Green synthesis and characterization of ZnO/CuO nanocomposite using leaf extract of *Acokantheraschimperri* and its antibacterial activity study

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A Thesis Submitted to the department of applied chemistry
School of applied natural science
Office of Post Graduate Study

Adama Science and Technology University
In Partial Fulfillment for the Degree of Master of Science in Applied chemistry (Industrial Chemistry)

Adama Science and Technology University

April, 2023
ADAMA, ETHIOPIA

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Declaration

This is to certify that this thesis entitled Green synthesis and characterization of ZnO/CuO nanocomposite using leaf extract of *Acokantheraschimperri* and its antibacterial activity study submitted in partial fulfillment of the requirements for degree of Master of Science in Applied Chemistry (Industrial chemistry) to the graduate program school of Applied Natural science Adama Science and Technology University is authentic work conducted by Ahmed Mohammed under the supervision of Aschalew Tedessa (PhD).

Ahmed Mohammed	_____________	____________________
Student	Signature	Date
Aschalew Tadessa(PhD)	_____________	____________________
Advisor	Signature	Date

Letter of approval

I hereby certify that the recommendations and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis entitled “Green synthesis and characterization of ZnO/CuO nanocomposite using leaf extract of *Acokantheraschimperri* and its antibacterial activity study” by Ahmed Mohammed.

Dr. Aschalew Taddesse ____________________ ____________________

Advisor Signature date

We, the undersigned, members of the Board of Examiners of the thesis defense by Ahmed Mohammed have read and evaluated the thesis “Green synthesis and characterization of ZnO/CuO nanocomposite using leaf extract of *Acokantheraschimperri* and its antibacterial activity study” and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the master degree in Applied Chemistry (Industrial Chemistry).

Board of Examiners

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Finally, approval and acceptance of the thesis is contingent upon submission of its final copy to the Office of Postgraduate Studies (OPGS) through the candidate‘s Department Graduate Council (DGC) and School Graduate Committee (SGC).

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Acknowledgement

First, I would like to express my praise to the great Lord, the most forgiving and the most kindly, who has granted me the potential to start and complete this study.

I would like to extend my honest appreciation and deepest gratitude to my advisor, Dr.Aschalew Tadesse for his intellectual advice, guidance, and tolerance in course of my study. I am deeply appreciative of the time, dedication and encouragement that he has significantly contributed to the completion of my work. It was indeed lucky for me to work under his supervision.

My thanks also go to ASTU chemistry laboratory technician especially who took part in this study. Of course thanks to all friends who facilitated the completion of this thesis particularly Getiye Behailu Haji Kadir and Kamil Abubaker for their continuous support in commenting the thesis constructively.

My deepest thanks to my family for always being there and providing support, encouragement and motivation as the situation required. Especially, I would like to give my special thanks to my beloved wife Misra Emamu and my father Mohammed Abdela who stood by me and encouraged me to accomplish this work and who had to give up their time and interests in order for me to reach this goal.

Finally, I take this opportunity to thank my fellow participants for all the good time we have spent together.

Contents

List of Abbreviation

NC   Nano composite

NP   Nanoparticle

UV/vis   UltraViolet visible

XRD   X-ray diffraction

SEM   Scanning Electron Microscope

FT-IR   Fourier transforms infrared

pH   parameter of hydrogen ion concentration

HNCs   Hetero structure nanocomposites

List of Figure

Abstract

Green synthesis of metal oxide nanoparticles and nanocomposite using plant extract as reducing and capping agent become the superior method in nanochemistry. In this study synthesis of ZnO, CuO nanoparticles and their nanocomposite (ZnO/CuO) were prepared using the phytochemical extracts from the leaf of Acokantheraschimperri. The physicochemical properties of those synthesized materials were resolute by using some characterization techniques including ultraviolet visible spectroscopy (UV-vis), X-ray diffraction (X-RD), Fourier Transform Infrared (FTIR) spectrometer and scanning electron microscopy (SEM). The X-RD result confirmed the presence of monoclinic CuO (Tenorite) and hexagonal ZnO (Zincite) of nanoparticles phase, which were both confirmed in the ZnO/CuO composite. The obtained particle sizes found for the ZnO, CuO nanoparticles and ZnO/CuO nanocomposite were 9.55nm, 3.34nm and 6.44nm, respectively. The electron microscopy of the ZnO, CuO nanoparticles, and ZnO/CuO nanocomposite showed a mixture of nanoscale possess plates, filled ellipse and agglomeration morphologies with some collection. The synthesized ZnO nanoparticle and ZnO/CuO nanocomposite were tested using disc diffusion method and the antibacterial activity of ZnO,CuO nanoparticles and ZnO/CuO nanocomposite prepared at different concentration (1 ,0.75, 0.5,0.25)gm/ml were compared toward four types of pathogenic bacteria, gram negative(Escherichia coli, Pseudomonas aerogenosa) and Gram positive (Staphylococcus aureus, Streptococcus pyogenes).1mg/ml concentration prepared CuO nanoparticle shows the highest antibacterial activity with inhibition zone of 22 mm against Streptococcus pyogenes. In conclusion, a result of greenly synthesized CuO nanoparticle shows significant antibacterial activity as compared with ZnOnanoparticle.

CHAPTER 1

1. Introduction

1.1. Background

Nanoparticle metal oxides represent a new class of important materials that are increasingly being developed for use in research and health related applications. Highly ionic metal oxides are interesting, not only for their wide variety of physical and chemical properties, but also for their antibacterial activity, although the in vitro antibacterial activity and efficacy of regular zinc oxides have been investigated (Nicole.J *et al*. 2007). The nanomaterial was exhibit distinct features when compared to their majority complements. Nanomaterial demonstration was very important potential for a range of applications due to their unusual properties, such as a high surface to volume ratio form size and composition (Bapat *et.al* 2019).

The ZnO nanoparticles have unusual properties, including optical and electrical conductivity, photochemical, photo catalytic, antiviral, antibacterial, antifungal and anticancer activities (Cheng B *et al.* 2017). Another metal oxide that has been used as a biocide for many years is copper oxide (CuO). This metal oxide is a p-type semiconductor with optical, thermal, electrical, catalytic, antifungal, antiviral and antibacterial activities (Kumar R *et al.* 2017).

Hetero structure nanocomposites (HNCs) have a wide range of applications in antibacterial activity (Kokilavani *et al.* 2021), photo catalysis (Iqbal *et al*. 2021), hydrogen production (Enesca and Andronic 2021), organic pollutant removal (Enesca and Andronic, 2021) and electrochemical sensor (Kokulnathan *et al.* 2021). Further, they are one of the greatest practical interest topics that have been studied by the scientific community recently (Iqbal *et al*. 2021, Draz *et al*. 2021, Biswal *et al*. 2021). HNCs can be fabricated by a combination of two or more materials such as metal oxide/metal oxide, metal sulfide/metal oxide, etc (Mendonc *et al.* 2021). These combinations of two or more materials lead to produce hetero structure nanocomposites with unusual properties that differ from the properties of individual compounds.

There are different methods to prepare those nanoparticles and nanocomposite. Presently, large number of physical, chemical and biological methods can be used to synthesize different types of nanoparticles (Shiny *et al.* 2019). However, most of the chemical and physical methods are expensive and involve the use of toxic chemicals that pose various biological risks. Therefore, the alternative method which are less toxic, ecofriendly and inexpensive are being discovered (Jagpreet *et al.* 2018). Synthesis of nanomaterials through biological systems supported by some biotechnological tools is an emerging asset of nanotechnology that provides safe, cost effective and ecofriendly synthesis process (Shinwari *et al*. 2017). On the other hand, green synthesis methods have been accepted as a promising technique to conquer the limitations accompanied with the abovementioned methods. In recent years, extensive efforts have been done for the application of plant extracts to fabricate different metal oxides and metallic nanoparticles, due to their availability, low cost and biocompatibility (Singh.P *et al.* 2016) and (Ahmed.S *et al.* 2016).

Several studies have successfully been controlled to synthesize ZnOnanoparticle using biotechnological tools such as bacteria, fungi, algae and plants (Agarwal *et al.* 2017). Among all biological systems, phytosynthesis of nanoparticles using plants has shown great potential and plant mediated nanoparticle synthesis is simple, ecofriendly and provides antibacterial assets (Gunalan *et al.* 2012, Iravani *et al.* 2015). A variability of metabolites such as terpenoids, polyphenols, sugars, alkaloids, phenolic acids and proteins have been reported to have metal ion reduction possessions (Parveen *et al.* 2016).

Copper oxides by themselves are hazardous and in order to remove this toxicity and make them suitable for human ingestion, they are refined complete a series of procedures that include treatment with plant extracts and some animal fluids. Thus, synthesizing nanoparticles from medicinal plants will benefit their application in the biomedical arena. Additionally, the natural antibacterial capabilities of plant extracts increase the qualities of green nanomaterials generated utilizing plants. It is vital to develop a diverse array of antimicrobials that are both affordable and readily available (Bhavyasree *et.al* 2022).

