# SYNTHESIS OF COPPER OXIDE NANOPARTICLES USING LEAF EXTRACT OF *BERSAMA ABYSSINICA* AND ITS APPLICATION FOR THE TREATMENT OF DRUG RESISTANT BACTERIA

**BY**

**SOLOMON BEKELE**

**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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**Major Advisor: - Bedasa Abdisa (PhD)**

**Co – Advisor: - Gemechu Deressa (PhD)**

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# APPROVAL SHEET

We, the undersigned, members of the board of examiners of the final open defense by Solomon Bekele have read and evaluated his thesis entitled **"SYNTHESIS OF COPPER OXIDE NANOPARTICLES USING LEAF EXTRACT OF *BERSAMA ABYSSINICA* AND ITS APPLICATION FOR THE TREATMENT OF DRUG RESISTANT BACTERIA"** 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.

**Submitted by:**

| | | |
|---|---|---|
| Solomon Bekele | _____ | _____ |
| Name of student | Signature | Date |

**Approved by**:

| | | |
|---|---|---|
| 1. Bedasa Abdisa (PhD) | _____ | _____ |
| Major Advisor | Signature | Date |
| 2. Gemechu Deressa (PhD) | _____ | _____ |
| Co – advisor | Signature | Date |
| 3. _____ | _____ | _____ |
| Chairperson | Signature | Date |
| 4. _____ | _____ | _____ |
| Internal Examiner | Signature | Date |
| 5. _____ | _____ | _____ |
| External Examiner | Signature | Date |

## 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: Solomon Bekele

Signature: _____

This M.Sc Thesis has been submitted for examination with our approval as thesis advisors.

Advisor Name: Bedasa Abdisa (PhD) Signature: _____

Co-advisor Name: Gemechu Deressa (PhD) Signature: _____

Date of submission: _____

## ADVISOR'S APPROVAL SHEET

This is to certify that the thesis entitled "**SYNTHESIS OF COPPER OXIDE NANOPARTICLES USING LEAF EXTRACT OF *BERSAMA ABYSSINICA* AND ITS APPLICATION FOR THE TREATMENT OF DRUG RESISTANT BACTERIA**" 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 Solomon Bekele Id. No GSU/0505/06, under our supervision. Therefore, we recommend that the student has fulfilled the requirements and hence hereby he can submit the thesis to the department.

| | | |
|---|---|---|
| Bedasa Abdisa (PhD) | _____ | _____ |
| Name of major Advisor | Signature | Date |
| Gemechu Deressa (PhD) | _____ | _____ |
| Name of Co-Advisor | Signature | Date |

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| | |
|---|---|
| CTAB | Cetyltrimethyl ammonium bromide |
| DSSCs | Dye-sensitized solar cells |
| EDX | Energy Dispersive X-ray spectroscopy |
| FT-IR | Fourier Transform-Infra-Red |
| JCPDS | Joint Committee for Powder Diffraction Standard |
| XRD | X – Ray Diffraction |
| NPs | Nanoparticles |
| PVP | Polyvinylpyrrolidone |
| rpm | revolution per minute |
| SEM | Scanning Electron Microscope |

## ABSTRACT

*Development of green technology is generating interest of researchers towards eco-friendly and low cost method for biosynthesis of nanoparticles. The present study focuses on the green synthesis and characterization of copper oxide nanoparticles using Bersama abyssinica leaf extract and monohydrated copper (II) acetate for antibacterial application on the drug resistant bacteria. The synthesized nanoparticles were characterized using Thermo Gravimetric Analysis (TGA), X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FT-IR), SEM and EDX. X-ray diffraction studies showed that the particles are monoclinic in nature and the size is found to be in the range 24.6–31.5 nm. The XRD analysis showed that for certain ratios, particle size were increased with increase precursor salt volume. The FT-IR analysis showed the formation of bond between copper and oxygen which confirm the formation of CuO nanoparticles. The SEM analysis indicates the spherical shape morphology for the CuO NPs and EDX spectrum showed the synthesized CuO NPs was pure. Furthermore, the synthesized CuO NPs exhibit significant antibacterial activity against pathogenic bacterial strains namely gram positive bacteria S.aureus & Bacillus subtilis and gram negative bacteria Escherichia coli but it shows resistance against gram negative Pseudomonas aeruginosa.*



**Keywords:-***Copper oxide NPs, Bersama abyssinica , XRD, SEM, EDX and Biosynthesis*.

# 1. INTRODUCTION

## 1.1. Background of the Study

The history of nanomaterials reaches back thousands of years. Archeologists found that already 3000 years ago in Egypt and China gold, silver, platinum and palladium nanoparticles were used in paint, even without the knowledge of the existence and the properties of these materials (Heiligta and Niederberger, 2013). The concept "Nanotechnology" was first proposed by Richard Feynman on December 29, 1959 in his talk entitled "There's plenty of room at the bottom" at the California institute of technology. Deriving its name from the Greek word "dwarf" meaning small, nanotechnology is commonly defined as the combined art of science and technology of small things (Akila and Ganesh, 2014). Nanotechnology is emerging as a rapidly growing field with its application in science and technology for manufacturing new material at the nano scale level (Vanden and Wildenberg, 2005). Nanotechnology involves synthesis, characterization and application of nanoparticles by controlling shape and size (Gratzel, 2001).

There are several definitions available from different international organizations for the terms "nanomaterials" and "nanoparticles". Conclusively, it can be said that nanoparticles are defined as objects having 1-100 nm dimensions in the nanoscale and that nanomaterials have an external or internal structures in the nanoscale (Borm et al, 2006). Nanoparticles usually ranging in dimension from 1-100 nm have properties unique from their bulk equivalent. With the decrease in the dimensions of the materials to the atomic level, their properties change. Nanoparticles have a larger percentage of atoms at their surface, which lead to high surface reactivity.

Nanoparticles have wide range of applications. In particular, metal oxide nanoparticles are receiving increasing attention in a large variety of applications. Metal oxide nanoparticles are of interest because of their unique optical, electronic and magnetic properties. The oxides of transition metals are an important class of semiconductors, which have applications in magnetic storage media, solar energy transformation, electronics, gas sensors and catalysis (Ramgir et al, 2013). Metal oxide nanoparticles have also applications in various other fields including molecular sensing, and medicine. However, the use of toxic chemicals on the surface of nanoparticles and non-polar solvents in the synthesis procedure limits their applications in

clinical fields. In this regard, these nanoparticles are synthesized under controlled conditions in order to give successful functionalities for specific applications.

Among transition metal oxides, attention have been paid to copper oxide because of their potential commercial applications in magnetic phase transitions, gas sensors, solar energy transformation, catalysis, and superconductivity (Guajardo et al, 2010; Mahmoud et al, 2015). Copper oxide nanoparticles are very important in medicinal field in which they act as antioxidant and antibacterial agents (Azam et al, 2012).

Copper oxide nanoparticles can be synthesized using a variety of methods including physical, chemical and biological techniques. Although different physical and chemical methods have been extensively used to produce nanocrystalline copper oxide, the stability and the use of toxic chemicals are subjects of paramount concern. Therefore, development of biocompatible, nontoxic and eco-friendly methods for nanoparticles synthesis deserves merit. The interest in this field has shifted toward green chemistry and bioprocess approach. These approaches focus on utilization of environment-friendly, cost effective and biocompatible reducing agents for synthesis of nanoparticles (Honary et al, 2012). Phytochemicals present in plants play a major role in nanoparticles production. The most common phytochemical present in plants that is expected to be responsible for nanoparticles formation is flavonoid (Makarov et al, 2014). However, there are only a very few reports on the use of yeast, fungi, bacteria or plant extract for synthesizing CuO NPs (Honary et al, 2012). Therefore, this work explored the use of *Bersama Abyssinica* leaf extract, which act as both reducing and stabilizing/capping agent for the synthesis of CuO nanoparticles.

*Bersama abyssinica* grows in Ethiopia is known locally as Azamer in Amharic and Lolchissa in Afaan Oromoo (Verdcourt, 1998). *B. abyssinica* has antibacterial activity and is a natural antibiotic (Kouadio, 2016). In Ethiopian traditional medical practices the leaves extracts of *Bersama abyssinica* are used for treating tumour, stomach disorders such as abdominal pain, diarrhea, cholera, intestinal worms, rabies, syphilis, gonorrhea and malaria ( Cos et al, 2006).
Recently resistance of microorganisms to antibiotics is steadily rising, with reports showing that quite a number of the recognized antimicrobial agents in existence have demonstrated resistance

by one species of microorganism or another (Raffi et al, 2010). This antibiotic resistance in pathogenic microorganisms has caused many premature deaths and has become a public health problem worldwide (Theuretzbacher, 2012).

Inorganic antibacterial agents, in particular metal oxides have improved stability under harsh processing conditions and antibacterial activity without photo-activation (Sawai, 2003). In this regard, synthesis of compounds such as nanoparticles with antimicrobial properties and has potentially applications in the fight against the ever growing number of antimicrobial-resistant pathogenic microorganisms must have particular attention. Therefore, in this work, attempts were made to utilize the leaf extract of *Bersama abyssinica* for the synthesis of CuO NPs and the potential antimicrobial activities of CuO NPs against some drug resistant pathogenic bacteria were evaluated.

### 1.2. Statement of the Problem

Infectious diseases caused by bacteria, fungi, viruses and parasites continue to pose a threatening challenge to public health [Cos, 2006]. During the last decade, a 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. A possible alternative is the synthesis of new inorganic compounds with antimicrobial properties. A copper oxide nanoparticle has the advantage of chemical and physical stability over common organics and antibiotics compounds. Therefore, this work is planned to synthesize copper oxide nanoparticles which are cost effective, from common and cheap precursors by green synthesis method and investigate the effect of CuO nanoparticles on microbial agent against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa*.

## 1.3. Objectives of the Study

### 1.3.1. General Objective

The general objective of this study is synthesis of CuO nanoparticles using aqueous extract of leaves of *Bersama Abyssinica* and to study the antimicrobial activity of CuO nanoparticles against selected drug resistant bacteria.

### 1.3.2. Specific Objectives

The specific objectives of this work are to:-

i. Synthesize CuO nanoparticles using aqueous leaf extract of *Bersama abyssinica* and copper acetate ($Cu(CH_3COO)_2.H_2O$).
ii. Characterize the synthesized CuO nanoparticles using XRD, SEM, EDX, and FT-IR.
iii. Investigate the antibacterial effect of the synthesized CuO nanoparticles against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa.*
iv. To study the effect of proportion of precursor salt and plant extract on size and antibacterial activity of CuO.

## 1.4. Significance of the Study

This study will provide important information about the synthesis strategy of CuO nanoparticles and their antimicrobial activity against some common antimicrobial-resistant pathogenic microorganisms.

## 1.5. Scope of the Study

The synthesis technique was limited to green synthesis of CuO NPs using *Bersama Abyssinica* leaf extracts, the characterization of the nanoparticles was limited to XRD, SEM, EDX, and FT-IR technique and its application was limited to *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa.*

## 2. LITERATURE REVIEW

### 2.1. Properties of CuO Nanoparticles

The properties of materials change as their size approaches the nanoscale. Further, the percentage of atoms at the surface of a material becomes more significant. Bulk materials possess relatively constant physical properties regardless of their size, but at the nanoscale this is often not the case. 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. At this scale, the surface-to-volume ratios of materials become large and their electronic energy states become discrete. This brings the unique properties of the nanomaterials such as electronic, optical, magnetic, and mechanical (Jamieson et al, 2007**)**. The chemical and physical properties of nanoparticles do not only depend on their size, but also on their composition which can be organic (polymers, lipids) as well as inorganic (metal, metal oxides, metalloid, metalloid oxides) or a mixture of both (Cupaioli et al, 2014). In addition, the shape affects the physical properties of nanoparticles. The size of nanoparticles and their shape define their surface area (Kittler et al, 2014). In comparison to bigger particles, the surface area of smaller particles is higher in relation to their volume. Therefore, the big advantage of nanomaterials is that only a low amount of material is required to create a very large surface.

Among various metal oxide nanoparticles, CuO NPs has attracted particular attention because it is the simplest member in the family of copper compounds and shows a range of useful physical properties such as high temperature superconductivity, electron correlation effects, and spin dynamics. The optical properties of CuO NPs are significantly influenced by the temperature, size and morphology (Zhang et al, 2014). The dimensions of CuO NPs also influence their magnetic properties. Moreover, the magnetic properties of CuO NPs strictly depend on their morphology (Yin M et al, 2005). Cupric oxide is a p-type semiconductor (Zhang et al, 2014) and has optical band gap of 2.43 eV, which is much larger than that of bulk CuO (1.85 eV) (Chand et al, 2014). CuO NPs has brownish black appearance and it belongs to the monoclinic crystal structural system (Chand et al, 2014). The unit cell of monoclinic structure of CuO NPs is shown in Figure 1.

