. As indicated in Table1-4 from zinc acetate dihydrate the diffraction peaks are observed at 2θ of (A): 31.75°, 34.41, 36.23, 47.53, 56.58, 62.83, 67.93 and 69.05° (B):31.83°, 34.46, 36.22, 47.55, 56.62, 62.86, 66.64,67.96 and 68.88° (C):31.84°, 34.40, 36.06, 47.51, 56.60, 62.83, 66.16 and 68.20,° (D): 31.74°, 34.40, 36.23, 47.52, 56.57, 62.84, 66.42,67.94 and 69.08° their corresponding crystal size is given in Table 1-5 respectively [62]. Even if the four ratios were prepared by the same procedure, there is a slight shift of 2theta position for certain peaks. This might be due to the different in ratios of zincacetate dihydrate precursors and plant extract used. The average crystalline size calculated using Debye-Scherrer equation of the diffraction peak was found to be 27.902nm, 10.27nm, 8.43nm, and 11.059nm.for each ratio given above the table respectively(A,B,C, and D

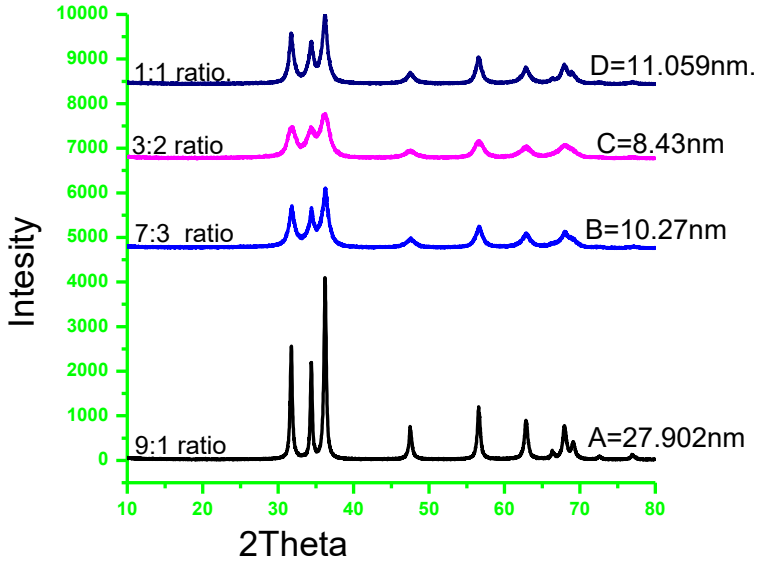


Figure8 XRD pattern of ZnO NPs synthesized from zinc acetate and Calpurnea aurea plant leaf extract (by different ratios A-D).

As we have observed from figure 8, the intense sharper peak is related to the larger crystalline size i.e as the peak sharpness increase the particle size increases, in the other hand, the shorter peak with larger width is smaller in size. From the above figure, 9:1 ratio shows sharper peak with larger size calculated (27.902nm) and the shorter with (3:2 ratio) larger width size have smaller crystalline size calculated (8.43nm).

Table 1.The data information of ZnONPs prepared by the ratio of (9:1) salt- plant extract calcinated at 400***℃***

Peak	2theta	D(A)	I/I1	Intensity	FWHM	Crystalline
1	31.2618	2.85890	5	133	0.2400	35.8723
2	31.7541	2.81569	61	1621	0.33210	25.95524
3	32.3605	2.76430	3	91	0.29500	29.5145
4	34.4086	2.60430	52	1375	0.29860	28.9442
5	36.2320	2.47731	100	2645	0.34910	24.98813
6	47.5255	1.91165	18	475	0.38340	23.62781
7	56.127	1.63740	5	136	0.34280	27.408
8	56.5829	1.62525	31	811	0.39040	27.6605
9	62.8399	1.47763	23	609	0.44580	21.79342
10	63.3632	1.46668	4	98	0.25720	37.88046
11	66.3708	1.40733	4	109	0.30290	32.706367
12	67.4220	1.38792	3	89	0.30000	33.22304
13	67.9339	1.37870	19	515	0.46400	21.54495
14	69.0536	1.35905	9	247	0.53840	19.51133
Average size of the particle						**27.902nm**

Table 2.The XRD data information of ZnONPs prepared by the ratio of (7:3) salt- plant extract calcinated at 400℃

Peak No	2theta	D(A)	I/I1	FWHM	Intensity	Crystalline size size)
1	30.5227	2.92642	5	0.70000	42	14.5230
2	31.8318	2.80900	66	0.95000	514	10.73531
3	34.4584	2.60065	60	1.04000	465	8.346600
4	36.2194	2.47814	100	1.05870	777	8.23936
5	46.3500	1.95736	3	0.88000	26	10.2485
6	47.5494	1.91074	15	1.30000	115	6.96906
7	56.6153	1.62440	36	1.02620	279	9.17668
8	57.9051	1.59125	4	0.5100	30	18.3724
9	61.6038	1.50428	4	0.64000	30	15.08204
10	62.8567	1.47728	22	1.18670	170	8.18776
11	66.6422	1.40225	7	1.32000	55	7.51674
12	67.9718	1.37820	26	1.16000	205	8.61935
13	68.8816	1.36203	16	1.33340	121	7.50052
Average size of the particle						10.27nm

Table 3 .The XRD data information of ZnONPs prepared by the ratio of (3:2) salt- plant extract, calcinated at 400***℃***

Peak No	2theta	D(A)	I/II	Intensity(count)	FWHM	Crystalline size
1	30.3429	2.9433	6	35	0.54660	15.71599
2	31.8461	2.8077	6	413	1.3613	6.33355
3	34.3985	2.6050	6	394	1.5200	5.70974
4	36.0619	2.4886	1	612	1.6900	5.15933
5	38.10353	2.3639	7	44	0.82450	8.83277
6	38.2751	2.3496	5	28	0.6410	14.98547
7	38.47500	2.3378	3	20	0.81340	9.62377
8	47.5069	1.9123	1	91	1.58500	5.71504
9	56.5930	1.6249	3	225	1.38500	6.62364
10	58.1650	1.58476	4	26	0.6400	14.82355
11	62.8334	1.47777	21	131	1.56660	6.20142
12	66.1624	1.41125	5	30	1.04000	9.51457
13	66.5623	1.4037	8	52	-	-
14	68.2001	1.37397	2	166	2.17670	4.59987
Average size of the particle						8.43nm

Table 4. The XRD data information of ZnONPs prepared by the ratio of (1:1) salt- plant extract calcinated a 600℃

Peak No	2theta	d/A	I/I1	Intensity(count)	FWHM	Crystal size
1	30.8623	2.89499	8	74	0.48000	10.2393
2	31.7732	2.81404	69	655	0.76600	11.2535
3	34.4191	2.60353	57	542	0.94140	9.20213
4	36.1804	2.48073	100	945	0.87490	9.96925
5	37.2759	2.41030	12	115	0.63000	13.8886
6	46.5899	1.94784	4	36	0.84000	10.7462
7	47.5494	1.91074	15	140	1.04000	8.71133
8	56.5720	1.62554	39	370	0.8309	11.3313
9	61.8237	1.49946	4	51	0.56000	17.2564
10	62.8334	1.47777	22	205	0.98000	9.91348
11	66.4223	1.40636	6	52	0.78660	12.5902
12	67.9691	1.37807	26	248	1.11870	8.93794
13	68.9216	1.36133	15	138	1.03340	9.73055
Average size						11.059nm

The above table 1-4 shows the XRD data information of ZnONPs prepared by different ratios such as (9:1, 7:3, 3:2, 1:1) salt precursor and plant leaf extract respectively. And based on these data the particle size of each ratio was calculated by using Debye Scherrer's equation.