Acokantherashemperri belongs to the family of *Apocynaceae*, was called "*keraru*" locally that is morphologically diverse. To the best of my knowledge, the use of *Acokantheraschemperri* leaf extract for the green synthesis of nanomaterials has not been reported in the literature. Moreover, there are no previously published studies on the antibacterial properties of ZnO, CuO nanoparticles ZnO/CuO nanocomposite containing leaf extract of *Acokantheraschemperri*.

In this study, green chemistry approach would be used to synthesize ZnO,CuOnanoparticles and ZnO/CuOnanocomposite containing leaf extract of *Acokantheraschemperri*. The formation of nanoparticles and nanocomposite was confirmed using ultraviolet-visible spectroscopy (UV-vis), X-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectrometry and scanning electron microscopy (SEM). Furthermore, to examine the anti-bacterial activities of the prepared nanomaterials were used against Gram negative (Escherichia coli, Pseudomonas aerogenosa and Gram positive (Staphylococcus aureus, Streptococcus pyogenes) bacteria.

1.2. Statement of the Problem

Capable of being passed to someone else by germ that enter the body are among the most popular challenges in medical field, industrial sectors, agricultural product, health care clinics and public health threat. Recent, traditionally most of the people accepted use for particular purpose of anti-bacterial drugs to lead the microbial. Moreover, lack of financial resources and existing scientific barriers are slowed the development of new antibiotics. Therefore, ZnO, CuO nanoparticles and ZnO/CuO nanocomposites were result from cautious thought nanomaterials having anti-bacterial effect and cost effectiveness. Many researchers have synthesized ZnO, CuO nanoparticles and ZnO/CuO nanocomposite by following, physical, chemical and biological methods that have been reported. Anyways, each of those methods were evaluated based on the different requirements needed by the researchers. However, most of the chemical methods were expensive, involve the use of toxic chemicals that pose various biological risks and limit the applications of chemically synthesized nanoparticles, especially in medical field but, chemical approach has been widely used to active fighting disease, this leads to environmental pollution and development of resistant strains. Physical synthesis methods were also energy intensive (high temperature and pressure) involves the use of costly equipment (Shiny *et al.* 2019). Biological methods component itself act as reducing and capping agent (redaction the overall cost of synthesis process), external experimental conditions like high pressure and temperatures were not required (leads to energy saving process), can be used at large scale production of nanoparticles, ecofriendly approach (toxic chemicals are not used) (Jagpreet *et al.* 2018). Due to this, biological method was hopeful alternatives than physical and chemical synthesized methods. Therefore, biological synthesis method of nanoparticles and nanocomposite can be a good alternative way to eliminate pathogenic bacteria. In this study a simple magnetic stirrer was applied to synthesize ZnO, CuO nanoparticles and ZnO/CuOnanocomposite by green synthesis method.

1.3. Significance of the Study

This work is probable to have its own influence on the green synthesis of the nanoparticles using locally obtainable plants and safe methods, so that there can be many credible ways for the green synthesis of metallic oxide nanoparticles as they have applications in many fields. Bacterial infections are challenge in various fields. To overcome the problem of bacterial infections, active metal nanomaterials are target matter. Based on this, pharmaceutical industries are looking to synthesize alternative nanoparticles for medicinal preparations. It was also trusted that, it could be used as one possible means of synthesizing ZnO,CuOnanoparticles and ZnO/CuOnanocomposite of desired quality with low cost and convenient methods of preparation so that the toxicity or hazardous nature of the chemical methods of nanoparticles and nanocomposite can be avoided. It was believed that, this work will have a positive impact on increasing the number of vacant research works with ZnO,CuOnanoparticles and ZnO/CuOnanocomposite synthesis and their antibacterial activity.

1.4.Objectives of the Study

1.4.1. General Objectives

The main objective of this thesis is to Synthesis, characterizes and test antibacterial activity of ZnO/CuO nanocomposite.

1.4.2. Specific Objectives

- To synthesis CuO, ZnOnanoparticles and ZnO/CuOnanocomposite with *Acokanthera schimperri* leaf extract as reducing and capping by using biological method.
- To characterize synthesized ZnO,CuO nanoparticles and ZnO/CuO nanocomposite by using UV/Vis spectrophotometer, XRD, FTIR Spectrometer and SEM techniques.
- To test antibacterial activity of ZnO, CuO nanoparticles and ZnO/CuO nanocomposite towards Gram negative (Escherichia coli, Pseudomonas aerogenosa and Gram positive (Staphylococcus aureus, Streptococcus pyogenes) bacteria.

CHAPTER 2

2. Literature Review

2.1 Historical aspects and growth of Nanotechnology and Nanoparticles

The term “Nano” comes from the Greek word dwarf which generally elaborates the particle of size roughly in the range of 1to100 nanometers. The theoretical concept of nanotechnology was first begun with lecture delivered by Richard Feynman in 1959. He gave a lecture titled “There's Plenty of Room at the Bottom”, suggesting the possibility of manipulating things at atomic level. He speculated on the possibility and potential of nanosized materials (Arrivalagan *et al.*2011,Daniel *et al.*2004). The term "Nanotechnology" was first defined by Norio Taniguchi of the Tokyo Science University in 1974 and Nanotechnology was shortened to “Nanotech”. However, the real burst of nanotechnology didn’t come until the early 1990s. In the early 1990s Huffman and Kraetschmer, discovered how to synthesize and purify large quantities of fullerenes (molecules composed entirely of carbon, in the form of a hollow sphere, ellipsoid, or tube are similar in structure to graphite, which is composed of a sheet of linked hexagonal rings, but they contain pentagonal (or sometimes heptagonal) rings that prevent the sheet from being planar). Shortly after a meeting of the Materials Research Society in 1992, Dr. T. Ebbesen described to a spellbound audience his discovery and characterization of carbon nanotubes. Using the same or similar tools as those used by Huffman and Kraetschmer, hundreds of researchers further developed the field of nanotechnology. No one knows how many products on the market today contain nanoparticles or are manufactured with the help of nanotechnologies (Arivilagan *et al.* 2001). Nanotechnology is a field of science which deals with design, production, manipulation and use of materials ranging in nanometers called nanomaterials(Roy *et al*. 2015). It is the application of science to control matter at the molecular level (Arivilagan *et al.* 2011). It is also the study and application of small object which can be used across all fields such as chemistry, biology, physics, material science and engineering (Shet *et al*. 2015). It has appealed many researchers from the above listed fields (Sundaramurthy *et al.*2015).

Generally, nanotechnology deals with structures sized between 1 to 100 nm, nanoparticles. It involves developing materials or devices within that size range (Arivilagan *et al.* 2011). Tremendous growth in nanotechnology has opened up novel fundamental and applied frontiers in materials science and engineering, such as, bionanotechnology, quantum dots, Surface-enhanced

Raman Scattering (SERS) and applied microbiology (Shet *et al.* 2015). The branch of nanoscience and technology is truly multi-disciplinary and is an emerging technology with full of promises to have an impact on virtually every spectrum of civilization including communications, computing, textiles, cosmetics, sports, therapy, automotives, environmental monitoring, fuel cells and energy devices, water purification, food and beverage industry, etc (Arivilagan *et al.* 2001, Barnejer *et al.* 2014).

Nanoscience and nanotechnologies are revolutionizing our understanding of matter and are likely to have profound implications for all sectors of the economy, including; agriculture and food, energy production and efficiency, the automotive industry, cosmetics, medical appliances and drugs, household appliances, computers, and weapons. Nanotechnology has the capacity to improve our ability to prevent, detect, and remove environmental contaminants in air, water, and soil in a cost effective and environmentally friendly manner (Arivilagan *et al*. 2001).

2.2 Nanoparticles or Nanomaterials

Nanomaterials (NMs) are defined as materials with at least one external dimensionlies in the size range of approximately 1-100 nm. While nanoparticles are solid particles with all three external dimensions at the nanoscale that can drastically modify physico-chemical properties compared to the bulk material(Jayalakshmi *et al.* 2014). Bulk materials possess relatively constant physical properties regardless of their size, but at the nanoscale this is often not the case. The nanoparticles possess unique physico-chemical, optical and biological properties which can be manipulated suitably for desired applications (Banerjer *et al.* 2014). They exhibit unique electronic, magnetic, optical, catalytic and medicinal properties as compared with the traditional and commercial bulk materials. This is due to its quantum size effect, large surface to volume ratio and their electronic energy states become discrete (Sylvia *et al.*2014, Banerjer *et al.* 2014). As particles are reduced from a micrometer to a nanometer size, the resultant properties can change dramatically. For example, electrical conductivity, hardness, active surface area, chemical reactivity and biological activity are all known to be altered (Ren *et al.* 2009). Because, as the material becomes smaller the percentage of atoms at the surface increases relative to the total number of atoms of the material bulk. This can lead to unexpected properties of nanoparticles which are partly due to the surface of the material dominating over the bulk properties. It is also known that with the decrease in the dimensions of the materials to the atomic level, their properties change.