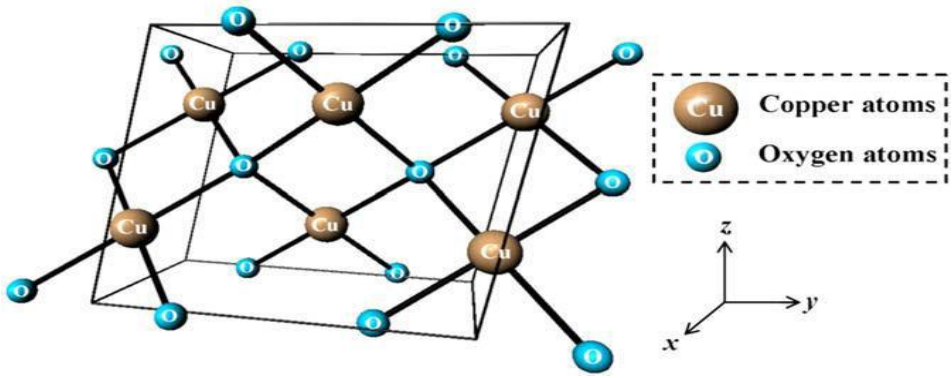



**Figure 1:** Schematic representation of a monoclinic CuO unit cell.

### 2.2. Synthesis of CuO Nanoparticles

Nanoparticles can be synthesized using a variety of methods including physical, chemical and biological techniques (Figure 2) (Rao et al, 2009).

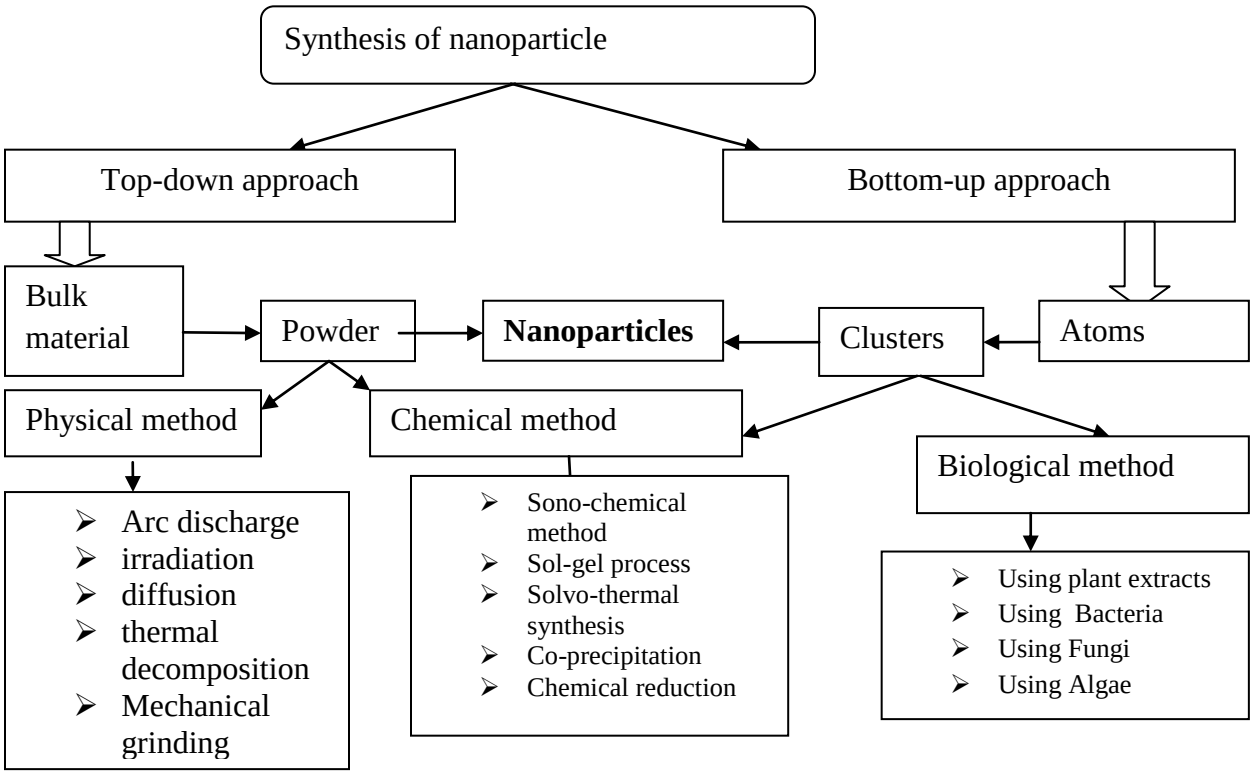



**Figure 2:** Schematic representation of synthesis methods of nanoparticles.

During nanoparticles, synthesis size and shape of NPs strongly depend on the growth medium parameters such as pH, solvent percentage, reflux temperature, calcination temperature and salt concentration (Gericke and Pinches, 2006).

### 2.2.1. Physical Methods

The physical method often called top-down approach, which includes methods like diffusion, irradiation, thermal decomposition and arc discharge etc. The thermal decomposition method is one of the significant top-down approaches. These approaches use larger initial structures or macroscopic units, which can be externally controlled in the processing of nanostructures. Mostly the top-down methods are not cheap and quick to manufacture (Rai et al, 2006). This method is slow and not suitable for large-scale production. The other drawback of physical method is that the physical method requires high temperature (>350$^{o}$C), whereas chemical methods require a temperature less than 350$^{o}$C. In most cases, the synthesis of nanoparticles using green technology requires temperatures less than 100$^{o}$C (Rai et al, 2006). Heat evolving from high temperature processes may cause heat rashes, heat cramps, heat exhaustion and heat strokes. Annealing process is responsible for the adsorption/decomposition on the surface, thus the structure and stoichiometric ratio of the material is changed. Moreover, the particle size and annealing temperature have a close relation, with increases of annealing temperature, particle size increases (Koudelk, 2004).

### 2.2.2. Chemical Methods

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 (Ren et al, 2009).

Copper oxide nanoparticle with different sizes, shapes and morphology were obtained by sonochemical method using copper(II) acetate as precursors, urea and sodium hydroxide as reducing agent and polyvinylpyrrolidone (PVP) as stabilizing polymer, followed by irradiation using high intensity ultra sound (Sambandam and Lee, 2012). In comparison, Karunakaran et al. synthesized two sets of samples by applying the sonochemical method. The first set was formed from CuO NPs and cetyltrimethyl ammonium bromide (CTAB) and the second from CuO NPs without CTAB, after sonication and calcination. The results revealed that in the absence of CTAB, the nanoparticles have irregular shape and particle agglomeration was favored. Karunakaran et al. reported that the presence of CTAB promotes the crystal formation of CuO (Karunakaran et al, 2013).

The sol-gel technique is a simple and relatively fast method and therefore it is widely used in the design of nanoparticles. This method is applied often as it ensures the rigorous control of the nanoparticle size. The method was optimized in order to obtain nanoparticles with dimensions ranging between 10 and 40 nm (Jayaprakash et al, 2014). Other chemical method such as micro emulsion method (Nassar et al, 2007), arc-submerged nanoparticle synthesis system (Kao et al, 2007), flame-based aerosol methods (Chiang et al, 2012), hydrothermal (Zhang et al, 2006) and solid-state techniques (Wang et al, 2009) are also used to synthesis nanostructured CuO. The production of nanoparticles through conventional chemical methods results in toxic byproducts that are environmental hazards. Additionally, these particles cannot be used in medicine due to health-related issues, especially in clinical fields.

Conventional chemical methods can be used to produce nanoparticles in large quantities with defined sizes and shapes in a shorter period. However, these techniques are complicated, costly, and outdated (Parashar et al, 2009).

### *2*.2.3. Biological Methods

The production of nanoparticles through conventional physical and chemical methods results in toxic byproducts that are environmental hazards. Size and shape of nanoparticles play crucial role for applications of NPs in different fields. Additionally, these many adverse effects have

been associated with chemical synthesis methods due to the presence of some toxic chemical absorbed on the surface. Eco-friendly alternatives to chemical and physical methods are biological ways of nanoparticles synthesis using microorganisms, enzymes, fungus and plants or plant extracts (Jayanta and Kwang, 2014). Green synthesis method is proved to be 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.

Biosynthesis involves using an environment-friendly green chemistry based approach that employs biological entities such as bacteria, algae and plants (Mohanpuria et al, 2008). Synthesizing nanoparticles via biological entities acting as biological factories offers a clean, nontoxic and environment-friendly method of synthesizing nanoparticles with a wide range of sizes, shapes, compositions, and physicochemical properties (Mohanpuria et al, 2008).

In comparison with microorganisms, the plant approach is more advantageous since it does not need any special, complex, and multi-step procedures such as isolation, culture preparation, and culture maintenance. In the case of a microorganism, culturing methods are very important. Hence, optimization of culturing parameters such as nutrients, light, medium pH and temperature can significantly increase enzyme activity (Iravani, 2011). Generally, biological synthesis using plants tends to be faster than microorganisms, is more cost-effective and is relatively easy to scale up for the production of large quantities of nanoparticles. In this regard using green methods in the synthesis of nanoparticles has increasingly become a need of the time.

Recently green synthesis of copper oxide nanoparticles using plants such as Azadirachta indica (Neem) leaf extract (Ansilin et al, 2016), Ixora Coccinea (Yedurkar et al, 2017), Aloe barbadensis (Sangeetha et al, 2012), Acalypha indica (Rajeshwari et al, 20140), T. arjuna bark extract (Yallappa et al, 2013), Calotropis gigantean leaf extract (Jitendra et al, 2015), Malvasylvestris leaf extract (Awwad et al , 2015), pyrus pyrifolia leaf extract (Sundaramurthy et al, 2015), Ocimum Sanctum leaf extract (Vasudev et al, 2013) have been reported.

### 2.3. Precursor Salts for Synthesis of CuO Nanoparticles

Various copper salts such as chloride, nitrate, sulfate, acetate were used to prepare CuO nanomaterial. However, particle size and uniformity of copper nanoparticles prepared from copper acetate seem better than those from inorganic copper salt (Sun et al, 2005). A reasonable explanation is that carboxylate groups are still adsorbed on the surface of the copper oxide nanoparticles, play the role of a surfactant, and suppress nanoparticles from growth and aggregating process. The other main necessary precursor for synthesizing CuO nanoparticles is the base agent which provides hydroxyl ion to react with copper salt and gives the $Cu(OH)_2$ precipitation. The most common used precursors are sodium hydroxide and potassium hydroxide (Sun et al, 2005).

### 2.4. Applications of CuO Nanoparticles

Metal oxide nanoparticles are important due to their applications in nanodevices, nanoelectronics, nanosensors, information storage, and catalysis. Copper oxide nanoparticle have useful application in gas sensors, batteries, dye sensitized solar cells, field emission emitters and fuel cells. Other applications of CuO NPs include antioxidant, antibacterial, anti-biotic, anti-fungal agent, catalysis and so on (Wang and Muhammed, 1999; Sankar et al, 2014).

#### 2.4.1. Application of CuO Nanoparticles in Dye-sensitized Solar Cells

The Dye-sensitized solar cells (DSSCs) have been known as promising renewable energy devices compared to commercial silicon based solar cells owing to their easy, low manufacturing cost and reasonably high overall light-to-electricity conversion efficiencies. Nanocrystalline $TiO_2$ has been used as an anode material and expensive platinum (Pt) based electrode as counter electrode for the fabrication of DSSCs. However, recent research advances on DSSCs have been focused on improving the electron transport and reducing the recombination rate using semiconductor materials other than $TiO_2$ (Jitendra et al, 2015). The high cost Pt could be replaced with the other cheaper materials to fabricate the less expensive counter electrode for DSSCs. In this regard, CuO is a p-type semiconductor and shows an excellent material for the fabrication of various efficient electronic and optoelectronic devices (Zhang et al, 2014).

Generally, CuO NPs are being considered as an alternative counter electrode material for the fabrication of DSSCs.

### 2.4.2. Application of CuO Nanoparticles for Catalysis

The enormous volume of environmental pollutants, non-degradable and carcinogenic natured colored dye effluents are discharged by the textile and paper industries. Moreover, to unique in their products most of the industry uses color dyes, without any treatment of coloring materials are liquidated in water leads to contamination of resources. Nowadays, a photo-catalytic method got the wide attention due to its effective de-colorization of dyes (Renu et al, 2014). Particularly heterogeneous catalysis supplies the opportunity for easy separation and recycling of the catalyst, easy product purification and possibly, continuous or multiple processing of compounds. In recent years, copper oxide nanoparticles are used in the form of heterogeneous catalysis in textile industry and water treatment, due to high surface-to-volume ratio and nanoparticles highly active surface. The nanoparticles used as a catalyst can be easily separated from the reaction mixture by simple centrifugation method and re-used without further disposal. CuO NPs is more efficient catalyst with respect to reaction time and yield than the others (Mahmoud et al, 2015).

### 2.4.3. Application of CuO Nanoparticles for Antimicrobial activity

Human beings have been using copper complexes for various purposes for centuries, such as water purifiers, algaecides, fungicides, and as antibacterial agent (Raoet al, 2009; Dongdong et al, 2010). In the field of medicine, nanoparticles are being explored extensively because of their size dependant chemical and physical properties. The size of nanoparticles is similar to that of most biological molecules and structures. This makes them an interesting candidate for application in biomedical research. The result of their integration in the field of medicine has led to their application mainly in targeted drug delivery, imaging, sensing, and artificial implants. Another interesting avenue for their exploration in medicine is their use as antimicrobials to target highly pathogenic and drug resistant microbes (Vinod et al, 2013). However, for the application of nanoparticles in medicine, biocompatibility is a highly desired trait. Biocompatibility is the materials ability to perform medically without exertion of undesired local or systemic effects. It is suggested that highly ionic nanoparticulate copper oxide have potential

application as antimicrobial agents as they can be prepared with extremely high surface areas and unusual crystal morphologies (Sanvicen and Marco, 2008).