The crystal structure of ZnO were determined by X-ray diffractometer in the range of 10° to 80° at room temperature. All the indexed diffraction peaks were match with the hexagonal phase ZnO reported in JCPDS card No 36-1451. The sharp intense peak obtained in all samples at 2θ for each ratio is given as follow.

Table 5 Lattice plane of ZnONPs prepared by the 4(9:1, 7:3, 3:2 and 1:1) ratios of salt & plant extract

9:1	$2\boldsymbol{\theta}$	31.75	34.41	36.23	47.53	56.58	62.83	67.93
	Lattice plane	100	002	101	102	110	103	112
7:3	$2\boldsymbol{\theta}$	31.83	34.46	36.22	47.55	56.62	62.86	66.64
	Lattice plane	100	002	101	102	110	103	112
2:3	$2\boldsymbol{\theta}$	31.84	34.40	36.06	47.51	56.60	62.83	66.16
	Lattice plane	100	002	101	102	110	103	200
1:1	$2\boldsymbol{\theta}$	31.84	34.40	36.23	47.52	56.57	62.84	66.42
	Lattice plane	100	002	101	102	110	103	200

The above lattice planes in table 5 confirm that these plane values are closely matched with wurtzite structure of ZnO (JCPDS Card No.36-1451). Additionally, no characteristic peaks other than the ZnO appears which in turn specify the high purity of the sample; the prepared samples are good crystalline in nature with wurtzite hexagonal structure.

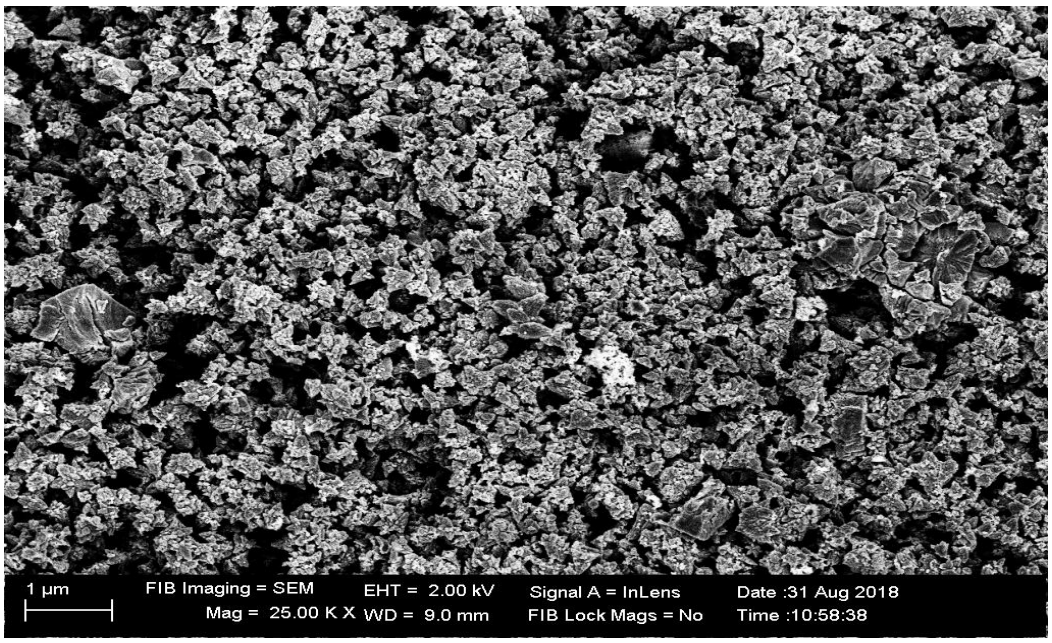
The difference in crystal size may be due to difference in ratios of precursor and plant extract used for making ZnONPs. All diffraction peaks of samples correspond to the characteristic hexagonal wurtzite structure of zinc oxide nanoparticles by comparison with the data from JCPDS card 36-1451with lattice parameters (A, a = 0.358nm, c = 0.620nmB,a = 0.324nm,c = 0.562nm C, a = 0.323nm,c = 0.560nm D, a = 0.325nm,c = 0.564nm). A definite line broadening of the diffraction peaks also resembles that the synthesized particles are in nanometer range and no characteristic peaks were observed other than ZnO peaks, confirming the high purity of the synthesized products [62, 66].

Table 6 d-spacing (d h k l) and Miller indices for 9:1 ratio for ZnO nanoparticle usin zinc acetate (9:1) ratio of salt and plant extract respectively.

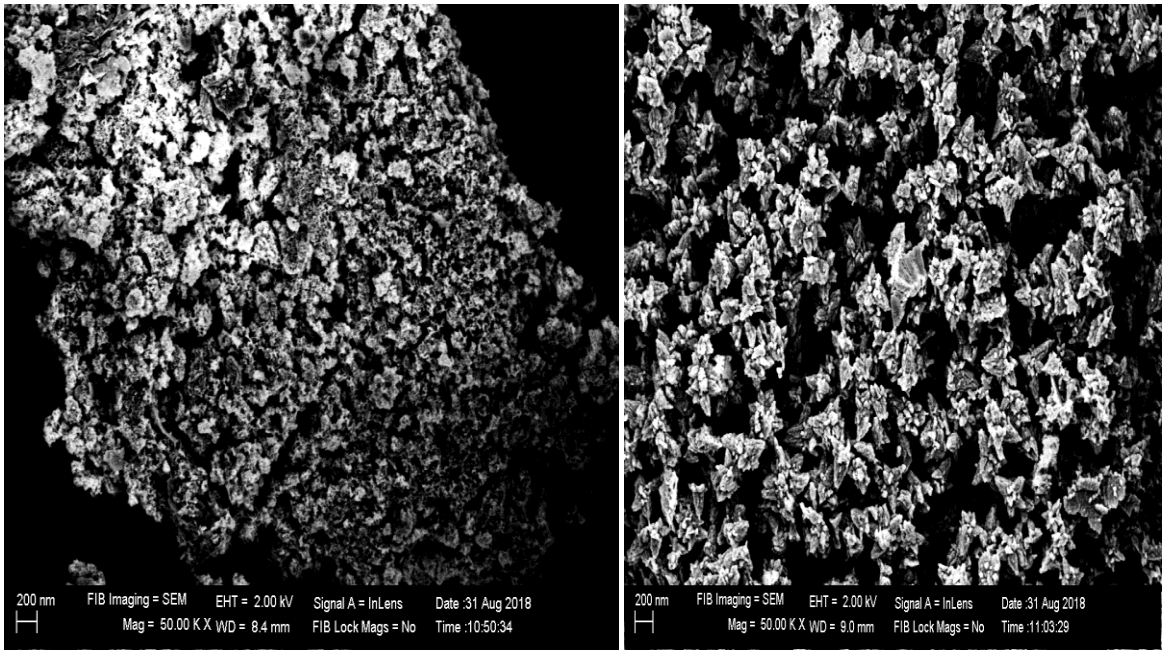
2θ (degree)	d – spacing (dh k l)	Miller Indices h k l
31.7541	2.81569	100
34.4086	2.60430	002
36.2320	2.47731	101
47.5255	1.91165	102
56.5829	1.62525	110
62.8339	1.37870	103
69.0536	1.35905	112