All nanoparticles, regardless of their chemical constituents, have surface area to volume ratios that are extremely high. This causes nanoparticles physical properties to be dominated by the effect of the surface atoms and capping agents on the nanoparticles surface. A particle with a high surface area has a greater number of reaction sites than a particle with low surface area, and thus, results in higher chemical reactivity. High surface area to volume ratio is important for applications such as catalysis. This increased reactivity due to surface area to volume ratio is widely taken advantage of in nature; one biological example is the body's digestive system. Within the small intestine, there are millions of folds and sub folds that increase the surface area of the inner lining of the digestive tract. These folds allow more nutrients and chemicals to be absorbed at the same time, greatly increasing our body's efficiency and the rate at which we digest food. Another example, a cube of sugar reacting with water as the water dissolves the outside of the sugar. The same size of sugar cube is cut into many little pieces. Each cut makes new outer surfaces for the water to dissolve. The smaller particles of sugar have same volume but have much more surface area. Almost all properties of nanoparticles are due to their small sizes. Nanoparticles, due to their extremely small size and large surface area, possess many interesting properties. Due to this they find novel applications in various areas of electronics, optoelectronic, magnetic, information storage, recording media, sensing devices, catalysis, chemistry, environment, energy, agriculture, medicine and drug delivery, communication technology, aircraft technology, heavy industry and consumer goods etc (Kurkure *et al.* 2016).

In recent years the interest in nanomaterials has increased dramatically due to their unique chemical and physical properties(Awwad *et al.* 2015). It is also obvious that research interest in nanomaterials has increased exponentially thanks to their unique chemical and physical features, different from those of their bulk materials, but not limited to diffusivity, electrical resistivity, electrical conductivity, strength and hardness, chemical reactivity and diverse and versatile biological activity (Saholi et al.2012). Nanoparticles are of great interest; because they act as bridge gap between the atomic/molecular structure to the material in bulk as they exhibit completely new or improved properties based on specific characteristics such as size, shape, distribution, ionic strength, capping agent and morphology (kurkure *et al.* 2012). And also on account of their potential applications which are strongly influenced by their size, morphology and structure (Thamilseva *et al.*2012). Nanoparticles have been influencing material science considerably. It seems this dominance will continue in the future years, because of fundamental and technological importance with implementing incessant researches in this field. Nanoparticles are considered as building blocks for the next generation of technology with applications in many industrial sectors (Caroling *et al.*2015). The nanoparticles are broadly grouped into organic and inorganic nanoparticles. The latter have gained significant importance due to their ability to withstand adverse processing conditions.

2.3 Noble metal nanoparticles or Metal Oxide nanoparticles

Noble metal nanoparticles have been the subject of focused research, due to their unique optical, electronic, mechanical and chemical properties that are significantly different from those of bulk materials. For this reason, metallic nanoparticles have found uses in many applications in different fields, such as catalysis, photonics, and electronics (Jayalakshmi *et al.*2014, Roy *et al.*2015). As the metal particles are reduced in size, bulk properties of the particles disappear to be substituted to that of quantum dot, following quantum mechanical rules. It can thus be easily understood that metal nanoparticles chemistry is different from that of the bulk materials (Naisari *et al*.2008).

Many kinds of nanoparticles, including metal nanoparticles, oxide nanoparticles, semiconductor nanoparticles, and even composite nanoparticles, have been widely used in electrochemical sensors and biosensors. Metal oxide nanoparticles are also important because of their superior electronic, electrochemical, paint/ink materials, catalytic and magnetic properties (Jayalakshmi *et al.*2014, Rahman *et al.* 2009). Metal oxide nanoparticles, such as Zink oxide and Copper oxide (CuO), have attracted attention mostly because of their antimicrobial and biocide properties and they may be used in many biomedical applications (Rahman *et al*. 2009). They have been receiving considerable attention for their potential applications in optoelectronics, nanodevices, nanoelectronics, nanosensors, information storage, and catalysis (Jayalakshmi *et al*.2014, Thamilseva *et al.*2012, Ahmed *et al*. 2014). Most of relevant recent studies on conductive inks have focused on noble metals exempt from significant oxidation, such as silver and gold nanoparticles (silver slightly oxidizes but its oxide is still a good conductor). In particular, silver with its high conductivity is of great interest and has led to much development with commercially available products. However, these noble metals are too expensive to be used in large quantities. In this context, copper and Zink is a good candidate material because it is highly conductive but significantly cheaper than Au and Ag. Copper nanoparticles synthesized in ambient atmospheric temperature and pressure inevitably have surface oxide layers because the ZnO and CuO phases are thermodynamically more stable than pure copper (Ahmed *et al.* 2014).

Hence, among various metal oxide nanoparticles, ZnO and CuO nanoparticles have attracted particular attention. Because, it is the simplest member of the family of copper compounds, easy to prepare in pure form and shows a range of useful physical properties such as high temperature superconductivity, electron correlation effects, and spin dynamics(Marabelli *et al*.1995, El-Trass *et al.* 2008).

2.4. Zink Oxide (ZnO) nanoparticle

Zinc oxide (ZnO) nanoparticles have become famous among researchers due to its use in various applications like anti-bacterial, anti-fungal, anti-cancer agents, gas sensors, chemical sensors, biosensors, superconductors, optoelectronic devices, and cosmetics. ZnO is a wide band gap semiconductor having high optical transparency and luminescence in visible and near ultraviolet range of spectrum (Aluri *et al.* 2016). several reports have demonstrated that ZnO nanoparticle can inhibit the growth rate of multi drug resistant both gram-positive and gram-negative bacteria. ZnO nanoparticles have high biocompatibility with mammalian cells and sufficiently higher antibacterial potency. Besides, ZnO is one of the most studied semiconductors having wide band gap energy (3.37 eV). Number of research groups has reported the photodynamic antibacterial activity of ZnO nanomaterial. It has been demonstrated that photodynamic antibacterial activity of ZnO nanostructures is mainly governed by reactive oxygen species (ROS) especially singlet oxygen and hydroxyl radicals (Jan et al. 2019).

2.5. Copper Oxide (CuO) nanoparticle

Copper oxide is a compound of two elements, copper and oxygen, which are d and p block elements in the periodic table respectively. When compared to the common copper oxide powder, nanocopper oxide shows better catalytic activity and selectivity. In a crystal copper ion is coordinate by four oxygen ions (Singh.J *et al*. 2016). Copper oxide (CuO) is one of potential p-type semiconductors and gains considerable attentions due to its excellent optical, electrical and magnetic properties (phiwdang *et al.* 2013). Copper oxide (CuO) is a narrow band gap (1.3 eV) semiconductor with good intrinsic and catalytic properties. CuO is considered as supporting to increase the efficiency of ZnO NPs in the formation of heterojunction. ZnO/CuO heterojunction is not the only solution, in addition the design of controlled morphology with dimensions of nanomaterials has been a promising strategy to enhance the efficiency (Bharathi *et al.* 2019).

2.6. ZnO/CuO Nanocomposite

Development of new material is the fundamental culture of human race since its birth. Thus in order to accomplish unique properties, two or more materials can be combined resulting in a new material called composite material. Composite materials are solids that comprised of two or more different elements or phases on the scale larger than the size of an atom. Since the birth of civilization the synthesis of composite material started its journey with the invention of mud bricks and concrete. In generally, composites are comprised of two types of materials, one is the called matrix or binder which encloses and binds together fragments of the other material, termed as reinforcement. If one of the constituent materials of the composite is nanometer dimension then, the composite is called nanocomposite (Nakshatra *et al.* 2016). ZnO (Zinc oxide) is a wide band gap n-type semiconductor material with 3.3 eV energy gap and high excitation binding energy of 60 eV (Ang *et al.* 2013). Copper oxide is an inorganic p-type semiconductor material having 1.85 eV direct band gap (Chen *et al.* 2012). The combination of ZnO/CuO semiconductor nanomaterial forms p-n hetero junction means that, electron donor acceptor pairs observed in between n type metal oxide and p-type metal oxide. Combination of narrow band gap semiconductors with wide band gap semiconductors will increase their sensing property (Allaf *et al.* 2014). According to another report, the smaller band gap energy was caused by the combined transition that enhanced from O_2 (2p) to Zn^{2+} ($3d^{10}$- 4s) to Cu^{2+} ($3d^9$). The swift heavy ion irradiation could decrease in the band gap with an increase in the ion fluency (Widiarti *et al.* 2017). Due to hetero junction, the ZnO/CuO has a better sensing properties compared to the ZnO nanoparticle or CuO nanoparticle (Li et al. 2010). The addition of CuO on ZnO produced a larger surface area of ZnO/CuO composite. According to another report, the large surface area of ZnO/CuOnano composites is affected antibacterial properties. The large surface area of composites can increase the anti-bacterial properties due to easier to absorb the UV light and generating the radical species. The ZnO/CuO composite could effectively kill the bacterial cells due to increased production of Zn^{2+}ions and reactive oxygen species (ROS) (Widiarti et al. 2017).

2.7. Synthesis Method of Nanomaterials

Synthesis of nonmaterial can be classified two types, bottom-up approach and top down approach, in bottom-up approaches include the miniaturization of materials components (up to atomic level) with further self-assembly process leading to the formation of nano structures. During self-assembly the physical forces operating at nano scale are used to combine units into larger stable structures. In top-down approaches use larger initial structures, which can be externally controlled in the processing of nanostructures (pejjai *et al.* 2018).