CuO nanoparticles were effective in killing a range of bacterial pathogens involved in hospital-acquired infections (Stoimenov, 2002). They are used in hospitals due to their antimicrobial ability to kill more than 99.9% of gram-positive and negative bacteria within 2 h of exposure if a suitable dose is applied (Madalina, 2016). Some advantages of using copper-oxide in hospital are: -

1. It is effective against both susceptible and antibiotic resistant microorganisms.
2. It has wide antifungal spectrum and antibacterial properties.
3. It inhibits bio-film or the development of microorganisms in attached communities on the surface of materials coated with CuO NPs.
4. It does not cause skin irritation or sensitization.

The efficacy of CuO NPs against antibiotic resistant microorganisms can be affected by size and concentration on the nanoparticles. Guy. A et al investigated that increasing the CuO particle size resulted in reduced antibacterial activity against *S. aureus.* For *E. coli*, however, the effect of particle size on the antibacterial activity was less pronounced (Guy.A et al, 2012).

### 2.5. *Bersama Abyssinica*

#### 2.5.1. Ethno Botanical Information of *Bersama Abyssinica*

*Bersama abyssinica* belongs to the family *Melianthaceae*; the genus *Bersama* made up of eight species, all found in Africa (Acharyulu et al, 2014). It grows in lowland bush savanna and gallery forests, from sea-level up to 2700 m altitude. It is distributed in Democratic Republic of Congo, Tanzania, Mozambique, Zambia, Zimbabwe, Angola, Nigeria, Ethiopia, Kenya, Sudan and Uganda. Ever green shrub to small tree up to 12 m tall; bark gray, brown. (Djemgou et al, 2010).The picture of leaves of *Bersama abyssinica* are shown in Figure 3.

**Figure 3**: Leaves of B. abyssinica (pictures taken by Solomon Bekele at Tullu Jebi March 2018).

### 2.5.2. Phytochemistry of *Bersama Abyssinica*

Phytochemicals are naturally occurring in the body parts of medicinal plants; such as, leaves, stems, barks and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoids, alkaloids and phenolic compounds. The presence of the secondary classes of compounds in a plant is responsible for medicinal activity of the plants (Krishnaiah et al, 2007). From phytochemical investigations, the *B. abyssinica* plant leaves contains alkaloids, glycosodes, flavonoids, steroids, phenols, tannins, triterpenene, anthraquinones, polysterols and coumarins (Mathewos et al, 2015).

### 2.5.3. Medicinal Uses of *Bersama Abyssinica*

Ethnobotany is the study of the relationship between medicinal plants and people with a particular emphasis on the traditional uses of those plants for the prevention and treatment of various diseases. Traditional uses of medicinal plant plays a significant role in many Asian and African countries. The genus *Bersama* is one among these medicinal plants used for treatment of

several diseases. From the pharmacological point of view, *B. abyssinica* is used in the treatment of various diseases: cancer, spasms, infections, male infertility, diabetes, diarrhea, cholera, intestinal worms, syphilis, gonorrhea, malaria and general fatigue (Kuete et al, 2008). Medicinal plants have been very intensively screened for their bioactivity in order to treat various disease and disorders in human.

### 2.6. Role of Plant Leaf Extract for Nanoparticles Synthesis

Due to the large number of phytochemicals present in plant extracts, many researchers believe that flavonoids can facilitate the formation of metal oxide nanoparticles (Makarov, 2014). Flavonoids are the most common phytochemical present in plants and expected to be responsible for nanoparticles formation (Makarovet al, 2014). The main phytochemicals responsible for the synthesis of nanoparticles have been identified as terpenoids, flavones, ketones, aldehydes, amides and carboxylic acids (Jaison et al, 2016). Plant parts like roots, leaves, stems, seeds, fruits have been utilized for the NPs synthesis as their extract is rich in phytochemicals which act as both reducing and stabilization agent (Akhtar et al, 2013). Plant leaf extracts serve as an excellent source for as metal oxide nanoparticles synthesis due to the presence of several phytochemicals that can be easily extracted (Jaison et al, 2016).

The mechanism for metal oxide nanoparticles synthesis through plant extracts is not clearly known. However, literatures suggest that plant phytochemicals were responsible for the formation of metal oxide nanoparticle. Initially, metal precursors will be reduced by the phytochemicals in the plant extracts. Oxygen either from the atmosphere or from the degrading phytochemical will bind the reduced metal ions. Metal oxide ions will bind to each other due to electrostatic attraction and form nanoparticles. The nanoparticles formed are then stabilized by certain phytochemicals to avoid agglomeration between them (Akhtar et al, 2013).

### 2.7. Drug Resistance Bacteria

Nowadays, bacterial resistance to antimicrobial drugs is an increasing health and economic problem. Over the years, the continued use of various antibacterial agents has led microorganisms to develop resistance mechanisms, which are the cause of resistance to one or

more drug (Agnė et al, 2011). Multidrug- resistant organisms are usually bacteria that have become resistant to the antibiotics used to treat them. This means that a particular drug is no longer able to kill or control the bacteria. Inappropriate use of antibiotics for therapy resulted in the selection of pathogenic bacteria resistant to multiple drugs. One of two mechanisms can occur multidrug resistance in bacteria. First, these bacteria may accumulate multiple genes, each coding for resistance to a single drug. Second type of resistance, namely multidrug resistance may also occur by the increased expression of genes that code for multidrug efflux pumps, enzymatic inactivation, and changes in the structure of the target.

Among the various clinically important bacteria, Staphylococcus aureus, Streptococcus pneumoniae, Enterococcus species, Pseudomonas species, and Klebsiella species represent major pathogens associated with a high incidence of infection that are resistant to treatment with antibiotics of many antimicrobial classes (Styers et al, 2006; Peterson, 2005).

Resistance to multiple drugs was first detected among enteric bacteria namely, *Escherichia coli*, *Shigella* and *Salmonella* (Levy, 2001). *Escherichia coli* is a gram- negative bacteria strain causes food poisoning, urinary tract infection, neonatal meningitis (Haritha, 2011). *Pseudomonas aeruginosa* is a common bacterium, Gram-negative opportunistic pathogen capable of infecting humans with compromised natural defenses and causing severe pulmonary disease. It is one of the leading pathogen associated with nosocomial infections. It has a vast arsenal of pathogenicity factors that are used to interfere with host defenses. Pathogenesis in *P. aeruginosa* facilitates adhesion, modulate or disrupt host cell pathways, and target the extracellular matrix. The propensity of *P. aeruginosa* to form biofilms further protects it from antibiotics and the host immune system. *P. aeruginosa* is intrinsically resistant to a large number of antibiotics and can be acquired resistance to many others, making treatment difficult (Alaa, 2015). *Staphylococcus aureus* is gram-positive bacteria responsible for endocarditic, skin and wound infections. Among these *Staphylococci*, the strains marked R (Resistant) are those of which there is a high probability of treatment failure, regardless of the type and dose of antibiotic treatment used (Kouadio et al, 2016). *Bacillus subtilis* has a rod shaped appearance and belongs to the family of the gram-positive bacteria causes pneumonia, meningitis and sepsis (Haritha, 2011).

Most antibiotics showed that lacked activity against *Escherichia coli*, although they were active against gram-positive bacteria. Gram-negative bacteria are in general more resistant to a large number of antibiotics agents than are gram-positive bacteria. The outer membrane of gram-negative bacteria contributes to this intrinsic resistance by acting as an efficient permeability barrier, because the narrow porin channels limit the penetration of hydrophilic solutes and the low fluidity of the lipopolysaccharide leaflet slows down the inward diffusion of lipophilic solutes (Plesiat and Nikaido, 1992).

# 3. MATERIALS AND METHODS

## 3.1. Chemicals and Solvents

Chemicals and solvents used in this project work were Copper(II) acetate monohydrate (Cu $(CH_3COO)_2.H_2O$) (99.7%) (Loba Chemie 107, India), Ethanol absolute (99.9%) (Ranchem Industry and Trading) and distilled water (Ranchem Industry and Trading). All chemicals were analytical grade and used without further purification.

## 3.2. Apparatus and Instruments

The laboratory apparatus used for this work were: Beakers, Conical flasks, funnels, volumetric flasks, mortar and pestle, sieve, Whatman filter paper no 1, crucible, spatula, analytical balance, Magnetic stirrer with hot plate, drying oven , tong and Muffle furnace.

## 3.3. Synthesis of CuO Nanoparticles

### 3.3.1. Collection of Plant Material

Young and disease free *B. abyssinica* leaves were collected from Tullu Jebi, Lode Hetosa Wereda, which is found in East Arsi zone, Oromia region, Ethiopia, after conducting field surveys. Then the *B. abyssinica* leaves were taken to Adama science and technology university laboratory for experimental works.

### 3.3.2. Preparation of the Leaf Extract

The collected leaves of the plant were washed many times with distilled water to remove the dust particles and after that, the leaves were dried for 8 days on absorbent paper in Adama Science and Technology University laboratory. The dried leaves were grinded into a fine powder using mortar and pastel. The aqueous extracts of sample were prepared by boiling 25 g of the collected leaves with 250 mL of distilled water at $60^oC$ for 30 minutes on a hot plate with magnetic stirrer until the color of the aqueous solution changes in to brown color (Ansilin et al, 2016). Then the

brown solution was cooled to room temperature and filtered using Whatman filter paper no 1. The extract separated from filtration was stored in a refrigerator at 4$^{o}$C in order to be used for further experiments. Generally, the schematic procedure for preparation of the plant leaf extract was shown in Figure 4.

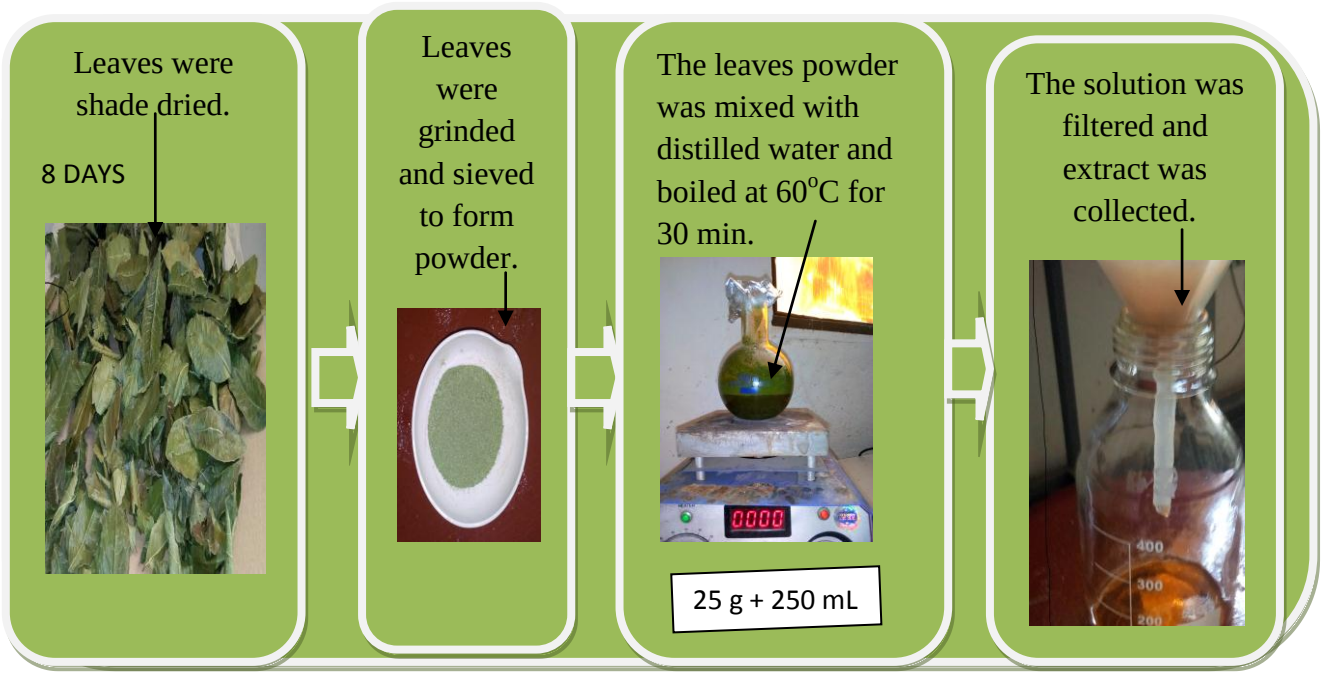



**Figure 4:** Schematic procedures for leaf extract preparation.

### 3.3.3. Preparation of Precursors

Monohydrated copper (II) acetate ($Cu(CH_3COO)_2.H_2O$), was used as precursor to synthesize CuO nanoparticles using aqueous leaves extract *B.Abyssinica*. 0.1 M copper acetate solution was prepared by dissolving about 4.99 g of copper acetate salt in distilled water using 250 mL volumetric flask.