4.3 Scanning Electron Microscopy (SEM) Analysis

The morphology of ZnO nanoparticles were studied using SEM images. The surface morphology of the synthesized samples was viewed through the high resolution scanning electron microscope. SEM images of ZnO NPs have different morphology as shown in Figure 9 (A-C). The SEM images of calcined ZnONPs shown in figure 9 (A-C) indicate the agglomeration of particles that increases with the concentration of zinc acetate. The morphology of ZnO NPs synthesized from zinc acetate in different mixing ratio of reactants (salt and plant extract) such as 9:1, 3:2 and 1:1 have agglomerated spherical shapes. The scanning electron microscope results confirmed that the grain sizes of the synthesized NPs were strongly dependent on mixing ratio of Zinc acetate & leaf extracts under the same reaction conditions.



A



B C

Figure 9 SEM images of ZnO nanoparticles A (1:1), B (3:2) and C(9:1) ratios

The SEM image results agreed with XRD results obtained, i.e. the crystal sizes in XRD with big size have also big shapes in SEM images. This indicates that ZnO NPs have successfully prepared using zinc acetate dihydrate precursor and plant extract within a nano range. The SEM images clearly show micro structural homogeneities and remarkably different morphology for ZnO powder.

4.4. Energy Dispersive X- Ray Spectroscopy.

The composition of the samples was determined by energy dispersive X-Ray spectroscopy (EDX) using scanning electron microscope. The energy dispersive X-ray analysis of all ZnO nano particles using zinc acetate dihydrate at different mixing ratios are shown in figures 10 to 15. It is evident from the X-ray patterns of all the samples in which Zn and O are found in the respective spectrum. In addition to that of Zn and O.the spectrum of other elements is observed. The observed spectrum of other elment may be from the plant matter. Quantitative analyses of the samples are put down in the tables. Also in the tables it was seen that as there is some impurities are found in it.

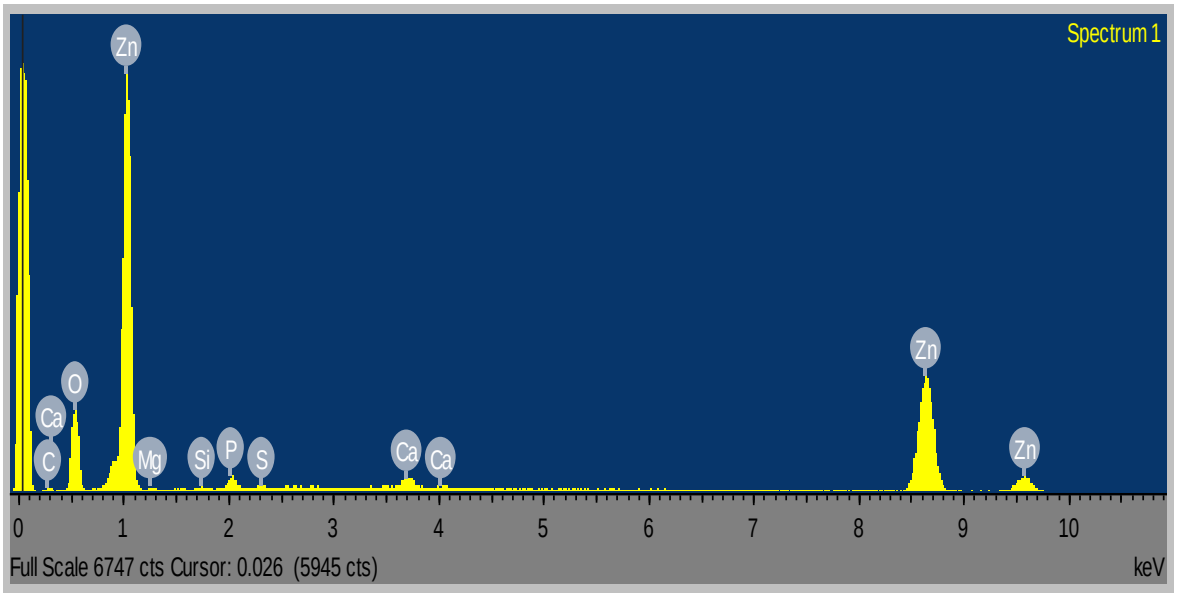


Figure 10 XRD spectrum of ZnONps(1:1)

Table 7 Quantitative analysis of ZnO(1:1)

Element	Wt%	Atomic%
Ok	20.96	45.37
Znk	71.10	37.67
Others	7.94	16.66

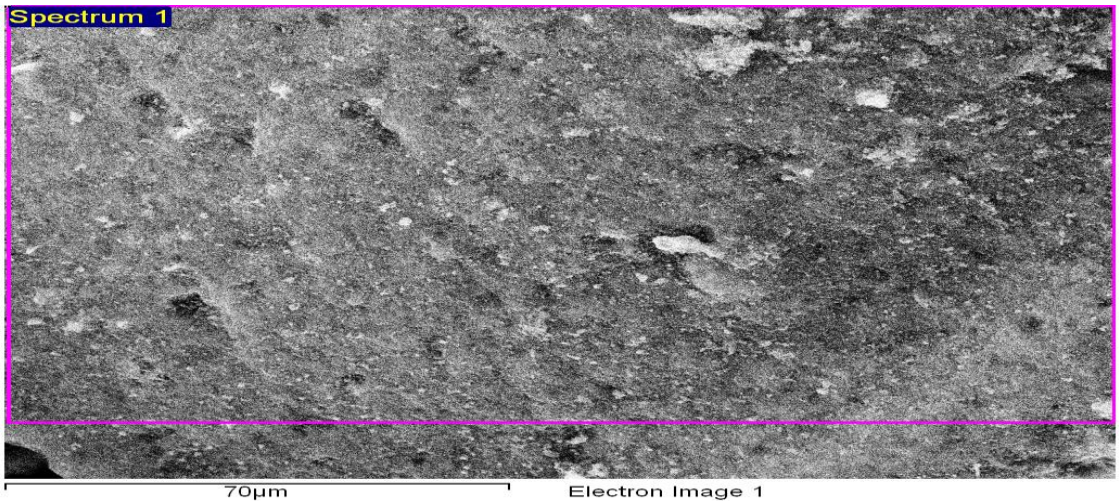


Figure 11 SEM images of selected area for ZnO NPs(1:1)