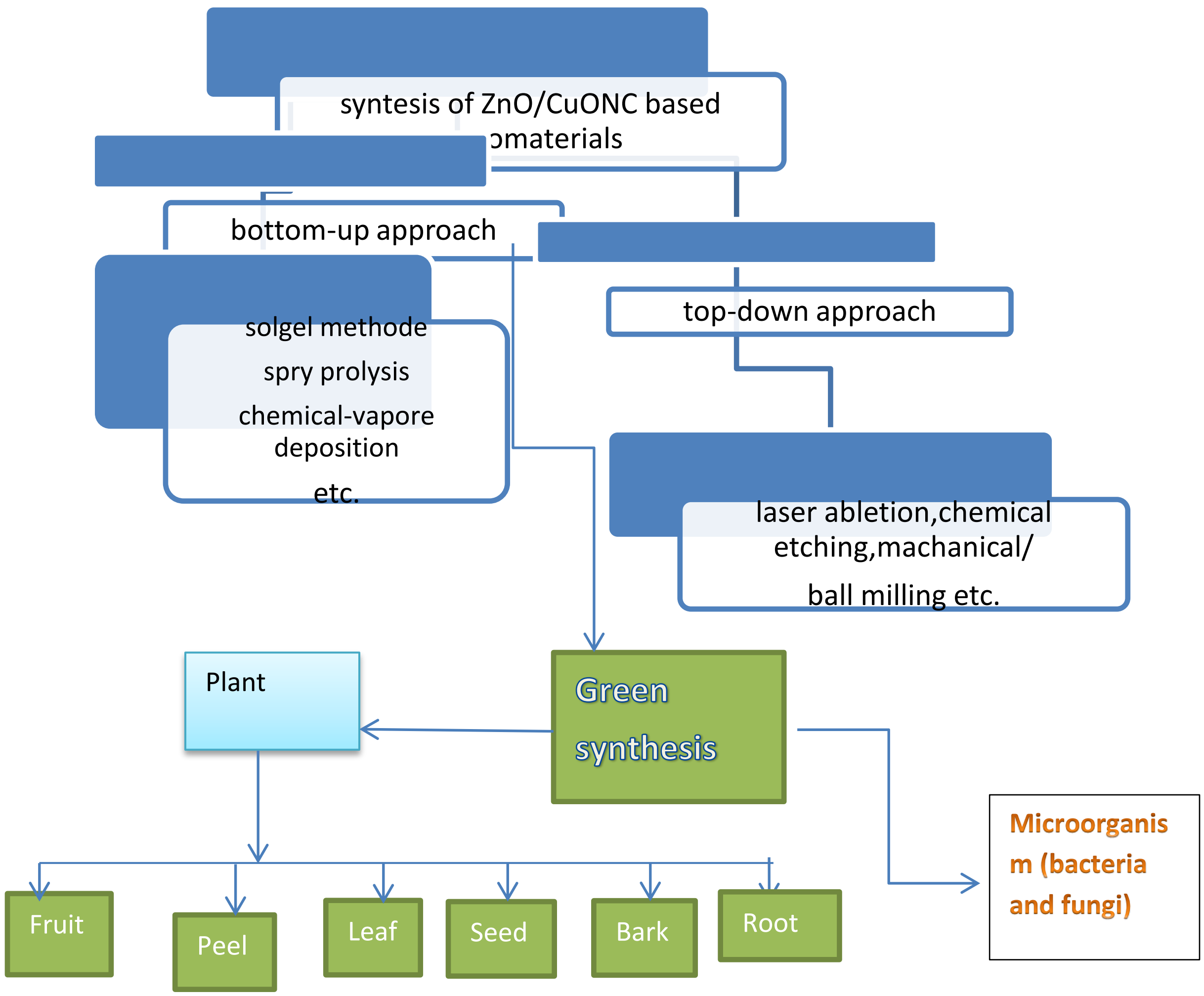


Figure 2.1 Flow chart representing synthesis of Zn/CuO composite (P.G. Bhavyasree *et al.*2022)

Recently, there are many experimental methods that had been reported to fabricate ZnO,CuO nanoparticless and ZnO/CuO nanocomposite. Even though those have addressed various issues of nanoscience in a more efficient way than the traditional methods of employing metal salts commercially available. Nanoparticles and nanocomposite are synthesized by physical and chemical methods. The disadvantages of physical synthesis methods are that they are energy intensive and expensive. Chemical synthesis methods involve the use of many toxic chemicals which limits the applications of chemically synthesized nanoparticle, especially in medical field and not environmental friendly. research for biocompatible, non-toxic and ecofriendly method of nanoparticle and nanocomposite synthesis lead to the development of biological synthesis route for production of nanoparticle and nanocomposite. Biological synthesis of nanoparticles and nanocomposite involves the use of biomaterials such as bacteria, fungi, yeast, virus, micro algae, macro algae and plant extracts. All of those biomaterials, plant extracts are considered more advantageous for the synthesis of ZnO, CuO nanoparticles and ZnO/CuOnanocomposite due to their availability, renewable nature, simplicity of process, rate efficiency, cost effectiveness and stability of synthesized nanoparticles and nanocomposite. Plant extracts contain numerous organic molecules like carbohydrates, proteins, vitamins, alkaloids, enzymes. These molecules can act as either potential reducing or stabilizing agents for the synthesis of nanoparticles, in addition to the eco friendliness and cost effectiveness. The plant extracts which have capable of synthesizing metal nanoparticles are to be selected (Shiny *et al*. 2019).

2.8. Application of Nanoparticles

Metallic nanoparticles are being used for diverse purposes, for medical treatments, using in various branches of industry production such as solar and oxide fuel batteries for energy storage, to wide incorporation into diverse materials of everyday use such as cosmetics or clothes, optical devices, catalytic, bactericidal, electronic, sensor technology, biological labeling and treatment of some cancers. Due to their exceptional properties including antibacterial activity, high resistance to oxidation and high thermal conductivity, nanoparticles have attracted considerable attention in recent years (Saba, 2015). ZnO nanoparticles show antibacterial activity against a broad spectrum of pathogenic bacteria and these nanoparticles adopt various mechanisms such as reactive oxygen species (ROS) generation, cell membrane integrity disruption, biofilm formation or enzyme inhibition (Tugba, 2019). Under UV irradiation, ROS such as superoxide ions, hydroxyl ions, singlet oxygen species and peroxide molecules are formed. The formed peroxide ions could easily penetrate through the cell membrane and result in cell death Penetration of ZnO nanoparticles results in cell death by the loss of phospholipid bilayer integrity and leakage of intracellular components of the cell. While the gram positive bacteria have a thick layer of peptidoglycan, teichoic acid and lipoteichoic acid in their cell membrane, gram negative bacteria have a triple layer of peptidoglycan. The different structure of cell membranes of these two types of bacteria results in a different mechanism of nanoparticle penetration through the cell membranes (Tugba, 2019).

2.9. Antibacterial activities of ZnOnanoparticles based nanomaterials

The rapid increase in bacteria mediated infectious diseases has been one of the most potential threats to human health and the cause of distressing millions of people around the world (kumar *et al.* 2019). However, antibacterial materials effectively protect human health from these deadly infectious diseases and abrupt misuse of traditional antibiotics has directed the path to evolve many drug-resistant bacteria, which leads to the irretrievable infections(Blair *et al*. 2015). The antibacterial materials are generally assessed against the typical group of the pathogenic bacteria such as Listeria monocytogenes, Staphylococcus aureus (S. aureus), Pseudomonas aeruginosa (P. aeruginosa), Salmonella typhimurium (S. typhimurium), Streptococcus mutans (S. mutans), Escherichia coli (E. coli), Staphylococcus epidermidis (S. epidermidis) Vibrio harveyi (V. harveyi), and Enterococcus faecalis (E. faecalis), which are the main cause for several human infections and are serious threats to health (Moritz *et al.* 2013) and(Hibbing *et al.* 2010). Antibacterial materials such as metal ions/oxides(Liu et al . 2008), Antibiotics (Pandey *et al.* 2011),quaternary ammonium compounds(Jia 2001), and antimicrobial peptides (Wang et al. 2006) have been used for the treatment of bacterial infections but suffering from several issues. Metal ions/oxides have a broad range of antimicrobial activities against fungal, viral, and bacterial pathogens, but they showed toxicity against some mammalian cells (Ran *et al.* 2010). Antibiotics and quaternary ammonium salt can develop resistance after prolonged use (Kummer 2004), where-as the development of pure antimicrobial peptides as antibacterial agents have very high cost (Luo *et al.* 2017). To conquer these problems, several non-traditional antibacterial materials such as metal nanoparticles (kumer.A *et al.* 2008), metal oxide nanoparticles (Wei *et al.* 2004).

Nanosized ZnO exhibits varying morphologies and shows significant antibacterial activity over a wide spectrum of bacterial species explored by a large body of researchers (Raghupathi.K. *et al*. 2011). ZnO is currently being investigated as an antibacterial agent in both microscale and nanoscale formulations. ZnO exhibits significant antimicrobial activities when particle size is reduced to the nanometer range, then nano-sized. ZnO can interact with bacterial surface or with the bacterial core where it enters inside the cell, and subsequently exhibits distinct bactericidal mechanisms (Seil.J *et.al* 2012)]. The interactions between these unique materials and bacteria are mostly toxic, which have been exploited for antimicrobial applications such as in food industry. Interestingly, ZnOnanoparticles are reported by several studies as non-toxic to human cells (Colon. G *et al*.2006).this aspect necessitated their usage as antibacterial agents, noxious to microorganisms, and hold good biocompatibility to human cells (Padmavathy.N *et al.* 2008) The various antibacterial mechanisms of nanomaterials are mostly attributed to their high specific surface area-to-volume ratios (Seil.J *et.al* 2009).