### 3.3.4. Synthesis of CuO Nanoparticles

Different samples of CuO NPs were prepared by taking different ratios of precursor salt solution and *B. abyssinica* leaf extract. General schematic producer for CuO Nanoparticles preparation was shown in Figure 5.

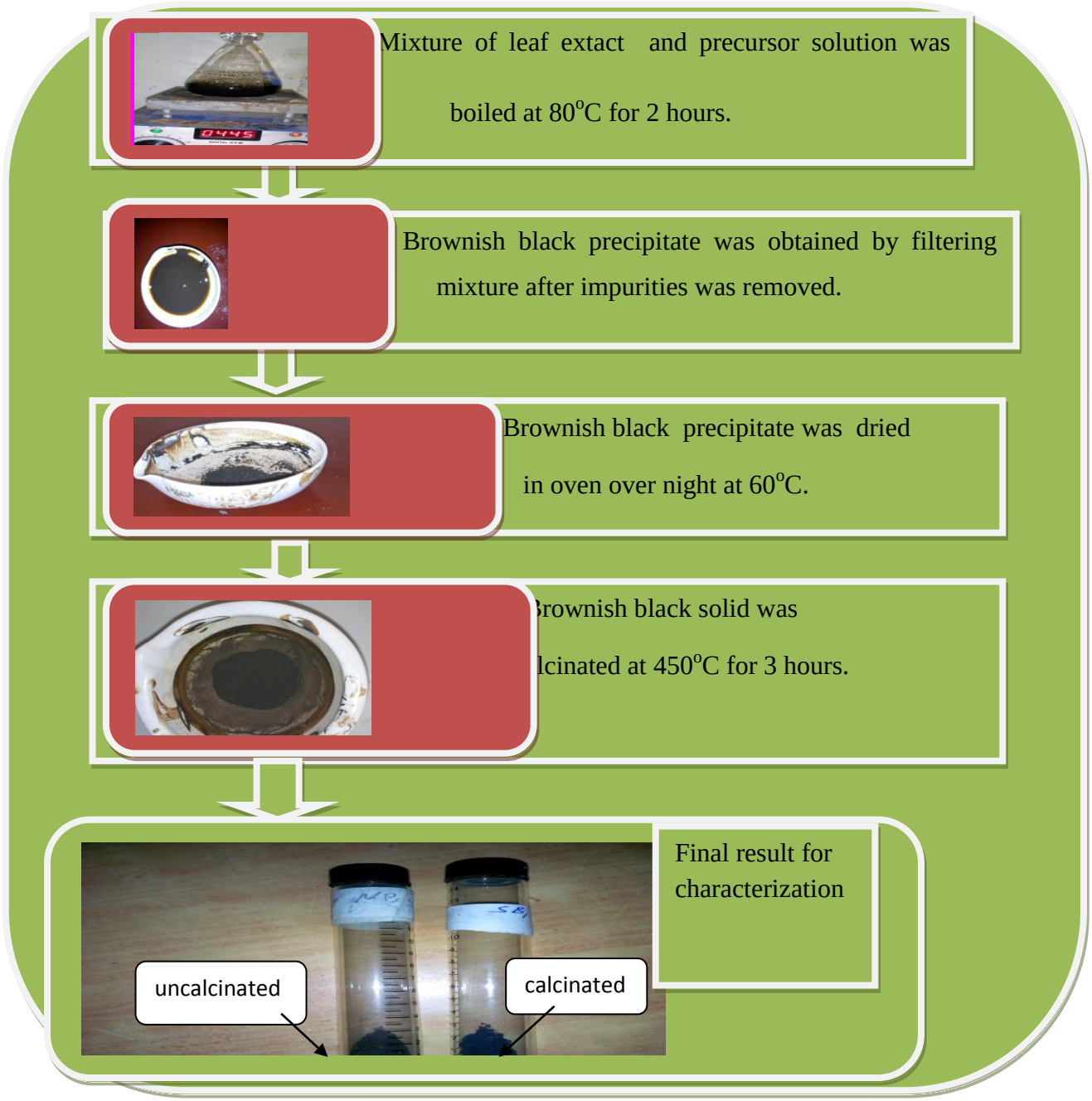



**Figure 5:** Schematic procedures for CuO NPs preparation.

CuO NPs synthesized using different ratios by volume of the plant leaf extract to precursor solution were labeled as follows:

Sample 1 (1:1 ratio): *B. abyssinica* leaf extract to $Cu(CH_3COO)_2.H_2O$ (50 mL : 50 mL )

Sample 2 (1:2 ratio): *B. abyssinica* leaf extract to $Cu(CH_3COO)_2.H_2O$ (50 mL : 100 mL)

Sample 3 (1:3 ratio): *B. abyssinica* leaf extract to $Cu(CH_3COO)_2.H_2O$ (50 mL : 150 mL)

Sample 4 (1:4 ratio): *B. abyssinica* leaf extract to $Cu(CH_3COO)_2.H_2O$ (50 mL : 200 mL)

Sample 5 (3:1 ratio): *B. abyssinica* leaf extract to $Cu(CH_3COO)_2.H_2O$ (150 mL : 50 mL).

Sample 6 (1:2 ratio uncalcinated): *B. abyssinica* leaf extract to $Cu(CH_3COO)_2.H_2O$ (50 mL : 100 mL)

Sample 1 (1:1 ratio)

To synthesis Sample 1 CuO NPs, 50 mL of *B. abyssinica* leaves extract was mixed with 50 mL of 0.1 M monohydrated copper (II) acetate ($Cu(CH_3COO)_2.H_2O$) in 250 mL conical flask. Then the mixture was boiled at 80°C with continuous magnetic stirring for 2 hours on a hot plate with magnetic stirrer until brown colour precipitate was formed (Yedurkar et al, 2017). After 2 hours, the solution mixture was cooled to room temperature and filtered using Whatman filter paper no 1. The brownish black precipitate was washed repeatedly with distilled water followed by 99.9% ethanol to remove the impurities. The precipitate was collected in a ceramic crucible and dried in an oven at $60^oC$ for overnight and grinded to fine powder using agate mortar. Then the brownish black powder was calcinated at $450^oC$ in Muffle furnace for 3 hrs. Finally, black colour powder was obtained. The final product obtained by the above method was stored in properly labeled plastic sample holder containers and used for further Characterization and antibacterial application. The same procedure was also applied for preparation of samples 2, 3, 4, 5 and 6.

### 3.4. Characterization Methods of Copper Oxide Nanoparticles

#### 3.4.1. Thermal Gravimetric Analysis (TGA)

Thermal gravimetric analysis (TGA) was carried out using a simultaneous DTA-TGA apparatus (DTG-60H, Shimadzu Co., South Korea) to determine the calcination temperature of the synthesized CuO NPs. About 18.475 mg of the uncalcinated CuO nanoparticles were placed in a platinum crucible on the pan of the microbalance and heated from room temperature to 1000°C.

### 3.4.2. X-ray diffraction (XRD)

The crystalline structure of the green synthesized copper oxide nanoparticles were investigated by the X-ray diffraction using X-ray diffractometer (XRD-7000, X-ray diffractometer, Shimadzu Co., South Korea). XRD spectrum was recorded from $10^0$ to $80^0$ with 2θ angles using CuK α radiation operated at 40 kV and 30 mA.

Debye Scherrer's formula was used to estimate the crystallite size of synthesized CuO NPs.

$$D = \frac{0.94\,\lambda}{\beta \cos\theta} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

Where, D is the Crystallite size, $\lambda = 1.54A^o$ for the X – ray source wavelength, β is the full width at half maximum (FWHM) of a peak, and θ is the diffraction angle.

The crystal structure lattice spacing of the synthesized copper oxide nanoparticles were determined using Bragg's equation based XRD spectrum result.

Bragg's equation

$$n\lambda = 2d \sin\theta \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

Where

$\lambda$ is wavelength.

d is lattice spacing.

$\theta$ is diffraction maximum.

n is order.

### 3.4.3. Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

The synthesized CuO nanoparticles were characterized by FT-IR Spectrophotometer Spectrum (Perkin Elmerbs) in order to analyze and detect surface functional groups in the wave number range of 4000 – 400 $cm^{-1}$. For the FT-IR characterization, the sample was mixed with solid KBr uniformly, properly which was compressed to settle down on a thin transparent film, and this transparent film was used for FT-IR analysis, which was kept in the chamber of the instrument

for scanning. FT-IR analysis was carried out to demonstrate the responsible phytochemicals during the green synthesized of CuO NPs and to confirm formation of Cu-O bond. 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. Hence FTIR is an important technique for identification and characterization of a substance.

### 3.4.4. Scanning Electron Microscopy (SEM)

In SEM characterization, nanoparticles powder was mounted on a sample holder followed by coating with a conductive metal. 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. The sample was 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.

### 3.4.5. Energy Dispersive X- Ray Spectroscopy

Energy dispersive X-ray spectroscopy (EDX) is an analytical technique used to determine the elemental analysis or chemical characterization of a sample through interaction 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, it may excite an electron from inner shell and create a hole over there. To compensate this vacancy an electron**s** 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.

### 3.5. Method for Antimicrobial Activity

Materials which were used for antimicrobial activities of the synthesized copper oxide nanoparticles were Nutrient broth, Nutrient agar, Agar-agar, Petri plates, cotton swabs, conical flasks, beakers, copper oxide nanoparticle samples, *Staphylococcus aureus, Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. Antibacterial activity of calcined nanoparticles were investigated against two gram positive bacteria *(staphylococcus aureus* and *Bacillus subtilis* ) and two gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa)* by agar well diffusion (6 mm in diameter) method. Agar well diffusion method is selected due to its simplicity and capacity to analyze multiple samples simultaneously and its ability to work well with defined inhibitors (Geyid et al, 2005).

#### 3.5.1. Preparation of Inoculum

Nutrient broth (1.3 g in 100 mL distilled water) was prepared in four different conical flasks and sterilized. Clinically isolated strain of *Staphylococcus aureus, Bacillus subtili, Escherichia coli* and *Pseudomonas aeruginosa* were added in to four different conical flasks, which contains the prepared Nutrient broth. These bacterial cultures inoculated in nutrient broth were kept on rotary shaker for 24 hours at 100 rpm (Mojtaba et al, 2017).

#### 3.5.2. Inoculation of Test Plate

Nutrient agar was prepared (5.6 g nutrient agar 0.5 g Agar Agar in 100 mL distilled water) and sterilized. The agar suspension within 15 minutes was used to inoculate plates by dipping a sterile cotton-wool swab into the suspension and remove the excess by turning the swab against the side of the container. Then the inoculum was spread evenly over the entire surface of the plate by swabbing in three directions. The plate was allowed to dry before applying to test antimicrobial activity of the NPs.

### 3.5.3. Agar Well Diffusion Method for Antimicrobial Activity Test

Antibacterial tests were carried out by the agar well diffusion method using the suspension of bacteria spread on nutrient agar. Antibacterial activity of green synthesized CuO nanoparticles were investigated against two gram positive and two gram negative bacteria by agar well diffusion method. The antibacterial activity of CuO nanoparticles prepared in different ratios was tested against these bacterial strains by using different concentrations of CuO NPs. About 50 mg, 100 mg and 200 mg of different samples of the synthesized CuO NPs were separately added to 10 mL of dimethyl sulfoxide (DMSO) in a 50 mL beakers and stirred for 2 hrs using magnetic stirrer. The wells were made on each Petri- plates by using sterile Cork borer having a diameter of 6 mm. Then 100 µL of each sample of CuO nanoparticles solutions and positive control (Gentamcin) were poured into wells on all plates with the help of micropipette. After different concentrations of CuO nanoparticles were added on to separate well of 6 mm diameter, the plate was allowed to dry. Then the plates were incubated at $37^{o}$C for 24 h in an incubator (Raja et al, 2015). 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 in millimeter (mm) of the inhibition zone (IZ) around the well against the test organisms by using a ruler.

# 4. RESULTS AND DISCUSSION

## 4.1. Thermal Gravimetric Analysis (TGA)

The synthesized nanoparticles were characterized by using thermo gravimetric analysis (TGA) to determine calcination temperature. The weight losses of the synthesized materials were analyzed. Figure 6 shows the thermo gravimetric analysis (TGA) and differential thermal analysis (DTA) of the uncalcined nanoparticles. 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 23.1 % up to 450$^{o}$C. The mass loss observed after 450$^{o}$C up to 1000$^{o}$C was low and not significant. This indicates that the synthesized nanoparticles are relatively thermally stable after 450$^{o}$C and as a result, this temperature was used for calcination of the synthesized nanoparticles.