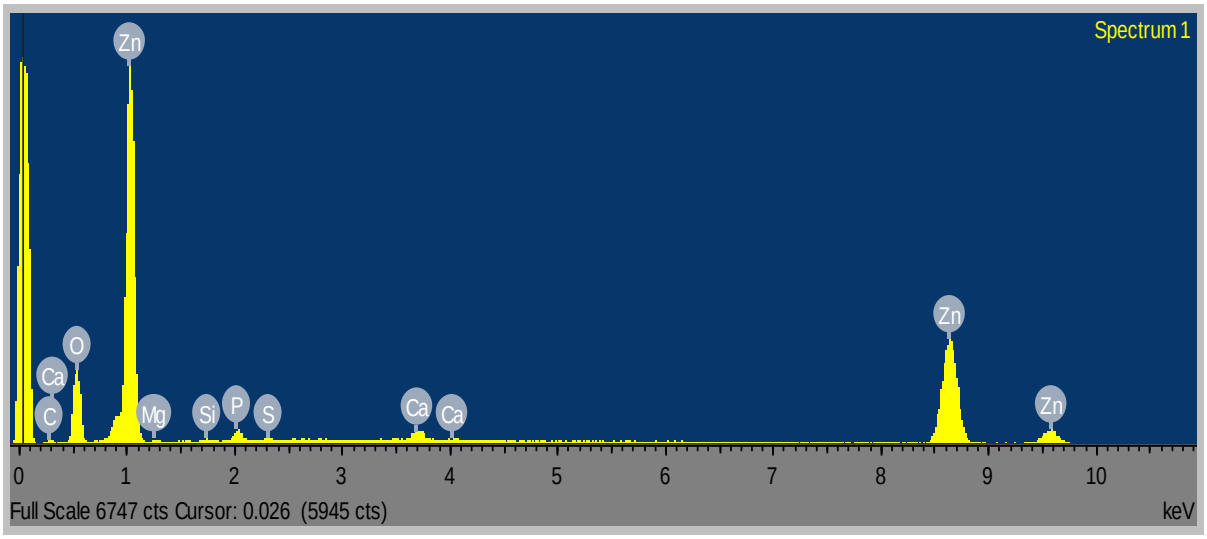


Figure 12 XRD spectrum of ZnO NPs(3:2)

Table 8 Quantitative analysis of ZnONPs (3:2)

Element	Wt%	Atomic%
Ok	20.30	42.03
Znk	69.69	35.31
Others	10.01	22.34

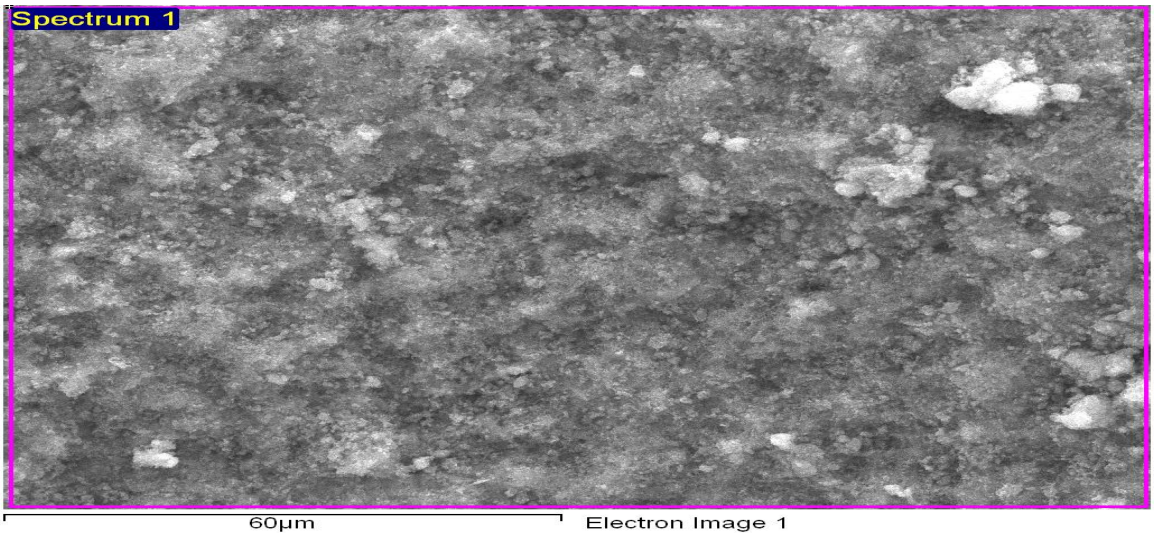


Figure 13 EM images of selected area for ZnO(3:2)

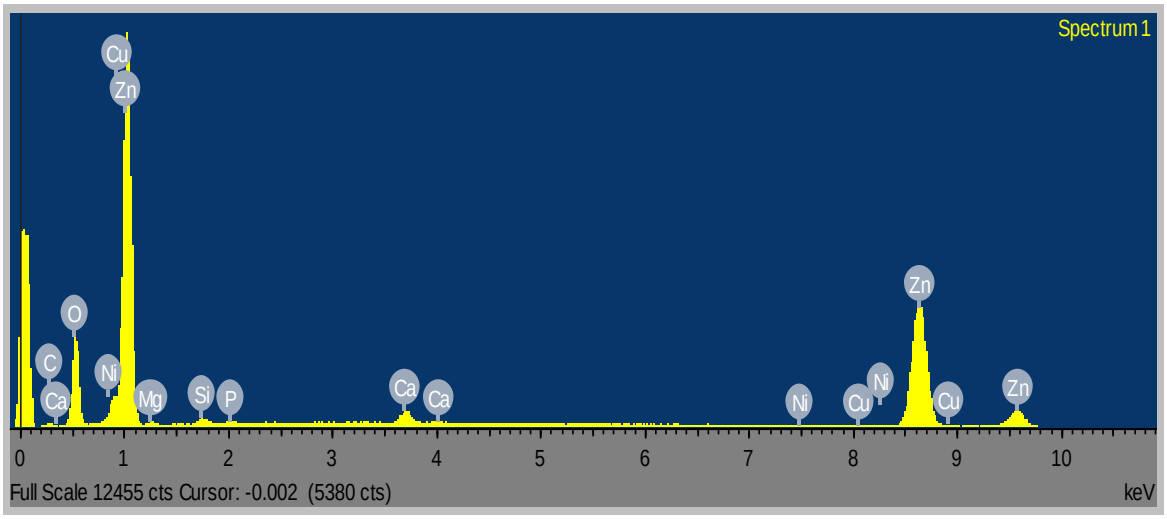


Figure14 EDX spectrum of ZnONPs (9:1)