2.10. Mechanism of antibacterial activity of ZnO/CuOnanocomposite

According to different researchers the exact mechanism behind is not known and needs to be further studied. But there are many hypothesized ways of explaining it. The antimicrobial activity of ZnO/ CuO nanocomposite is linked with ions that are released from nanoparticles. The activity is further enhanced by its small size and high surface area to volume ratio that it allows them to interact closely with microbial membranes. Typically, antimicrobials kill bacteria by binding to some vital compounds of bacterial metabolism, there by inhibiting the synthesis of functional biomolecules or impeding normal cellular activities. Nanoparticles attached to the microbial surfaces can decrease both cell mobility and nutrient flow between the cell‟s exterior and interior compartments (Navarro *et al.* 2008). Differentiating copper from other trace metals, results in the production of hydroxyl radicals that subsequently bind with DNA molecules and lead to disorder of the helical(zigzag) structure by cross-linking within and between the nucleic acid strands and damage essential proteins by binding to the sulfhydryl amino and carboxyl groups of amino acids. This denatures the protein and makes the enzymes ineffective. It inactivates cell surface proteins necessary for transport of materials across cell membranes, thus affecting membrane integrity and membrane lipids. Copper and Zink ions inside bacterial cells also disrupt biochemical processes. Based on all of previous studies, the denaturing effect of Zink-copper ion on proteins and enzymes in microbes gives ZnO/CuO its antimicrobial characteristics. The mechanism of toxicity of ZnO/CuO nanocomposite in the cellular membrane was suggested to follow a Trojan horse-type mechanism (Figure 2.2). If this nanocomposite was soluble they can penetrate the membrane cancelling the barrier function of the membrane. After entering the cells, nanoparticles are able to dissolve at acidic intracellular pH (4.5), and metal ions produce pores in the membrane (Rodhe *et al.* 2015). It is also assumed that the high affinity of metallic nanoparticles towards sulfur and phosphorus is the key element of the antimicrobial effect. Due to the abundance of sulfur-containing proteins on the bacterial cell membrane, metallic nanocomposite (such as ZnO/CuO nanocomposite) can react with sulfur containing amino acids inside or outside the cell membrane, which in turn affects bacterial cell viability. The general understanding is that metallic nanoparticle of typically less than 20 nm diameters get attached to sulfur-containing proteins of bacterial cell membranes leading to greater permeability of the membrane, which causes the death of the bacteria.

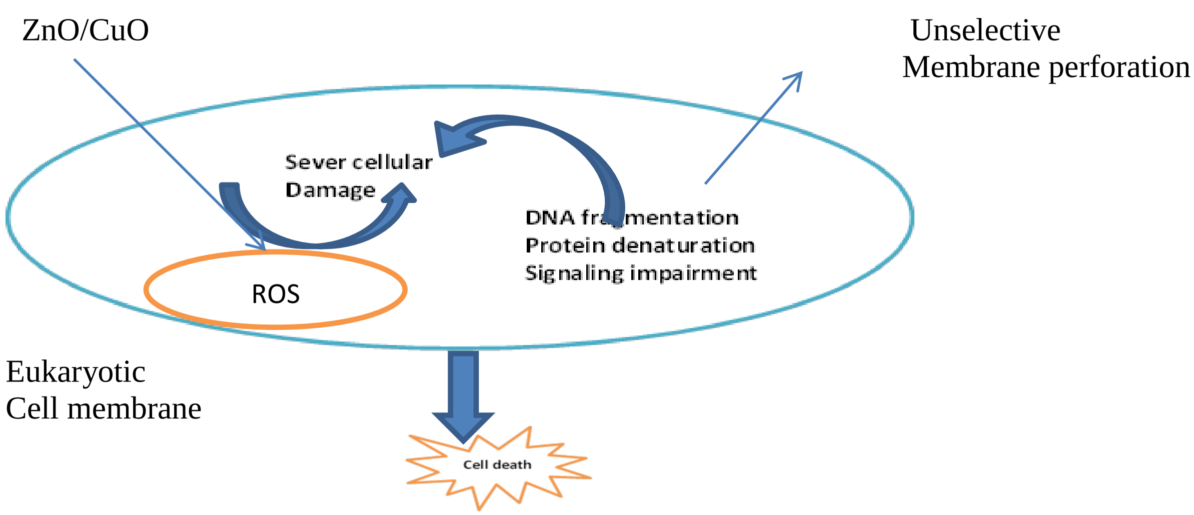


Figure 2.2 Toxicity mechanism of ZnO/CuOnanocomposite in eukaryotic cell (Kiflom. 2018),

In this work antibacterial activity of ZnO, CuO nanoparticles and ZnO/CuO nanocomposite were examined by disc diffusion method for only four bacterial species. These were quantitatively done by measuring the clear zone of inhibition. Generally, both Gram-positive and Gram-negative bacterial strains are thought to present a major public health problem (Rodhe et al. 2015). But the antibacterial activity of the ZnO,CuO nanoparticles and ZnO/CuO nanocomposite were examined against two gram negative and two gram positive bacteria's.

CHAPTER 3

3. Materials and Experimental Methods

3.4. Chemicals and reagents

[Zn ($CH_2COOH)_2$. $2H_2O$](98% Sigma-Aldrich, and [Cu(CH_3 $COOH)_2$.H_2O](99.9% Alti chem) as precursors, Dimethyl sulphoxide as a solvent (99.5% Adis Abeba Karakore), sodium hydroxide (5M NaOH) for estimated pH of the sample, CH_3COOH 99.8% for washed prepare sample. All aqueous solutions in the synthesis were prepared with deionized water from ASTU chemistry laboratory class was used in this research to prepared ZnO, CuOnanoparticles and ZnO/CuO nanocomposite.

3.5. Instrument and Apparatus

Instrument that used in this study were, pH meter, Hot air oven, XRD (XRD-7000S Shimadzu) with Cu Kα radiation, UV-Vis spectrometer(JASCO V-670), Fourier transform (FTIR, Thermo Nicolet Model: 6700) spectrophotometer, SEM (JEOL, JSM6510 LV) instrument, magnetic stirrer, centrifuge, Petri dish, agar incubator.

3.6. Experimental procedures

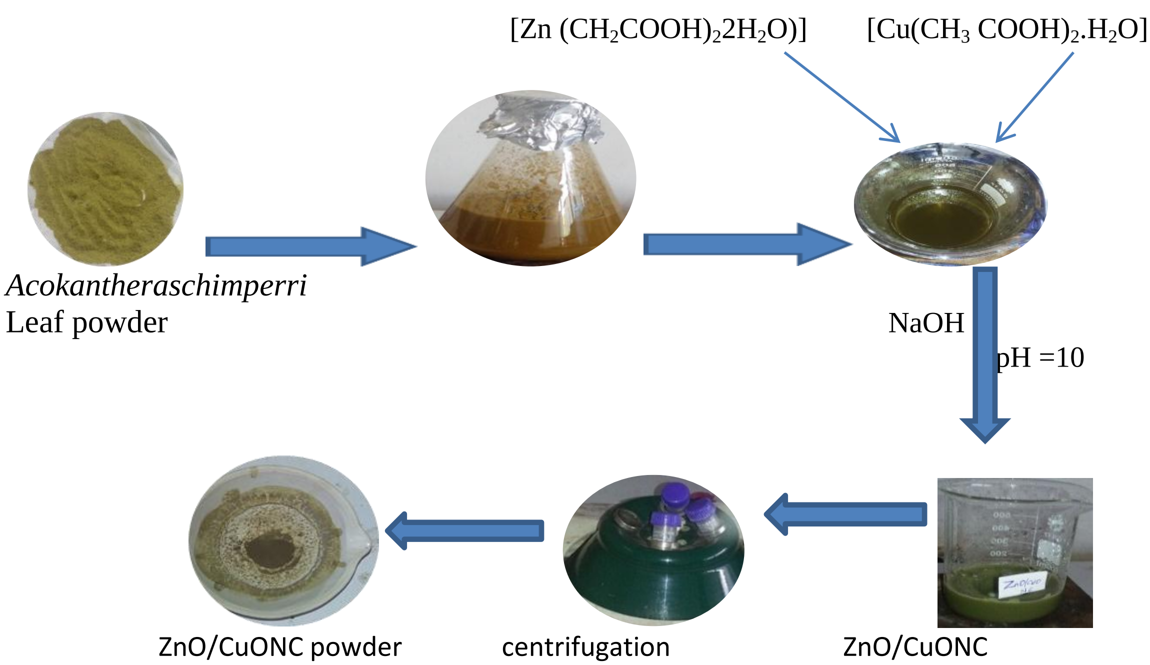
3.6.1. Preparation of the extract

The green Acokantheraschimperri leaf was collected from Dilfakar Mountain Park in Arsi Dodota zone after conducted the field surveys. The collected green leafs were completely washed by tap water and deionized H_2O and dried at room temperature in a dust free condition for 10 days. Dried leafs were crushed into fine powder by grinding mill. Afterward, 20 gm of the powder was mixed with 150 ml of deionized H_2O and stirred at 60 °C for 60 min. The leaf extract was obtained by filtering the mixture and stored in refrigerator to be used in the green synthesis of nanomaterials.