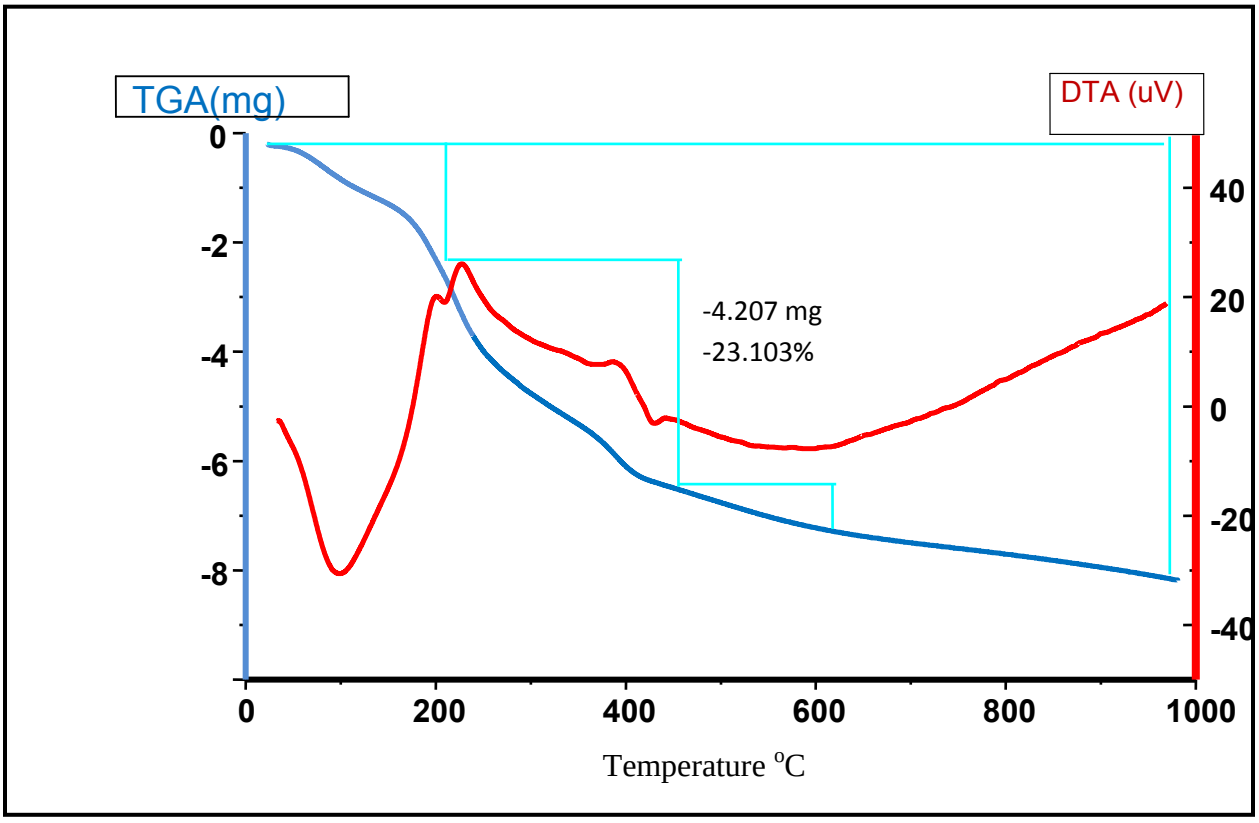



**Figure 6:** Thermo gravimetric analysis and differential thermal analysis of the uncalcined nanoparticles.

### 4.2. X-ray Diffraction (XRD) Analysis

CuO NPs with different particle sizes were synthesized from different ratios of precursor and plant extract. The crystal structure of CuO NPs was determined by using X-ray diffractometer in the range of $10^{o}$ to $80^{o}$ at room temperature. Figure 7 (a , b, c, d and e) shows the XRD patterns of calcinated CuO NPs synthesized from 1:1, 1:2, 1:3, 1:4 and 3:1 ratios by volume of *B. abyssinica* leaves extract to monohydrated copper (II) acetate respectively.

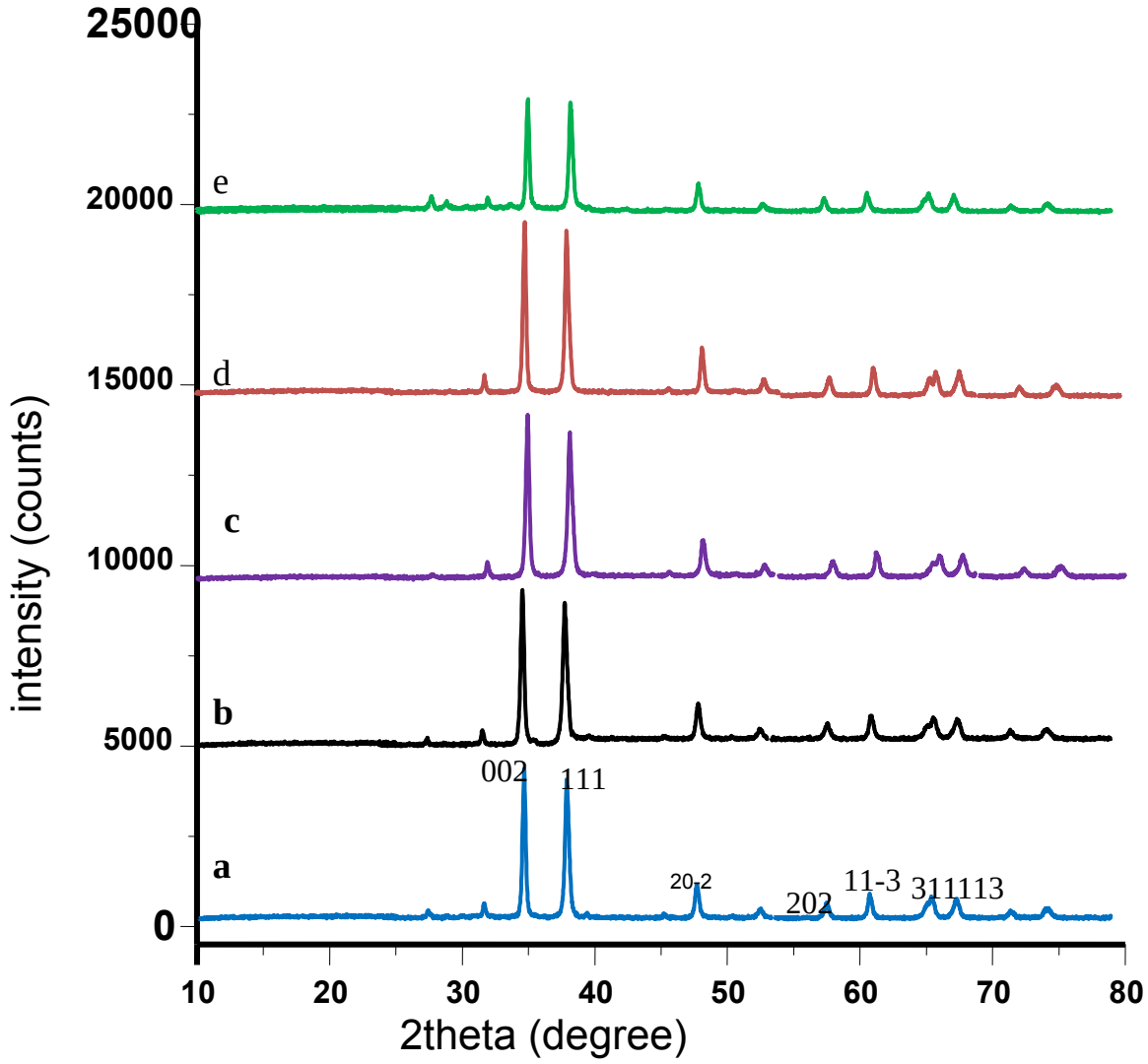



**Figure 7:** XRD patterns of Calcinated CuO NPs a) 1:1 (b) 1:2 (c) 1:3 (d) 1:4 e) 3:1 ratios by volume.

As indicated on Figure 7a the peaks of calcinated CuO NPs synthesized from *B. abyssinica* leaves extract and monohydrated copper (II) acetate in 1:1 observed at 2θ of 35.538$^{o}$, 38.773$^{o}$ , 48.739$^{o}$, 58.296$^{o}$, 61.562$^{o}$, 66.262$^{o}$ and 68.075$^{o}$ were assigned to planes of (002), (111), (20-2) (202), (11-3), (311) and (113) respectively. The sharp and narrow diffraction peaks observed from XRD patterns indicates crystalline structure nature and phase purity of the synthesized nanoparticles. The 2θ values are in good agreement with those of powder copper oxide obtained from the international center of diffraction data card JCPDS-048-1548, confirming the formation of a crystalline monoclinic structure CuO NPs (Yi-Huang et al , 2017). There is a slight shift of 2θ position for other peaks CuO NPs synthesized from other ratios even though the same materials and the same procedures were used. The 2θ values of peaks of calcinated CuO NPs synthesized from *B. abyssinica* leaves extract and monohydrated copper (II) acetate in 1:2, 1:3, 1:4 and 3:1 were also match with monoclinic structure of CuO NPs reported in JCPDS-048-1548.

The XRD pattern of uncalcined NPs synthesized from *B. abyssinica* leaves extract and monohydrated copper (II) acetate in 1:2 ratio was shown in Figure 8. XRD analysis shows that the 2theta values obtained at 36.42$^{o}$, 42.29$^{o}$ and 61.34$^{o}$ were assigned to the lattice planes of (111), (200) and (220) respectively. The observed diffraction reflections are comparable with JCPDS No. 005-0667 and were attributed to $Cu_2O$ NPs (Lily and Mary, 2015). The formation of $Cu_2O$ NPs confirmed that the plant extract serve as reducing agent and stabilize the particle durning the synthesis process. The result formed after calcination was CuO NPs. Therefore, CuO NPs were synthesized through two consecutive reactions reduction followed by oxidation. Generally, calcination process was very important to remove organic impurities and make the reaction complete for formation of CuO NPs from monohydrated copper (II) acetate *using B.abyssinica* leaves extract.

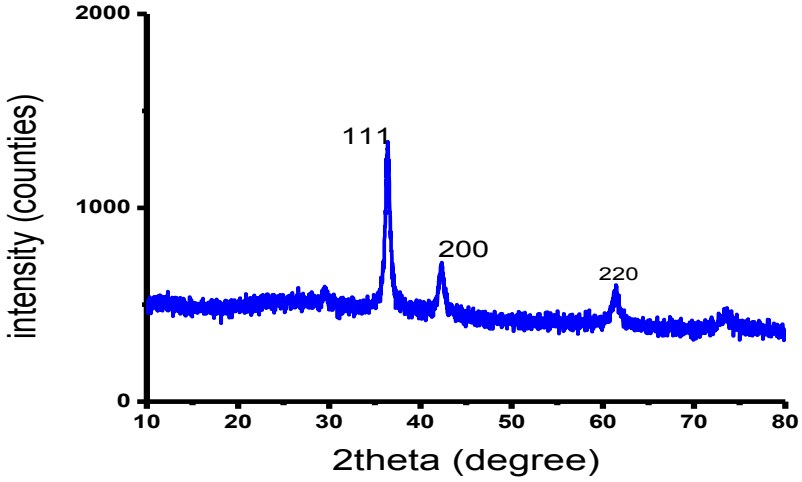



**Figure 8:** XRD pattern of uncalcined NPs.

CuO NPs peaks and their corresponding crystal size were given in Table 1. The average crystalline size for the synthesized CuO NPs was calculated using Debye- Scherrer equation. The average crystalline size for calcinated CuO NPs was found between 24.6 nm and 31.5 nm. The difference in crystal size may be due to difference in amount of leaf extract used as stabilizing agent for preparation of CuO NPs. XRD results were showed that the average crystallite size of CuO nanoparticles in 1:2 ratio has a smaller particle size. The average crystallite size of CuO nanoparticles synthesized from 1:3 ratios was greater than 1:2 ratios but smaller than 1:4 ratios. Generally, in the three ratios the particle size of the synthesized nanoparticles increases with increase the amount of the precursor salt solution. This difference may be due to the greater amount of leaf extract used during synthesis process resulting in more capping agents that in turn effectively stabilize the synthesized nanoparticles.