Table 9 Quantitative analysis ofZnONPs (9:1)

Elements	Wt%	Atomic%
Ok	20.87	45.75
Znk	71.68	38.45
Others	7.45	15.8

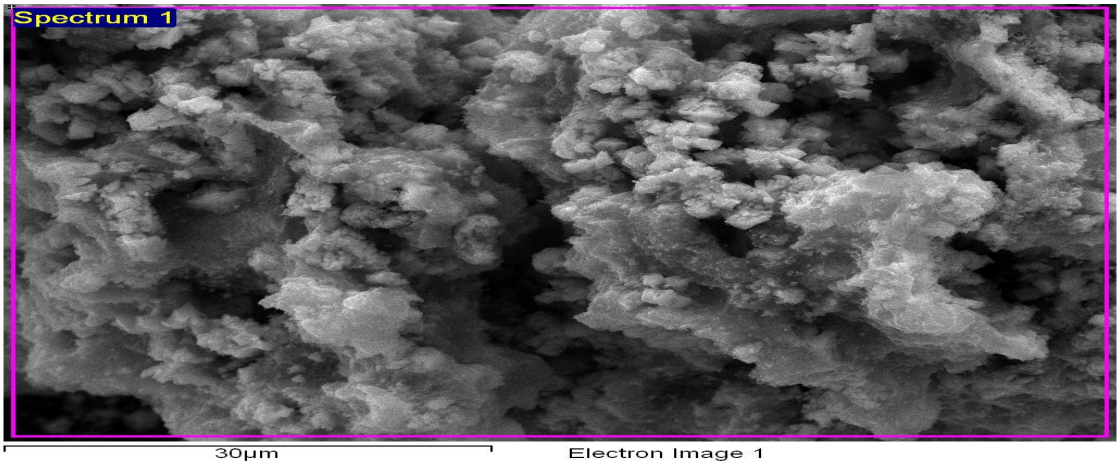


Figure 15 SEM images of selected for ZnO(9:1)

The EDX spectrum shows that in all samples of ZnO nanoparticles which were synthesized using zinc acetate at different ratios zinc and oxygen signals have been detected, and also suggesting tha t there were some other elements were detected as impurity.

4.6. UV – Visible Spectroscopy Analysis

The UV-Vis spectra of calcined ZnO NP prepared by using zinc acetate and *C.aurea* leaf extract with (1:1), (3:2) and (9:1) ratios were shown in figure 16. The absorption peak of the as-prepared ZnONPs was found at around 454 nm, 466nm and431nm and its absorption feature indicates that the ZnO NPs were monodispersed in nature. The excitonic absorption peak observed due to zinc oxide nanoparticles lies much below the band gap wavelength of bulk zinc oxide 388 nm

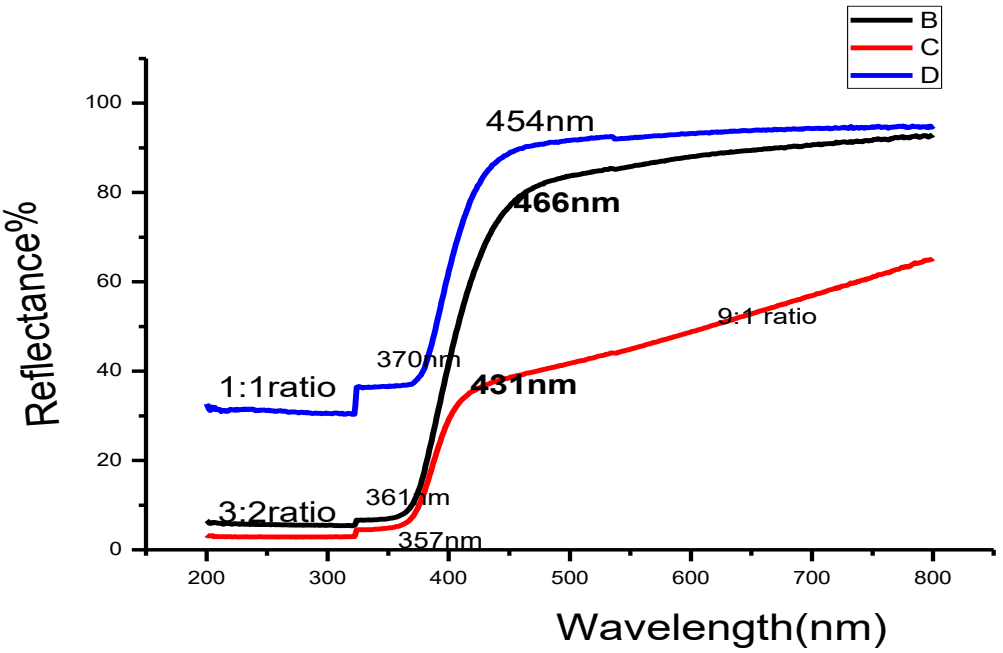


Figure 16Diffuse reflectance spectra of synthesized ZnO nanoparticlesUV-Vis absorbance of calcined ZnO NPs synthesized using zinc acetate dihydrate and Calpurnea aurea leaf extract (1:1),(3:2)and (9:1).

To determine the optical band gap of synthesized ZnO NPs, their reflectance spectra were measured. As seen in Figure 16, the reflectance spectra show a strong decrease around 457nm, 475 nmand431nmfor (1:1), (3:2) and (9:1) respectively. This decrease is related to the electron transitions occurring in the optical band gap. In order to determine the precise value of the band gap, the reflectance values were converted to absorbance by applying the Kubelka–Munk function.As a result, the direct band gap of ZnO NPs was estimated from the plot of (F(R)hυ)2 versus the photon energy (hυ), and the value was 3.883eV,4.374eVand3.858eVrespectevely. The reported band gap values of 3.37 eV for ZnO and 3.24 eV for ZnO pseudo-sphere particles obtained by the precipitation route. This slight variation in band gap values could be attributed to the different levels of oxygen vacancies in the ZnO NPs [67].

4.5. Fourier Transformed Infrared (FT-IR) Spectroscopy Analysis

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

The vibrational phonon and the quality of ZnO NPs were characterized by Fourier Transformed Infrared (FT-IR) in the wave number range of 400-4000 cm^{-1}. Measurements of ZnO NPs were performed by putting the powder of ZnO NPs on KBr plate.