3.6.2. Synthesis of Nanomaterials

A simple magnetic stirrer was applied to synthesize nanomaterials. For synthesis of the ZnO nanoparticle the method followed by (Ramin *et al.* 2018) with slight modification 5.578 g of Zinc(II) acetate di hydrate was dissolved in 80 ml of deionized H_2O and mixed with 20ml of leaf extract under stirring for 60min at room temperature. After solution mixed with a continuous stirring, in order to adjust pH of the solution to 10 an aqueous solution of NaOH(5M) was drop-wisely added into the solution. After few time, the nearly white powdered precipitates were formed in the mixtures and then centrifuged for 30 min. at 2500 rpm. The usual clear liquid was decanted and the precipitate was thoroughly washed with distilled H_2O and ethanol repeatedly. Finally, pellets were transferred to Petri dishes for complete dryness in oven at 60 °C overnight (Naseer *et.al* 2020). The green synthesized of CuO nanoparticle, was also prepared with the same procedure, except 5.07gm of copper (II) acetate monohydrate was dissolved in the solution with a 20:80 v/v ratio of the extract to deionized H_2O.

For preparation of the ZnO/CuONC 1.2545g of Zinc (II) acetate di hydrate and 0.1254 g of copper (II) acetate were dissolved in 80 ml of deionized H_2O and mixed with 20ml of leaf extract under stirring for 60min at room temperature. After solution mixed with a continuous stirring, in order to adjust pH of the solution to 10, an aqueous solution of NaOH(5M) was drop wisely added into the solution. After the incubation period, the precipitates were formed in the mixtures and then centrifuged for 30min. at 2500 rpm. The supernatant was decanted and the precipitate was thoroughly washed with distilled water and ethanol repeatedly. After washing with deionized H_2O and ethanol repeatedly, it was oven-dried at 60°Cfor 24 hr (Naseer *et.al* 2020).



Scheme 3.1. schematic diagram for preparation of the samples in the presence of *Acokantheraschimperri* leaf extract.

3.7. Characterization techniques

The UV-visible absorption spectra of the samples were taken in the range between 250 to 600 nm at room temperature. UV–visible spectral characterization of the nanomaterials was performed using a UV–vis spectrophotometer (JASCO V-670). In the investigation of optical properties, the samples were ultrasonically dispersed in DMSO before poured the suspension in quartz cuvettes to run the measurement. The X-RD is one of a crucial tool to determine the crystalline structures and phase composition of materials. In this research, the crystallinity and crystal phase of the obtained powder was investigated by an X-ray diffraction (X-RD) instrument (XRD-7000S Shimadzu) with Cu Kα radiation. The FTIR is a technique was used thermo Nicolet Model: 6700) to obtain the infrared spectrum absorption or emission of a solid prepared sample. The measurements of sample would be performed by putting the powder on the KBr plate. The morphology and shape analysis of the samples carried out by SEM (JEOL, JSM6510 LV), Where a small amount of the powder taken and characterized. The surfaces of synthesized sample under study were scanned with an electron beam. The interaction between the electrons and the sample gives rise to different signals (the emission of electrons and photons) which gathered and analyzed bring together image surface on the observed sample.

3.8. Antibacterial activities of nanomaterials

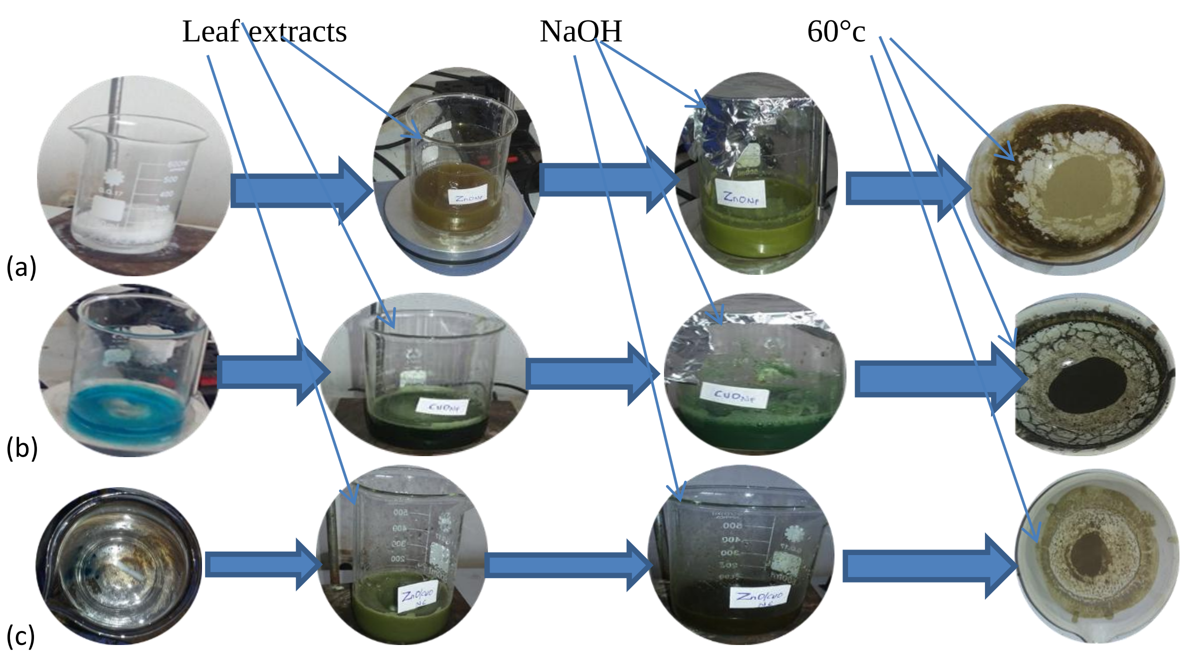
All the equipment and tools used in these experiments were autoclaved for 24hr at 37°C. The gram positive (streptococcus pyogenes and Staphyloccocus aureus) and gram negative (Pseudomonas aerogenosa and Escherechia coli) bacteria strains were cultured in NB and incubated at 37°C overnight before using. The pathogenic strain was used for study which Standard microbial techniques were followed for media preparation. Mueller Hinton Agar (HIMEDIA, MUMBAI) plate was prepared and inoculated with 24 hrs. Antibacterial activity was determined by following standard disc diffusion technique. Suspension of bacteria culture was cotton swabbed on Muller Hinton Agar (HIMEDIA, MUMBAI) Petri plates and (1, 0.5, 0.25 and 0.125)mg/ml different concentration of sample solution was placed above the swabbed culture plate. Growth inhibition zone produced by the substrate coated with the nanocrystalline thin film was measured as zones of inhibition in millimeters.

CHAPTER 4

4. Results and Discussion

4.1. Color observation and analysis

In this study ZnO, CuOnanoparticles and ZnO/CuOnanocomposite were prepared by using biological method in the presence *Acokantherashemperri* leaf extraction with slight modification (ramin *et al*. 2018). Leaf extract used as capping, stabilizing and reducing agent. After addition of *Acokantheraschemperri* leaf extracts to the metal salt solution, the colors of the prepared sample suspension were changed. Figure 4.1(a) shows the formation of biosynthesized ZnOnanoparticle was confirmed by a color change after the addition of *Acokantheraschemperri* leaf extract was added to the zinc acetate dehydrates. The color change from dark green to turbid white was, due to surface Plasmon significance (Fatim *et al*.2015). An observed nearly white precipitate indicates ZnOnanoparticle, but after oven dried at 60°C for 24hr orange of ZnOnanoparticle was observed this would be shows, *Acokantheraschemperri* leaf extraction was an effected on the color of ZnOnanoparticle. Figure 4.1(b) shows CuO salt solution from blue to dark green and after oven dried at 60°C for overnight black powder was observed, which indicated CuOnanoparticle. Figure 4.1(c) shows ZnO/CuO solution from near colorless to brown and after oven dried at 60°C again brown powder was observed which indicate ZnO/CuOnanocomposite.



Figures 4.1 color of synthesized ZnO, CuO and ZnO/CuO nanomatherials.

4.2. Characterization

The UV–Vis spectra gave useful information about the electronic absorption characteristics of the fabricated samples. Figure 4.2 displays the UV–vis spectra in the range of 250–600 nm for the ZnO, CuO and ZnO/CuO samples. Depending on the synthesis method metal oxide nanomaterials show some shifts in their UV-Vis absorbance. As can be seen from this figure two peaks of maximum absorption CuO nanoparticle were displayed. One is strong at about 255nm with another weak peak at 280nm (Berra.et al.2018), which are recognized the formation of copper oxide. This may be due to the increasing number of nanoparticles formed as a result of copper ions reduction (Ahmed *et al*.2012) which may be caused by the complete enlacement of the copper ions by the extract. As declared in the experiment part, the change in color from green to dark. This change in color may be owing to the excitation of surface Plasmon absorption of copper oxide (Jayakumarai *et al*.2015), which was caused by a coupling between the conduction electrons fluctuation modes and the incident electromagnetic radiation (Berra.D *et al.* 2018). ZnO also shows two absorption peaks at 265 and 395 nm. However, the composite ZnO/CuO shows only one absorption peak at 267 nm.