**Table 1:** Different angles, FWHM values, Miller indices, d-spacing and Average crystal sizes for different samples of CuO NPs.

| Sample 1 ( 1:1 ratio) | | | | Sample 2 ( 1:2 ratio) | | | |
|---|---|---|---|---|---|---|---|
| 2theta degree | FWHM degree | d (A°) dhkl | Miller Indices (h k l) | 2theta degree | FWHM degree | d(A°) dhkl | Miller indices (h k l) |
| 35.538 | 0.275 | 2.524 | 0 0 2 | 35.517 | 0.298 | 2.524 | 0 0 2 |
| 38.773 | 0.329 | 2.321 | 1 1 1 | 38.723 | 0.366 | 2.321 | 1 1 1 |
| 48.739 | 0.304 | 1.867 | 2 0 -2 | 48.785 | 0.357 | 1.867 | 2 0 -2 |
| 58.296 | 0.375 | 1.582 | 2 0 2 | 58.228 | 0.406 | 1.583 | 2 0 -2 |
| 61.562 | 0.268 | 1.505 | 1 1 -3 | 61.546 | 0.32 | 1.505 | 1 1 -3 |
| 66.262 | 0.342 | 1.409 | 3 1 1 | 66.242 | 0.459 | 1.409 | 3 1 1 |
| 68.075 | 0.392 | 1.376 | 1 1 3 | 68.032 | 0.511 | 1.376 | 1 1 3 |
| Average crystal size 29.2 | | | | Average crystal size 24.6 | | | |
| Sample 3 ( 1:3 ratio) | | | | Sample 4( 1:4 ratio) | | | |
| 35.505 | 0.305 | 2.526 | 0 0 2 | 38.682 | 0.295 | 2.527 | 0 0 2 |
| 38.71 | 0.371 | 2.324 | 1 1 1 | 48.77 | 0.284 | 2.326 | 1 1 1 |
| 48.795 | 0.35 | 1.865 | 2 0 -2 | 58.194 | 0.358 | 1.866 | 2 0 -2 |
| 58.224 | 0.402 | 1.583 | 2 0 2 | 66.254 | 0.279 | 1.584 | 2 0 2 |
| 61.544 | 0.332 | 1.506 | 1 1 -3 | 67.974 | 0.404 | 1.506 | 1 1 -3 |
| 66.254 | 0.449 | 1.41 | 3 1 1 | 53.427 | 0.324 | 1.41 | 3 1 1 |
| 68 | 0.473 | 1.378 | 1 1 3 | 65.788 | 0.414 | 1.378 | 1 1 3 |
| Average crystal size 24.9 nm | | | | Average crystal size 30.8 nm | | | |
| sample 5( 3:1 ratio) | | | | | | | |
| 35.546 | 0.265 | 2.749 | 0 0 2 | | | | |
| 38.789 | 0.308 | 2.524 | 1 1 1 | | | | |
| 48.717 | 0.324 | 2.32 | 2 0 -2 | | | | |
| 58.328 | 0.294 | 1.868 | 2 0 2 | | | | |
| 61.559 | 0.273 | 1.71 | 1 1 -3 | | | | |
| 66.242 | 0.276 | 1.581 | 3 1 1 | | | | |
| 68.094 | 0.368 | 1.505 | 1 1 3 | | | | |
| Average crystal size 31.5 nm | | | | | | | |

The inner spacing for the synthesized nanoparticles were calculated using Bragg's equation and compared with results of d– spacing ($d_{hkl}$) obtained using software. Results of d– spacing ($d_{hkl}$) and miller indices (h k l) for CuO nanoparticles obtained using an interactive powder diffraction data interpretation and indexing program version 2.2 software was shown in Table 1. The d-value for the major peaks were calculated using Bragg's equation and matched with the d-value data given in the literature for monoclinic CuO NPs crystals (Sundaramurthy and Parthiban, 2015).

### 4.3. Fourier Transformed Infrared (FTIR) Spectroscopy Analysis

The FTIR spectra for calcined CuO NPs was shown in Figure 9a. FT-IR spectra peaks were appears at 3427 $cm^{-1}$, 1626 $cm^{-1}$, 1051 $cm^{-1}$ and 530 $cm^{-1}$. In the spectrum, the wide absorption band around 3427 $cm^{-1}$ is due to O-H stretching vibrations. The absorption band around 1626 $cm^{-1}$ is because of C=C stretching of aromatic ring. C-O stretching vibrations are observed around 1051 $cm^{-1}$. There is sharp peak observed at 530 $cm^{-1}$ in the spectrum of calcined CuO nanoparticles, which is the characteristic of Cu-O bond formation (Sundaramurthy and Parthiban, 2015).

The FTIR spectra for uncalcined product was shown in Figure 9b. The spectrum-depicted bands at 615 $cm^{-1}$ was assigned to M–O stretching of Cu-O (M–O) (Sundaramurthy and Parthiban, 2015). The broad and strong absorption band at 3394 $cm^{-1}$ was corresponds to O–H stretching of alcohols. 2927 $cm^{-1}$ C–H stretching alkanes , 1570 $cm^{-1}$ C=O stretching and 1410 $cm^{-1}$ and 1076 $cm^{-1}$ C−O stretching . As compared to calcinated CuO NPs, the FT-IR spectra of uncalcined product displayed a number of absorption peaks. This might be due to chemically bonded functional groups of leaf extract used as a capping agent during the synthesis of the materials, which were not removed by washing with distilled water and ethanol.

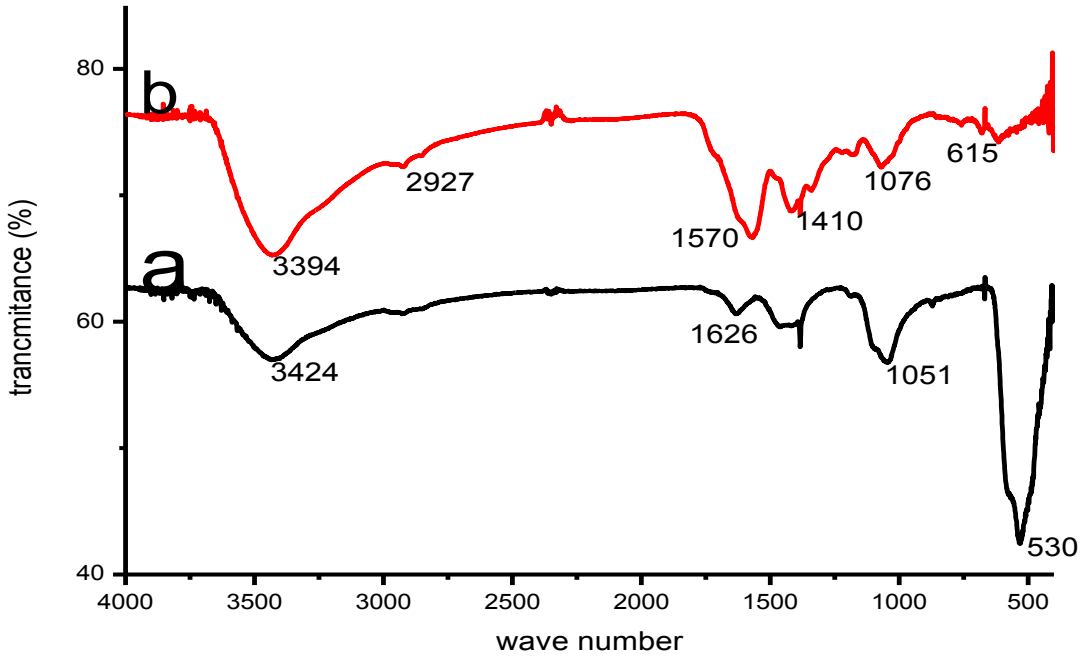
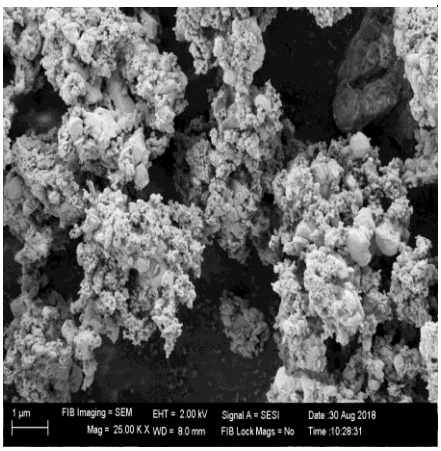


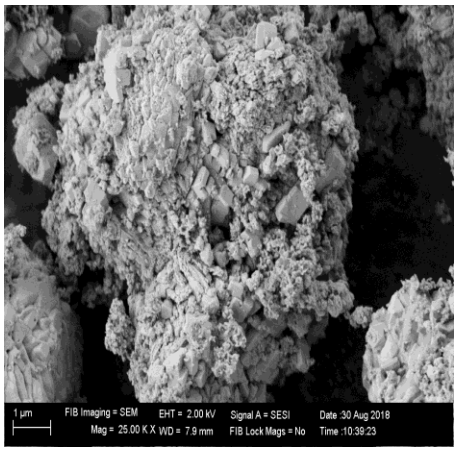

**Figure 9:** FT-IR spectra for a) calcinated CuO NPs b) uncalcinated NPs

### 4.4. Scanning Electron Microscopy (SEM) Analysis

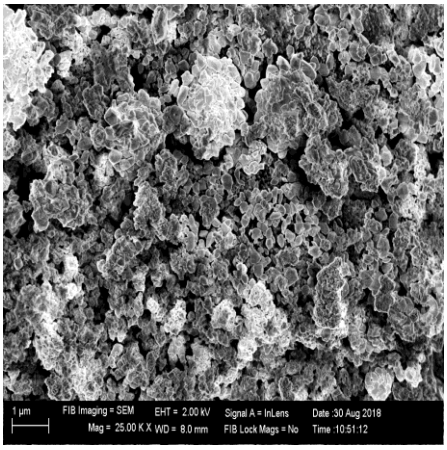
SEM images of the synthesized CuO NPs have different morphology as shown in Figure 10 (a-f). The SEM images of calcinated CuO NPs shown on Figure 10(a - e) indicate the agglomeration of particles. The morphology of most of CuO NPs synthesized from different mixing ratios of copper acetate monohydrate and leave extract such as 1:1, 1:2, 1:3, 1:4 and 3:1 have agglomerated spherical shapes. The SEM image of CuO NPs in 1:1 and 1:2 shows that irregular shaped particles which are non-uniformly distributed. On the other hand CuO NPs in 1:3 were uniformly distributed throughout the structure. Therefore scanning electron microscope results confirmed that the shapes of the synthesized NPs were dependent on mixing ratio of precursor salt and leaf extracts under the same reaction conditions. The morphology of uncalcined NPs as indicated in Figure 10f was not clearly identified. The SEM image results were indicated that calcination affect the morphology of the synthesized CuO NPs.



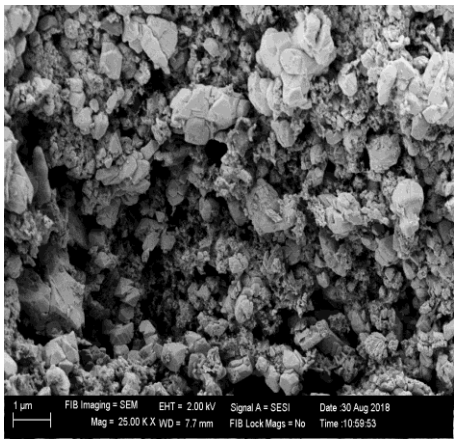

**a**



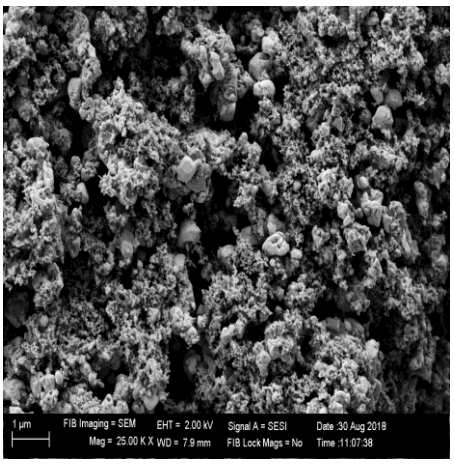

**b**



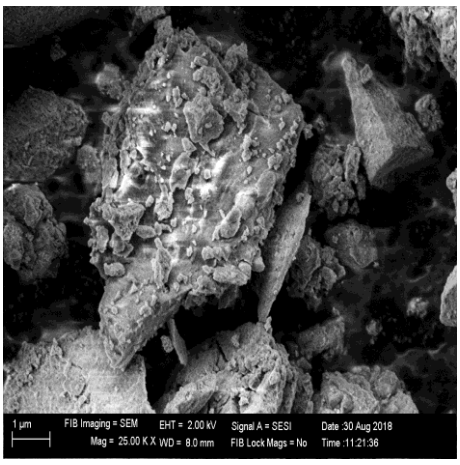

**c**




**d**




**e**

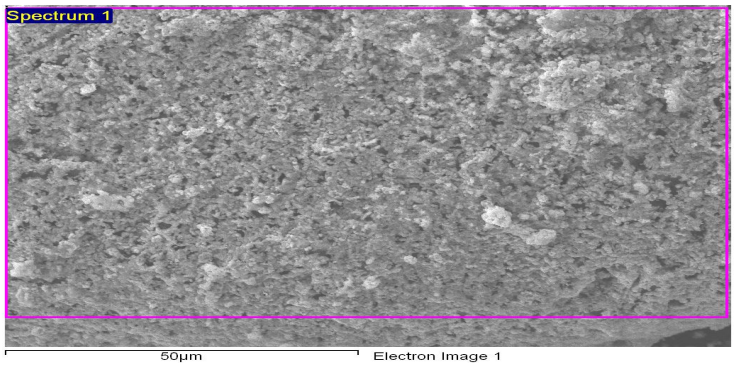



**f**

**Figure 10**: SEM images of CuO Nanoparticles (a) 1:1 (b) 1:2, (c) 1:3, (d) 1:4 (e) 3:1 and (f) uncalinated.

### 4.5. Energy Dispersive X-Ray Spectroscopy (EDX) Analysis

The energy dispersive X-ray analysis of all CuO nanoparticles synthesized using different mixing ratios was shown in Figures 11 to 16. It is evident from the X-ray patterns of all the samples in which Cu and O were found in the respective spectrum. In addition to copper and oxygen, it was observed that there are some additional impurities present in the spectrum. The impurities found in the synthesized materials were increased with increases in the volume of the leaf extract.