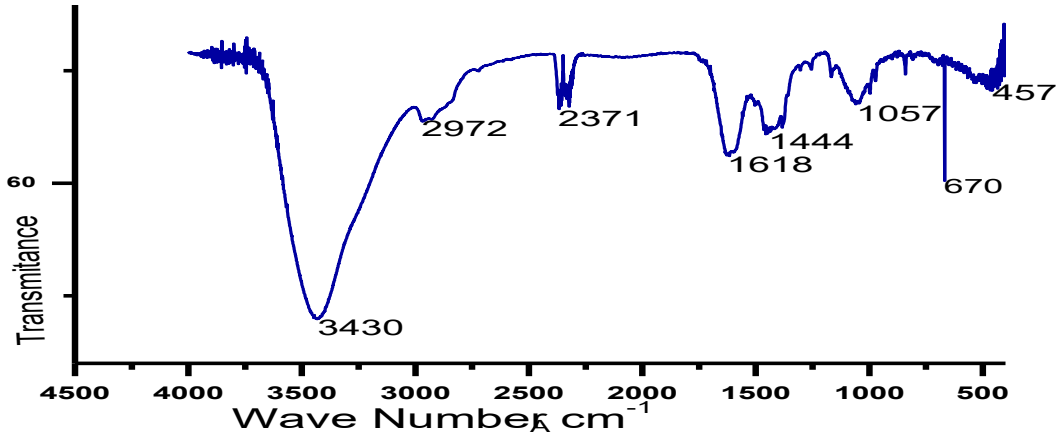


Figure 17 FT-IR patterns for uncalcinated ZnO nanoparticles synthesised using zinc acetate dihydrated and Calipurnea aurea leaf extract.

Figure 16 .Shows the FT-IR spectra of the green synthesized ZnO NPs using zinc acetate before calcinations. FT-IR analysis of the ZnONPs revealed the presence of peaks at 3430, 2972,2371, 1618, 1444, 1057 and 457cm^{-1} (Figure 16).In the spectrum, the wide absorption around 3430 cm^{-1} and 1618cm^{-1} is due to O-H stretching vibrations. As observed from Figure 16, the intense absorption peak at 457cm^{-1} is related to the stretching vibrations of Zn–O bond. Because the stretch for ZnO nanoparticles were found around 400-800 cm^{-1}.Medium absorption in the region 1617-1411cm-1 often implies an aromatic ring. The weak absorption near 2900-2750 cm^{-1} indicates the

presence of aromatic aldehydes [68]. Strong intensity around 1444 cm^{-1} is due to ∂-CH_2 bending vibration.

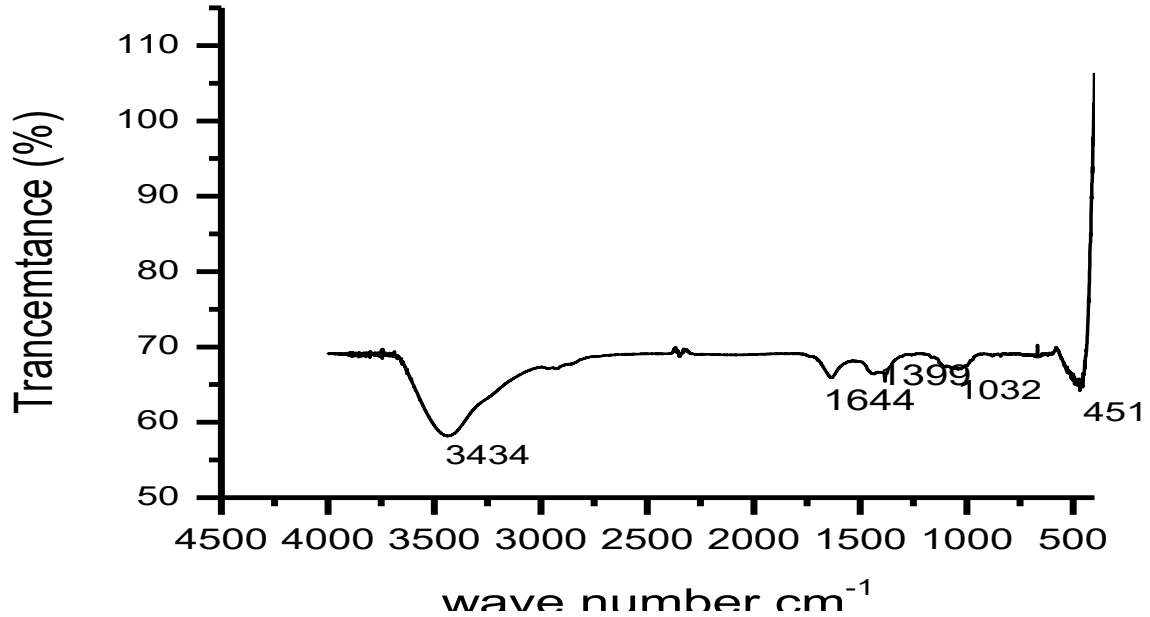


Figure 18 FT-IR pattern for ZnO nanoparticles synthesized using zinc acetate dihyderate andCalpurnea aurea leaf extract after calcination

Figure17. shows the FT-IR spectra of the green synthesized ZnO NPs using zinc acetate after calcination. In the spectrum, the wide absorption around 3434 cm^{-1} is due to O-H stretching vibrations. As observed from Figure17, the intense absorption peak at 451 cm^{-1} is related to the stretching vibrations of Zn–O bond. As compared to calcined ZnO NPs the FT-IR spectra of uncalcined ZnO NPs (Figure 16) displayed a number of absorption peaks. This might be due to chemically bonded functional groups of *Calpurnea aurea* leaf extract used as a capping agent during the synthesis of the materials which was not removed by washing with distilled water and ethanol

4.6. Antibacterial Activity of ZnO NPs

The aim of the present study is to determine the antibacterial activity of ZnO nanoparticles against Gram-positive and Gram-negative bacteria. Staphylococcus aureus (S.aureus), and k.pneumoniae were used as test microorganisms respectively. The antibacterial activity perfor-

mance of ZnO nanoparticles was done by using disc diffusion method. The effects of particle size and concentration on the antibacterial activity of ZnO nanoparticles were studied using this method. These tests were performed in nutrient broth and nutrient agar following standard methods. This method is well documented and standard zones of inhibition have been determined for susceptible and resistant values. The zone of inhibition increases with the increase in zinc oxide nanoparticle concentrationor/ and decrease in particle size. In Table 10 , zone of inhibition (mm) of the tested samples of ZnO NPs against two different bacterial strains were given.

Table 10 Zone of inhibition (mm) of ZnO NPs against gram positive and gram negative bacterial strains.