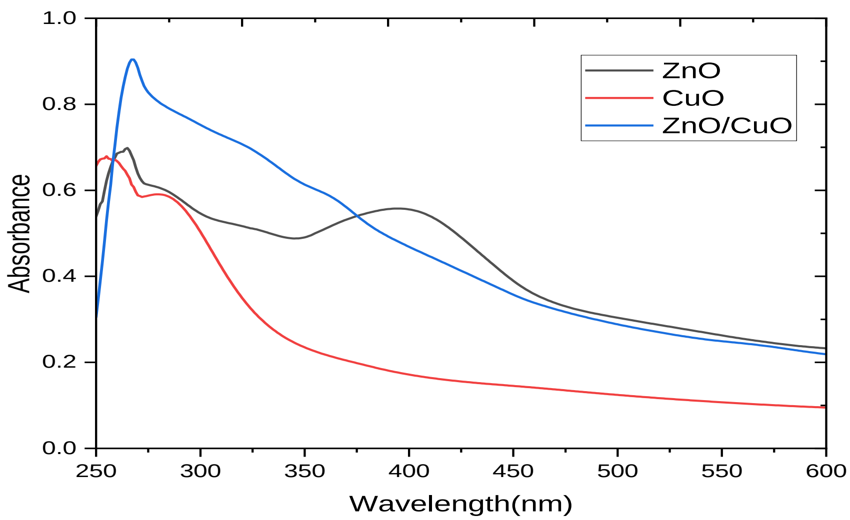


Figure: 4.2 Uv-Vis absorbance of ZnO, CuO, ZnO/CuO

The phase identification and the crystallinity of the obtained ZnO, CuO nanoparticles and ZnO/CuOnanocomposite were confirmed using the XRD and the obtained diffractograms are presented in Figure 4.3 From the diffraction pattern of the ZnO/CuO composite, the peaks recognized at 2θ=31.71°(100),34.38°(002),36.19°(101),47.37°(102),56.56°(110),62.75°(103),66.36°(200), 67.9° (112) and 76.10°(201) shows consistent with the hexagonal phase of the Joint Committee on Powder Diffraction Standard(JCPDS) number 00-036-1451 ZnO(Zincite) nanoparticles (Shaban *et al.* 2022),(Jamil *at al.*2021) and (ER *et al.* 2022). Similarly, the identified peaks at 2θ= 36.71°(110), 38.96 °(111), 42.45°(200),48.79°(202), 61.71°(-113) and 76.43°(220) were also consistent the match agreeably obtained from the International Center of Diffraction Data card (JCPDS-80-1916) confirming the monoclinic crystallinity CuO(Tenorite) nanoparticle(Veisi *et al*. 2021). Comparing the obtained diffraction peaks of both ZnO and CuO nanoparticles with the synthesized ZnO/CuO nanocomposite, there was an increase intensity peaks which is indicative high crystallinity, thus The (1 0 1) peak has the most intensity which proposes that most of the crystals are conquered by the (1 0 1) planes. Furthermore, as seen in this figure the intensities of the peaks associated with the ZnO in the ZnO/CuO diffraction pattern appeared higher than those of CuO nanoparticle. This is evocative of a larger percentage of the bimetallic structure of zinc–copper nanoparticles and high crystallinity(Cao et al. 2021). However, the lower crystallization and intensity of the CuO nanoparticle peak in the composites have been accompanying with the covering role of ZnO nanoparticle on the formed CuO nanoparticle, which consequently keeps the copper cover from further oxidation (wanjin *et al* 2020). The CuO and ZnO nanoparticles, which are often made from copper acetate and zinc acetate precursor salts, were reported to show good purity (Rangel *et al.*2020 and padmavath *et al*. 2008). Hence, no impurity was found in the nanoparticles and their composite. Furthermore, from the diffraction patterns of the nanocomposite, the crystallite size, D, was calculated using Scherrer's equation (Equation (1).

$$D = \frac{0.9\lambda}{FWHM \cos\theta} \quad \ldots\ldots\ldots\ldots 1$$

Where **λ** is the copper K wavelength at 1.5418 Å, the full width at half maximum (FWHM) of the peak with the highest intensity (2ɵ = 36.19°) and the angle parameter (θ) was estimated using the Origin Pro8. The obtained particle sizes found for the ZnO,CuOnanoparticles and ZnO/CuOnanocomposite samples were 9.55nm, 3.34nm and 6.44nm respectively. Due to the presence of different functional groups in the *Acokanthraschemperri* leaf extract, including OH, C-O and C-C, the size of the synthesized nanoparticles and nanocomposite in aqueous solution of

Acokantheraschemperri leaf extract was decreased in comparison with the ZnO (W) sample (Ramin *et al.* 2018). Similar results have been reported for the ZnO particles during fabrication in the presence of different extracts (Bayrami *et al.* 2017), (Ambik.S et al. 2015) and (Anbuvannan.M *et al.* 2015).

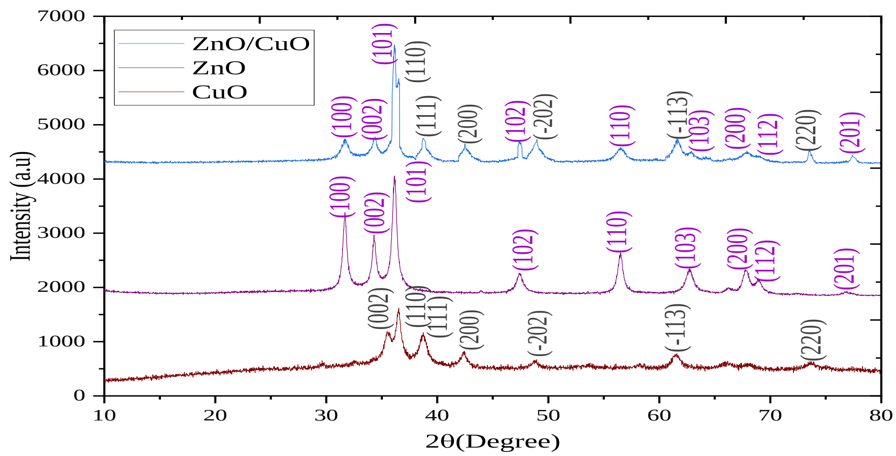


Figure 4.3 XRD spectra of CuO, ZnO, and ZnO/CuO

FT-IR instrument was performed to determine the functional groups of the synthesized samples in the range of 400–4000 cm^{-1}. As shown in figure 4.4 the broad band observed in the range of 400–900 cm^{-1} indicates the metal–oxygen (M–O) stretching mode (Aghaei *et al*. 2020, Shaban *et al.* 2022). The peak at 589 shows CuO stretching mode of vibration. The peak at 666.34 cm^{-1} shows the ZnO stretching vibration. ZnO/CuO samples exhibited a peak at 631 cm^{-1}.

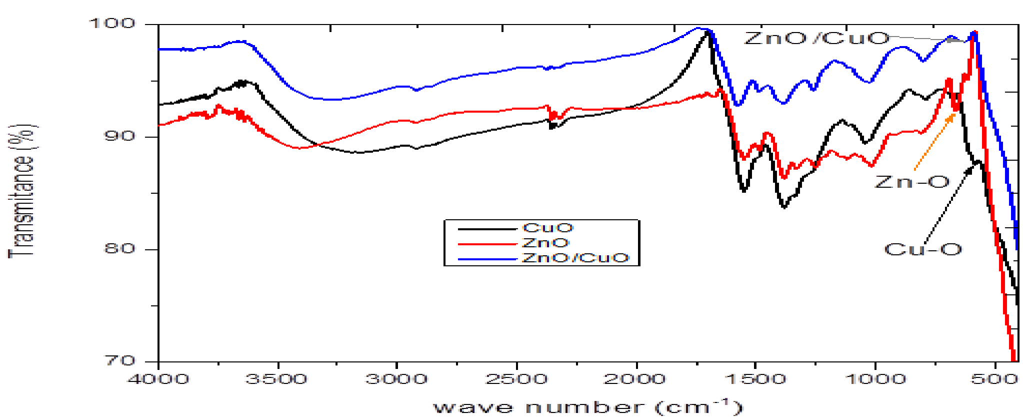
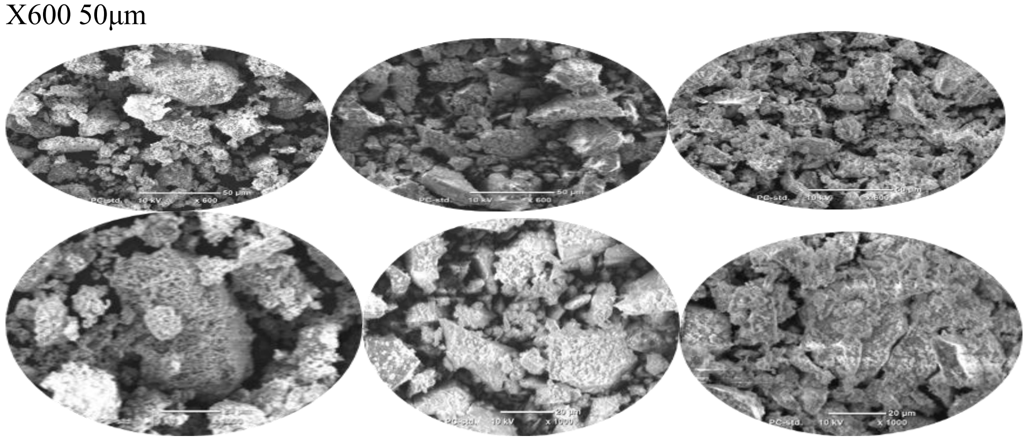