Quantitative analysis of CuO (1:1).

| Element | Wt% | At% |
|---|---|---|
| OK | 21.39 | 42.62 |
| CuK | 64.55 | 32.40 |
| Others | 14.06 | 24.98 |

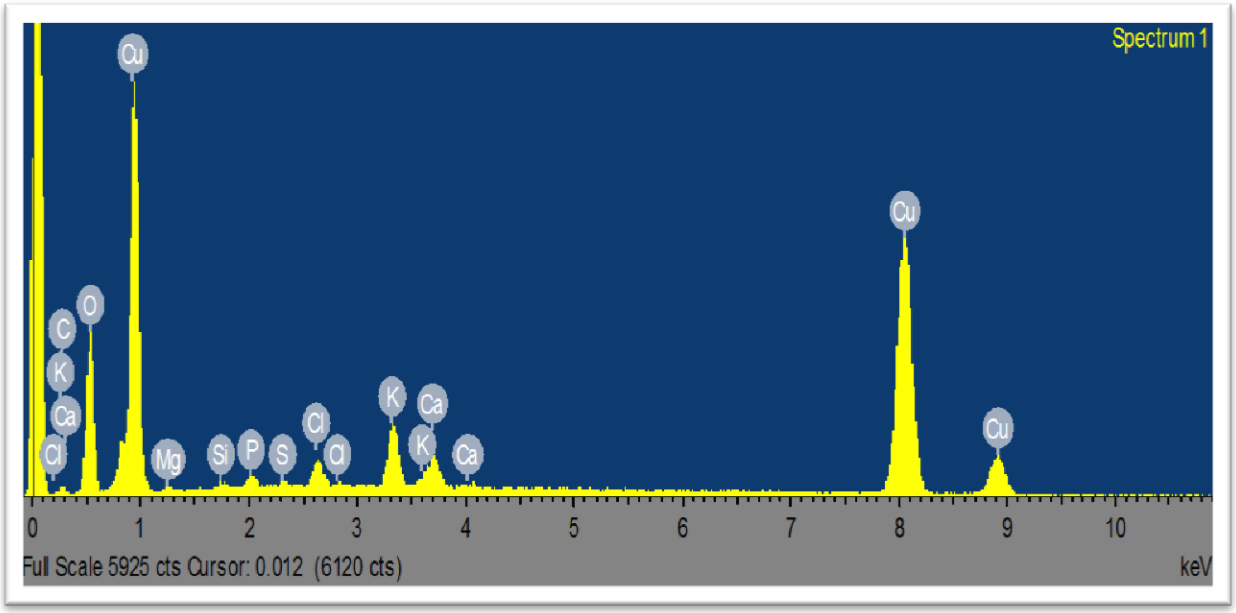
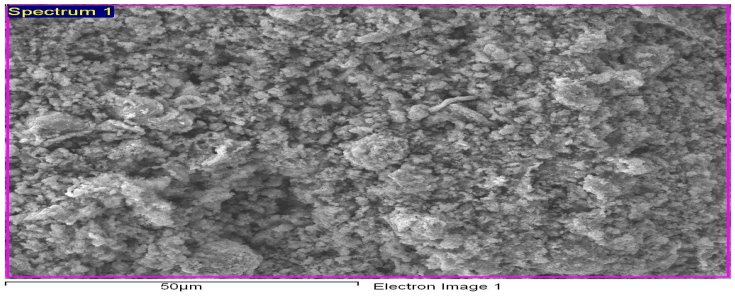



**Figure 11:** EDX spectrum of CuO NPs (1:1).




Quantitative analysis of CuO (1:2).

| Element | Wt% | At% |
|---|---|---|
| OK | 18.57 | 38.50 |
| CuK | 69.44 | 36.24 |
| Others | 11.99 | 25.26 |

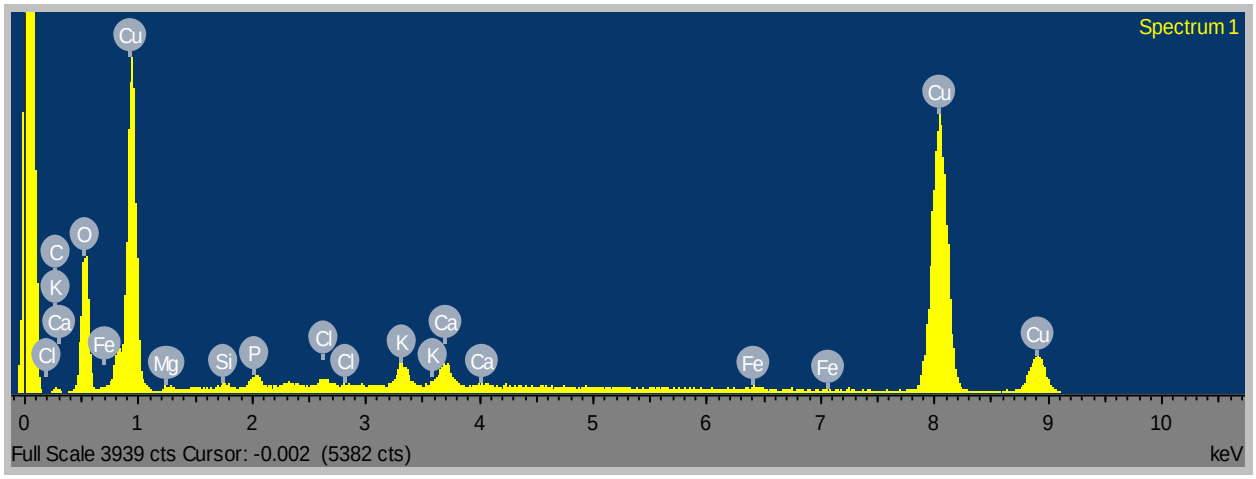
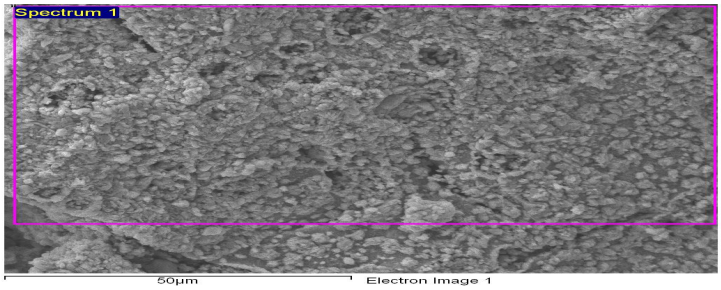



**Figure 12:** EDX spectrum of CuO NPs (1:2).




Quantitative analysis of CuO (1:3).

| Element | Wt% | At% |
|---|---|---|
| OK | 30.73 | 59.67 |
| CuK | 53.30 | 26.06 |
| Others | 15.97 | 14.27 |

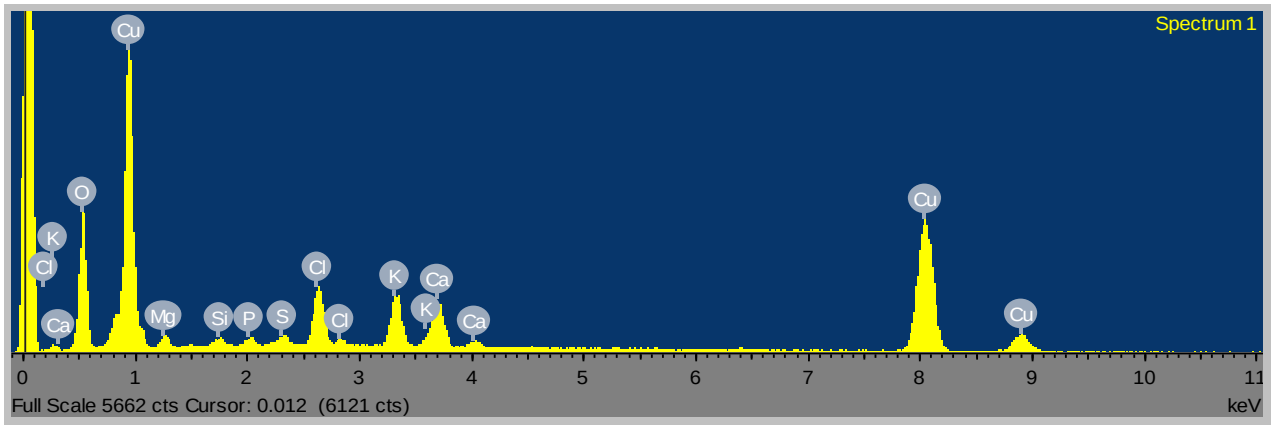
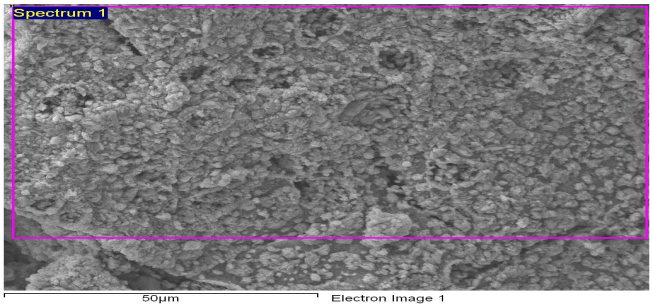



**Figure 13:** EDX spectrum of CuO NPs (1:3).




**Quantitative analysis of CuO (1:4).**

| Element | Wt% | At% |
|---|---|---|
| OK | 11.46 | 31.57 |
| CuK | 85.88 | 59.60 |
| Others | 2.66 | 8.83 |

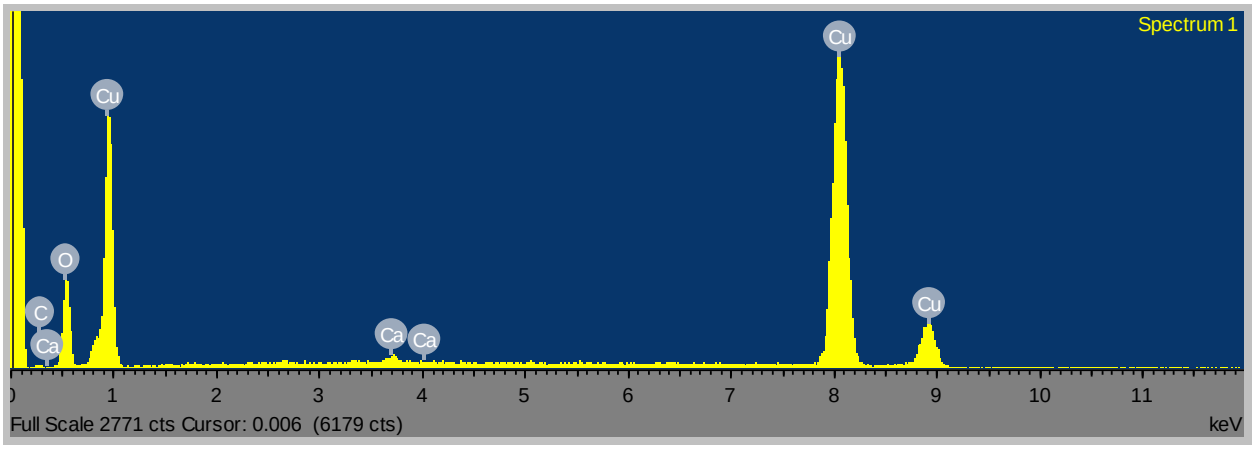
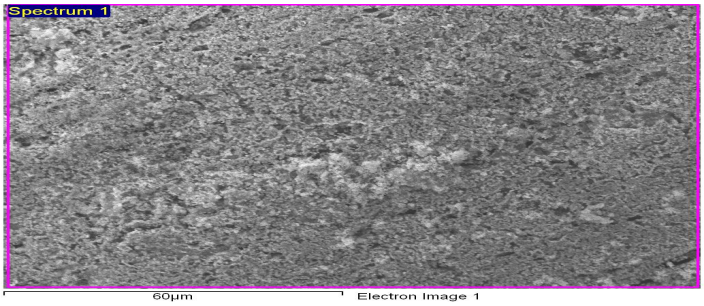



**Figure 14:** EDX spectrum of CuO NPs (1:4).




Quantitative analysis of CuO (3:1).

| Element | Wt% | At% |
|---|---|---|
| OK | 24.09 | 43.94 |
| CuK | 56.62 | 26.01 |
| Others | 19.29 | 30.05 |

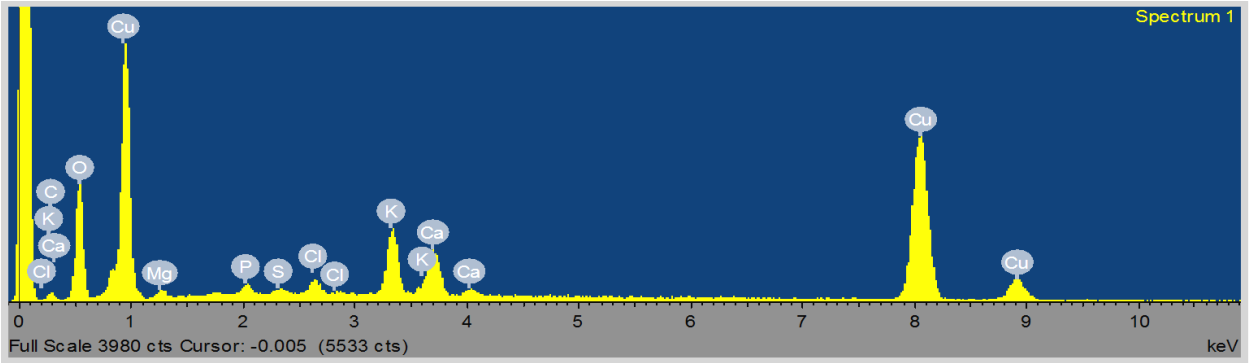
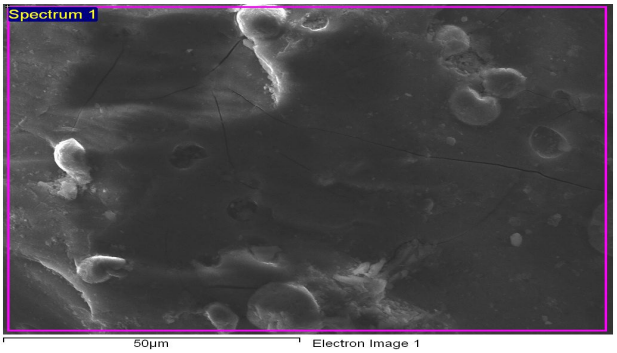