	Zone of inhibition (mm)(Diameter)	
Samples	Gram positive bacteria	Gram negative bacteria
	Staphylococcusaureus(ATCC25923)	***Klebsiella pneumonia (ATCC700603)***
A (9:1)	8mm	6mm
A (9:1)	9mm	8mm
A (9:1)	9mm	8mm
B (7:3)	9mm	8mm
B (7:3)	10mm	9mm
B (7:3)	9mm	8mm
C (3:2)	10mm	8mm
C (3:2)	10mm	6mm
C (3:2)	8mm	6mm
D (1:1)	13mm	10mm
D(1:1)	13mm	11mm
D(1:1)	10mm	8mm
Un calcinated ZnO NPs	15mm	13mm
Plant extract	6mm	6mm

According to Table 10 ZnO NPs using zinc acetate dihydrate are sensitive to gram positive bacteria (Staphylococcus aureus) and gram negative bacteria (*k.pneumonia*). The results showed that ZnO nanoparticles have antibacterial inhibition zone of 8-to-13mm for gram positive bacteria and 8-to-10 mm for gram negative bacteria at the same concentration of 20 mg/mL against S. aureus

and *k.pneumonia,* respectively. The presence of an inhibition zone clearly indicated the antibacterial effect of ZnO nanoparticles. This indicates that ZnO NPs are more sensitive to gram positive bacteria. ZnO NPs using zinc acetate are sensitive to S.aureus but *k.pneumonia* show *some* resistant to ZnONPs.

The uncalcineted ZnO NPs show more antibacterial effect than the calcinated ZnO nanoparticles. This may be due to the presence of other chemicals (ions) that are not removed before calcinations. The plant extract didn't show any antibacterial activity

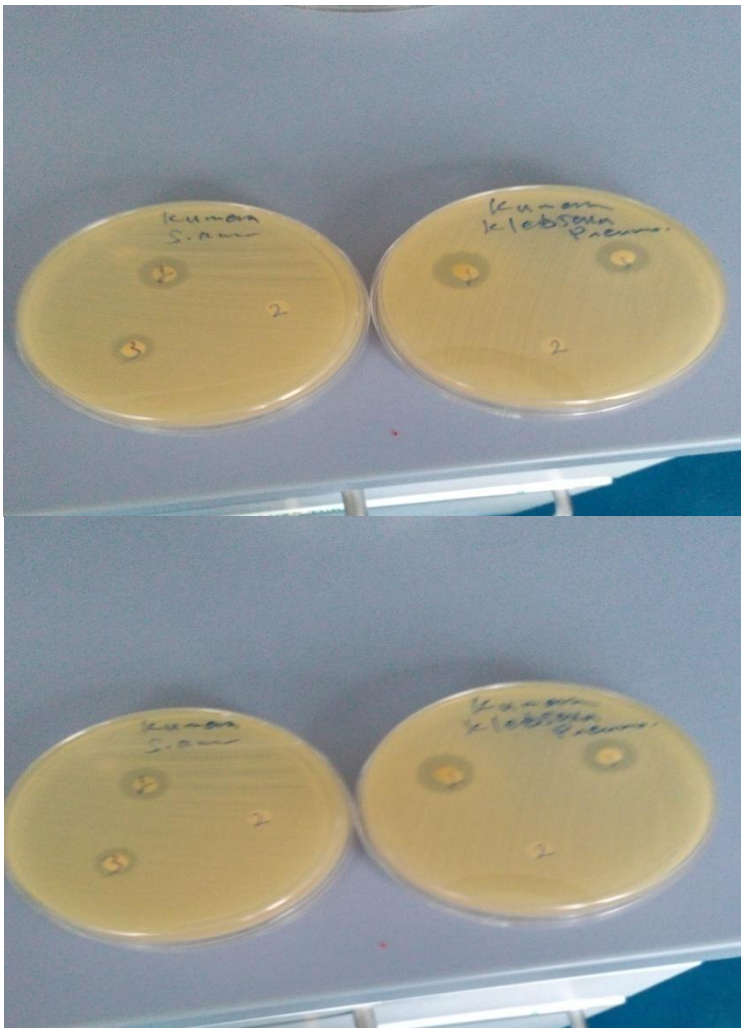


Figure 19 Zone of inhibition of ZnO nanoparticles against Staphylococcus aureus, and k.pneumonia.

From the above results, the size of ZnONPs using zinc acetate dihydrate have smaller particle size based on the concentration, combining ratio and calcination temperature. This means that as the

combining ratio of the plant extract increases by volume the particle size was decreased for the same concentration of the precursor salt for the same duration of drying time. But, when the concentration of the precursor salt increase and also the drying time increase the particle size increases even if the volume of the salt solution and the plant extract is the same. The smaller size of ZnO nps shows greater zone of inhibition indicating that they have greater antibacterial effect on gram positive bacteria and less on gram negative bacteria (*k.pneumonia*) but, Gram-negative bacteria seemed to be more resistant to ZnO nanoparticles than Gram-positive bacteria. It was found that the antibacterial activity of ZnO nanoparticles increased with decreasing particle size and increasing powder concentration. The anti-bacterial activity ZnOnps was found lower than reference antibiotic topical dosage forms.

5. CONCLUSION

The main objective of the study was to synthesize ZnO nanoparticles using *Calpurnea aurea* leaf sextract and to evaluate the antibacterial activity ZnONPs. ZnO NPs was successfully synthesized by the biological synthesis method using aqueous leaf extracts and characterized by advanced techniques. The SEM image analyses revealed as the ratio of the leave extract and zinc acetate affects the grain size of synthesized zinc oxide nanoparticles. The EDX studies also confirmed the composition of the synthesized nanoparticles were Zinc and oxygen and other elements.

The XRD study confirms the formation of hexagonal wurtzite structure and the crystallite size 8.43-27.3nm of the synthesized ZnO nanoparticles while the vibrational stretching around 451 cm-1 (Zn-O), synthesized ZnO with (1:1) ratio of ZnO nanoparticles showed significant antibacterial activity against Gram positive and Gram negative bacteria, including Staphylococcus aureus, and *Klebsiella pneumonia.* All the synthesized nanoparticles ratios have not equal sensitivity towards these drugs resistant bacteria. ZnO nanoparticles have significantly higher antibacterial effect on S. aureus indicating that the synthesized ZnO NPs are more sensitive to gram positive bacteria. Compared with Gram-positive bacteria, Gram-negative bacteria are more resistant against antibodies because of their impenetrable cell wall. From the result obtained it suggested that the green synthesis is a better choice due to its low cost, available, safe to handle and possess a broad viability of metabolites eco-friendliness rather than other method of preparation of ZnO nanoparticles.