Figure 4.4 FTIR spectra of ZnO, CuO, and ZnO/CuO composite

The morphological properties (shape and roughness) of the nanoparticles were reported to influence their applications, which make the microscopic investigation a useful technique in establishing the nature of a nanomaterial (Elemik *et al.* 2020). The morphological properties of the nanoparticles and their composite were studied using scanning electron microscope (SEM) at high and low magnifications. The SEM micrographs of ZnO nanoparticle in Figure 4.5a shows solidly fixed and filled ellipse with aggregation structures that are randomly distributed dissimilar to earlier report (Saravanan *et al*.2013). On the other hand, the SEM micrograph of CuO nanoparticle in Figure 4.5b showed that they are mostly composed mixtures of nanoscale possess plates that are aggregated, with interconnected thin shavings similar to other report (Buledi *et al*. 2021). Additionally, from the obtained micrograph of the ZnO/CuO nanocomposite in figure 4.5c shows contain several numbers of solidly crowded particles without a definite shape, which is caused by agglomeration, Moreover for the sample prepared in the presence of the extract, mean particle sizes have been increased,

X600 50µm



x1000 20µm (a) (b) (c)

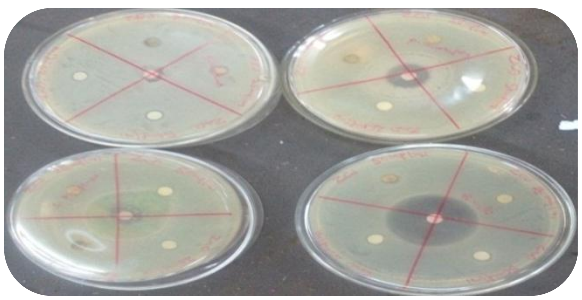
Figure 4.5 SEM micrograph images reveal the morphological of the prepared (a)ZnO, (b)CuO, (c)ZnO/CuO showing nanoparticles at different magnifications

4.3. Antibacterial activity result

Compound	Conc (mg/mL)	Inhibition Diameter (mm)±SD											
		S. aureus			Streptococcus pyogenes			Pseudomonas aerogenosa			E. coli		
		Trial	2	3	1	2	3	1	2	3	1	2	3
CuO	+ve	20	19	17	22	20	18	21	18	18	19	20	24
	1	15	19	11	18	22	11	15	19	7	15	17	19
	0.5	11	8	10	8	8	7	10	17	9	9	10	15
	0.25	8	10	6	7	6	6	10	11	12	7	9	10
	0.125	8	10	6	7	6	7	10	10	8	6	8	8
ZnO	+ve	15	8	6	17	13	16	15	8	9	10	6	6
	1	11	6	6	13	9	14	11	7	6	8	7	6
	0.5	12	9	6	10	7	6	12	6	7	7	8	6
	0.25	7	6	6	10	11	9	6	6	6	6	6	6
	0.125	9	6	8	9	10	11	7	6	8	7	6	6
ZnO/CuO	+ve	15	9	7	19	18	15	20	18	16	19	18	17
	1	14	10	6	18	17	12	19	17	15	18	17	15
	0.5	12	9	7	17	16	12	14	11	9	14	10	8
	0.25	11	9	7	14	11	9	13	10	9	12	8	6
	0.125	10	9	7	13	10	8	11	10	9	11	8	6

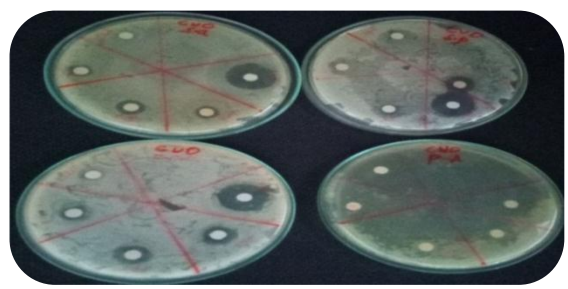
Table 1: the bacterial culture showing the antibacterial activity of synthesized Nanoparticle.

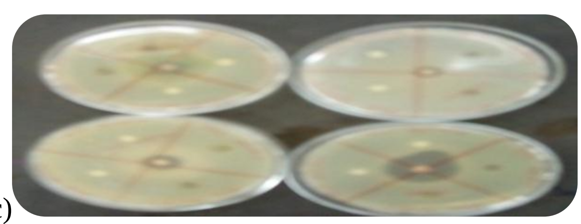
In this study, the antibacterial activity of CuO, ZnO nanoparticles, and ZnO/CuO nanocomposite was determined by using disk diffusion methods with measured inhibition zone. The inhibition zone of CuO nanoparticle prepared was the highest diameter toward streptococcus pyogenes and Staphyloccuce. aureus (gram +ve) bacteria indicating the most antibacterial activity when compared with both ZnOnanoparticle and ZnO/CuOnanocomposite was measured 22mm and presented in table 1, these results were compared to the standard control, this is due to the smallest size of the synthesized CuO nanoparticle, that means the smaller nanoparticles have larger surface areas, which result in higher probability of being in bit with and passing through the bacterial cell membrane than with larger nanoparticles. However the Escherichia Coli and Pseudomonas aerogenosa (gram negative) bacteria has smallest inhibition zone with CuOnanoparticle on trial 1 compared to ZnO/CuOnanocomposite of the other pyogenic bacteria, which indicates its resistance toward the synthesized nanoparticles. In other word, the antibacterial activity of copper nanoparticle composited with other nanoparticle was greater than that CuO alone. because, during composited there is more direct interaction between Cu nanoparticle and antibacterial strain leading to more penetration and rupture of the bacteria. The enhanced hunting potential of the prepared ZnO/CuO nanocomposite may have emerged due to the increased surface-to-volume ratio and small particle size estimated for the ZnO/CuOnanocomposite in the XRD analyzed (Vibitha *et al*. 2020). Another report shows bacterial properties of the nanoparticles, which confirmed with ZnO/CuO nanocomposite exhibited better hunting potential than the individual metal oxide nanoparticles CuO, and ZnO (Jerry.O *et al*.2020). It could be noted from the results presented in table were as the concentration of the prepared nanoparticle increases, the hunting proficiency also increases (Vibtha et al. 2020).



(a)

(b)





(c)

Figure 4.6 the bacterial culture showing the antibacterial activity of (a) ZnO, (b) CuO and (c) ZnO/CuO nanocomposite.

5. Conclusion

In the present study, the ZnO, CuO nanoparticles and ZnO/CuO nanocomposites were fabricated by the biological method using in the presence of *Acokantheraschemperri leaf* extract and their antibacterial activities were investigated against gram positive (streptococcus pyogenes and Staphyloccus aureus) and gram negative (Pseudomonas aerogenosa and Eschericiacoli) bacteria. The XRD and SEM analyses displayed that the ZnO nanparticle synthesized in the presence of the leaf extract have smaller size in comparison with the sample prepared in water and the antibacterial activity measurements showed that the ZnO (W) sample exhibited a weak antibacterial activity against both bacteria compared to the sample prepared in water according to (Ramin *et al.* 2018) reported. As known, different functional groups presented in the leaf extract of *Acokantheraschemperri*, was stabilize particles of ZnO during their growth, which is responsible for stabilization and formation of smaller particles of the ZnO (ext) sample in comparison with the ZnO (W) sample (Elumalai *el al.* 2015, Siripireddy *et al*. 2017). Similar results have been observed during fabrication of ZnO nanoparticle in the presence of various plant extracts (Vanathi *et al.*2014, Ramesh *et al*.2015). Interestingly, the ZnO/CuO nano composites exposed high antibacterial activities against both gram negative (Pseudomonas aerogenosa and Escherciacoli) bacteria, due to the increased surface to volume ratio and small particle size estimated for the ZnO/CuO nanocomposite in the XRD analyzed(Vibitha *et al.* 2020). The biosynthesis of nanoparticles and nanocomposite using leaf parts of *A. schemperri* extract have been reported, these were simple, cost-effective, rapid and ecofriendly way capable of synthesizing within few hours at room temperature. In this method, plant extracts played as capping, stabilizing and reducing agent. The synthesized nanoparticles may be further explored for preparation of nano medicines and other products.

Due to the reassuring characteristics of the prepared nanocomposites and their favourable antibacterial effectiveness against tested bacteria, the green synthesized ZnO/CuOnano composite can be introduced as a potential skillful antiseptic.

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