Recommendation

Since green synthesis of ZnO NP is the best anti bacterial activity& more other applications, environmentally friendly and easy to handle, inexpensive to prepare. *Then,*

- more attention must be given to carry out more researches on this area.
- it needsmore and more announcement since it is a part of green chemistry

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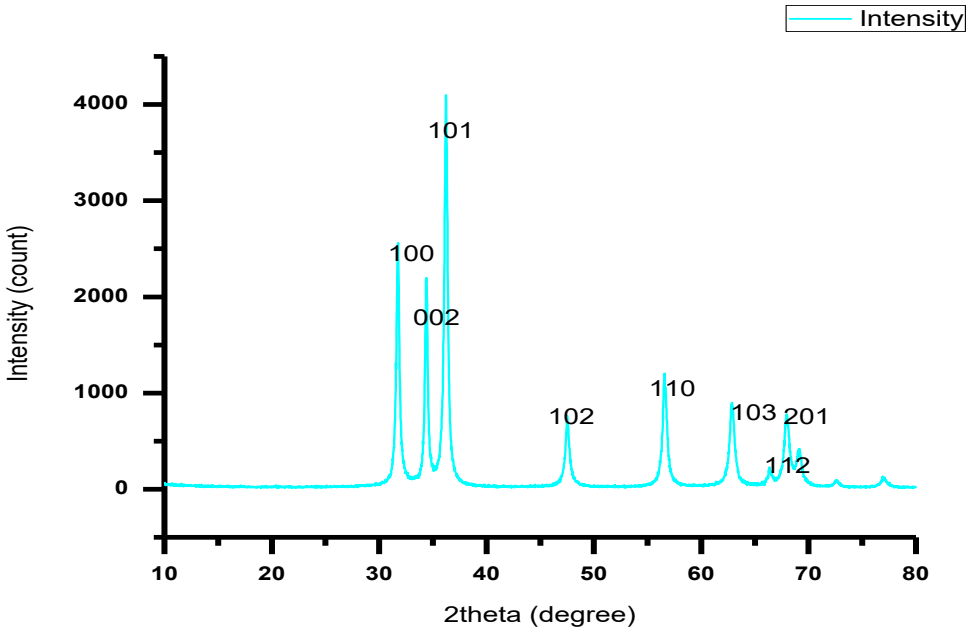
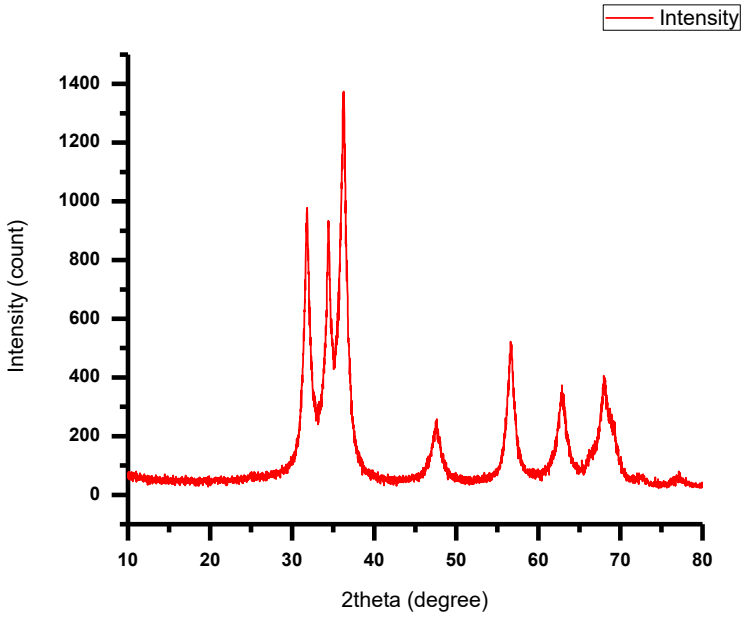
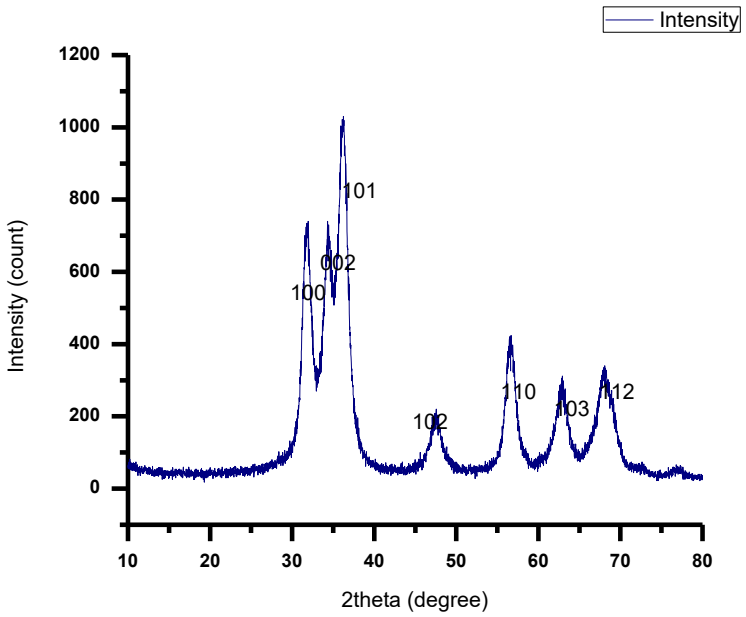
APPENDICES



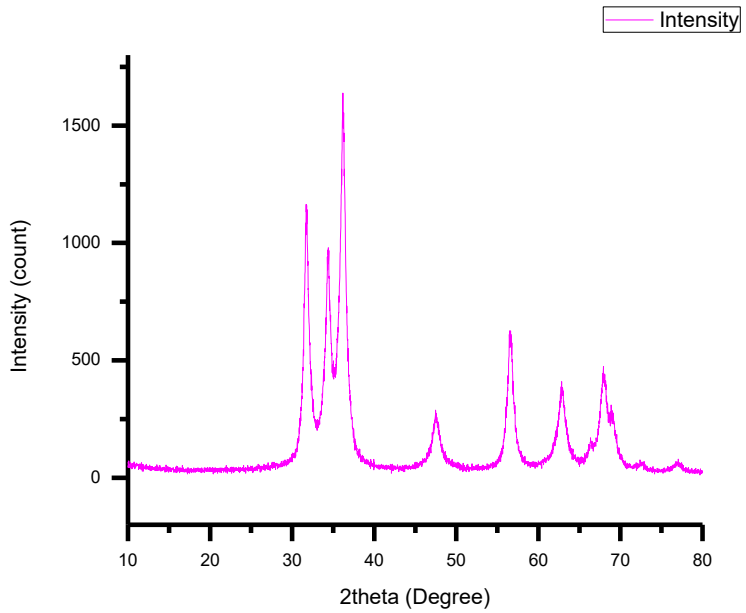
Appendix -1; XRD patterns for ZnO nanoparticles prepared by the ratio of salt and leaf extract (9:1)



Appendix -2; XRD patterns for ZnO nanoparticles prepared by the ratio of salt and leaf extract (7:3)



Appendix -3; XRD patterns for ZnO nanoparticles prepared by the ratio of salt and leaf extract (3:2)



Appendix -5; XRD patterns for ZnO nanoparticles prepared by the ratio of salt and leaf extract (1:1) calcinated at 600℃