**Figure 15:** EDX spectrum of CuO NPs (3:1).




Quantitative analysis of uncalcinated NPs.

| Element | Wt% | At% |
|---|---|---|
| OK | 43.46 | 44.65 |
| CuK | 19.39 | 5.02 |
| Others | 37.15 | 50.33 |

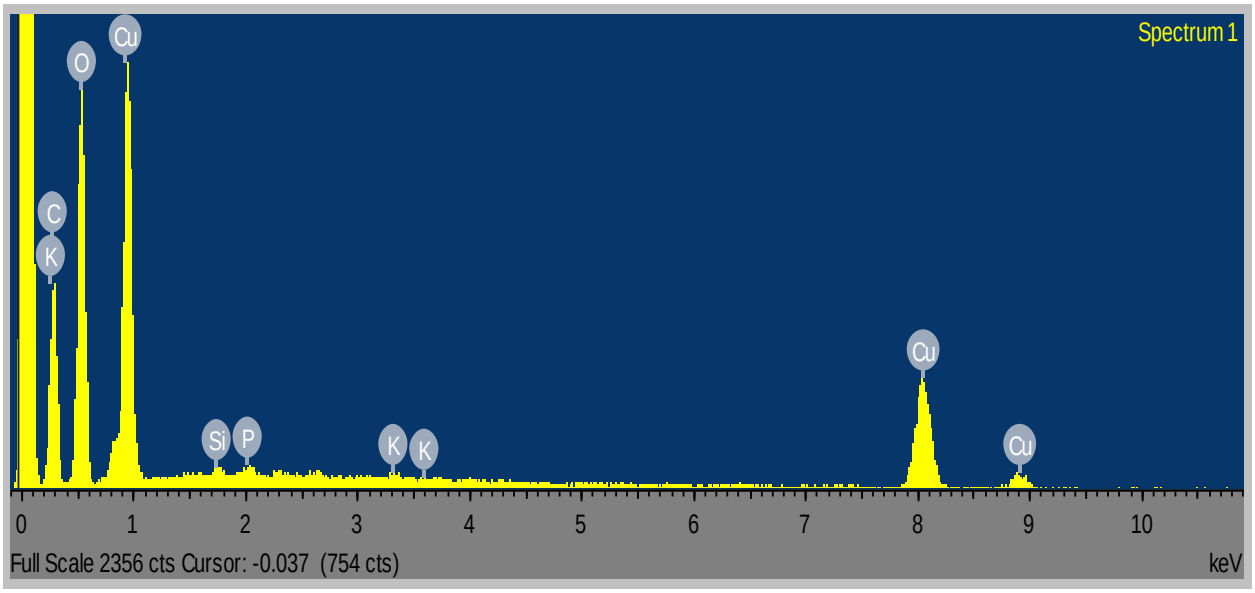



**Figure 16:** EDX spectrum of CuO (uncalcinated).

### 4.7. Antimicrobial Activity of CuO NPs

The antibacterial properties of the calcinated and uncalcinated Nps were evaluated against gram-negative bacteria *E. coli* and *P.aeruginosa* and gram positive bacteria *S. aureus and B. subtilis* using agar well diffusion method. The effects of particle size and concentration on the antibacterial activity of CuO nanoparticles were studied using this method. These tests were performed in nutrient broth and nutrient agar following standard methods. Zone of inhibition in mm of the tested samples of CuO NPs against four different bacterial strains were given in Table 2.

**Table 2:** Zone of inhibition (mm) of CuO NPs against four bacterial strains.

| Samples | ratio of extract to precursor | Zone of inhibition(mm)(Diameter) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $\frac{5mg}{mL}$ | $\frac{10mg}{mL}$ | $\frac{20mg}{mL}$ | Gentamcine | $\frac{5mg}{mL}$ | $\frac{10mg}{mL}$ | $\frac{20mg}{mL}$ | Gentamcine |
| | | gram positive bactria | | | | | | | |
| | | *S.aureus* (ATCC25923) | | | | *Bacillus subtilis* (ATCC6633) | | | |
| S1 | 1 to 1 | 6 mm | 6 mm | 7 mm | 16mm | 6 mm | 6 mm | 9 mm | 14mm |
| S2 | 1 to 2 | 6 mm | 11 mm | 12 mm | | 11 mm | 12 mm | 13 mm | |
| S3 | 1 to 3 | 6 mm | 10 mm | 11 mm | | 9 mm | 11 mm | 13 mm | |
| S4 | 1 to 4 | 6 mm | 6 mm | 6 mm | | 6 mm | 6 mm | 6 mm | |
| S5 | 3 to 1 | 8 mm | 11 mm | 12 mm | | 6 mm | 6 mm | 6 mm | |
| | | gram negative bactria | | | | | | | |
| | | *Escherichia coli (ATCC 25922)* | | | | *Ps. Aeruginosa (ATCC 7553)* | | | |
| S1 | 1 to 1 | 6 mm | 6 mm | 6 mm | 18mm | 6 mm | 6 mm | 6 mm | 14mm |
| S2 | 1 to 2 | 6 mm | 6 mm | 6 mm | | 6 mm | 6 mm | 6 mm | |
| S3 | 1 to 3 | 6 mm | 6 mm | 6 mm | | 6 mm | 6 mm | 6 mm | |
| S4 | 1 to 4 | 9 mm | 12 mm | 14 mm | | 6 mm | 6 mm | 6 mm | |
| S5 | 3 to 1 | 9 mm | 12 mm | 14 mm | | 6 mm | 6 mm | 6 mm | |

As indicated in Table 2 the synthesized CuO NPs are sensitive to gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. CuO NPs synthesized using *B. abyssinica* and copper acetate in $S_1$(1:1), $S_2$(1:2) and $S_3$(1:3) shows antibacterial activity against gram positive bacteria *S.aureus* and *Bacillus subtilis* bacterial strains. CuO NPs synthesized in 1:2 ratios has greatest zone of inhibition 12 mm and 13 mm at concentration of 20 mg/mL against *S.aureus and B.subtilis* respectively than 1:1 and 1:3 ratios. This indicates that low size CuO NPs has effective antibacterial activity against *S.aureus* and *Bacillus subtilis*. Generally, for the synthesized CuO NPs in size range 24.6 nm to 30.8 nm antibacterial zone of inhibition was

decreased with increasing particle size for gram- positive bacteria. The results showed that CuO nanoparticles synthesized from 1:1, 1:2 and 1:3 has greater zone of inhibition at concentration of 20 mg/mL against *Bacillus subtilis* than *S. aureus.* This shows that the CuO NPs are more sensitive to *B. subtilis* than *S. aureus.*

CuO NPs are partially sensitive to gram-negative bacteria (*E. coli*) and but they are resistant to gram negative bacteria *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa has* the tendency to form bio-films, which further protects it from antibiotics (Alaa, 2015). The resistance of *P. aeruginosa than E. coli* to CuO NPs may be due to ability of *P. aeruginosa* form bio-film easily.

CuO NPs at concentration of 20 mg/mL has highest zone of inhibition against different bacterial strains than the other two concentrations. Similarly, CuO NPs at concentration of 15 mg/mL has higher zone of inhibition against different bacterial strains than CuO NPs of 10 mg/mL concentration. Therefore, the antibacterial activity of the synthesized CuO nanoparticles was increased with increases in NPs concentrations, which are similar with other report (Mojtaba et al, 2017).

As indicated in Table 2, most samples of CuO NPs shows antibacterial activities against the two gram-positive bacteria. However, most samples shows resistance to the gram negative bacteria. Generally, gram-negative bacteria seemed to be more resistant to the synthesized CuO NPs than gram-positive bacteria. The outer membrane of gram-negative bacteria contributes to this intrinsic resistance by acting as an efficient permeability barrier (Plesiat and Nikaido, 1992). The details of zone of inhibition (mm) for the above bacterial strains are given in Figure 17, 18, 19 and 20.

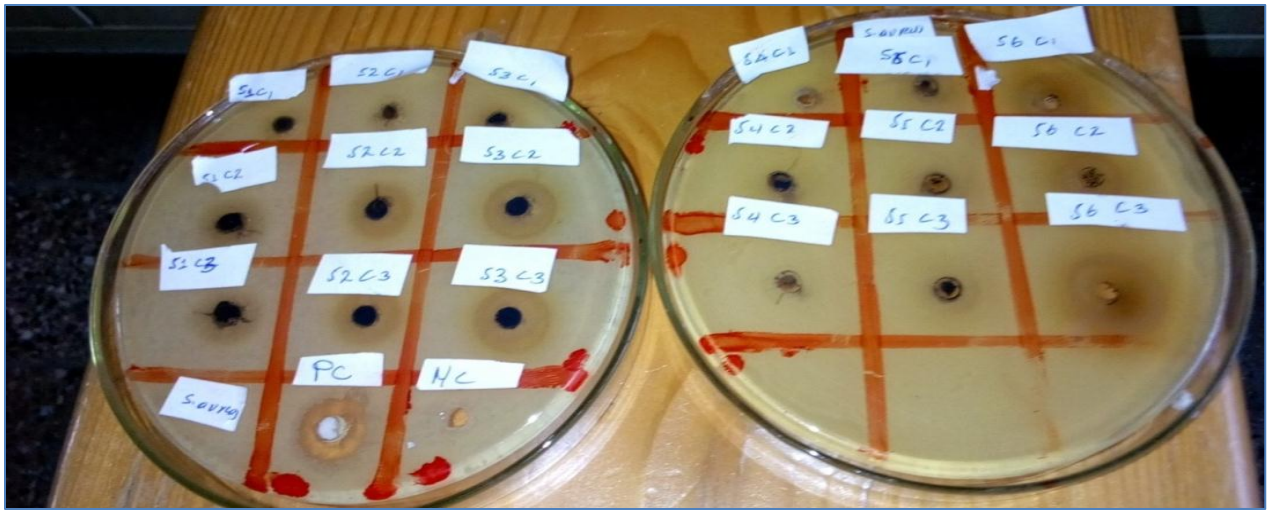



**Figure 17:** Zone of inhibition of CuO nanoparticles against Staphylococcus aureus.

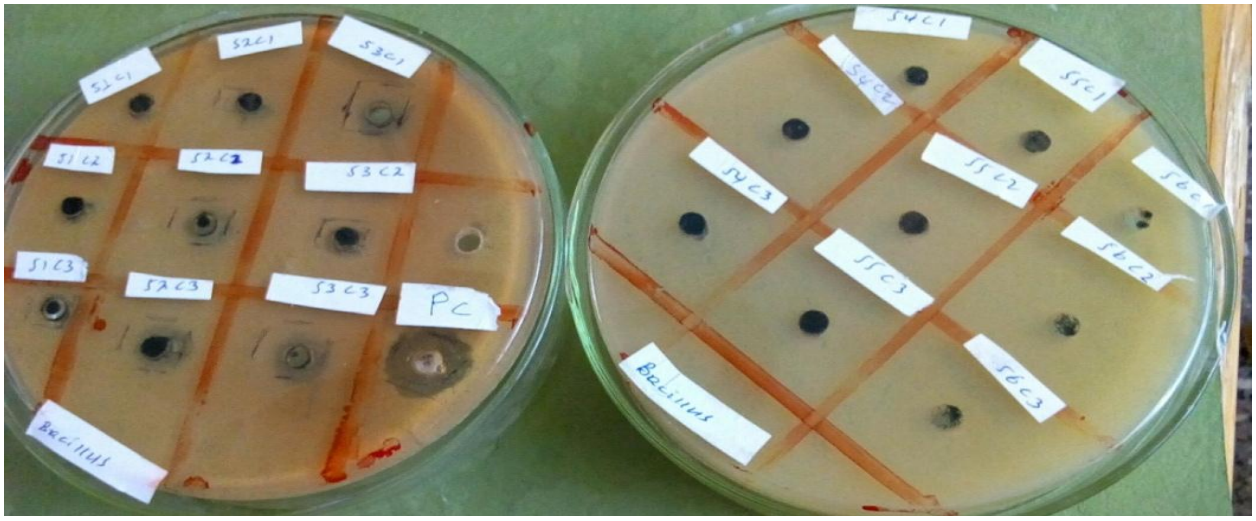



**Figure 18:** Zone of inhibition of CuO nanoparticles against Bacillus subtilis.

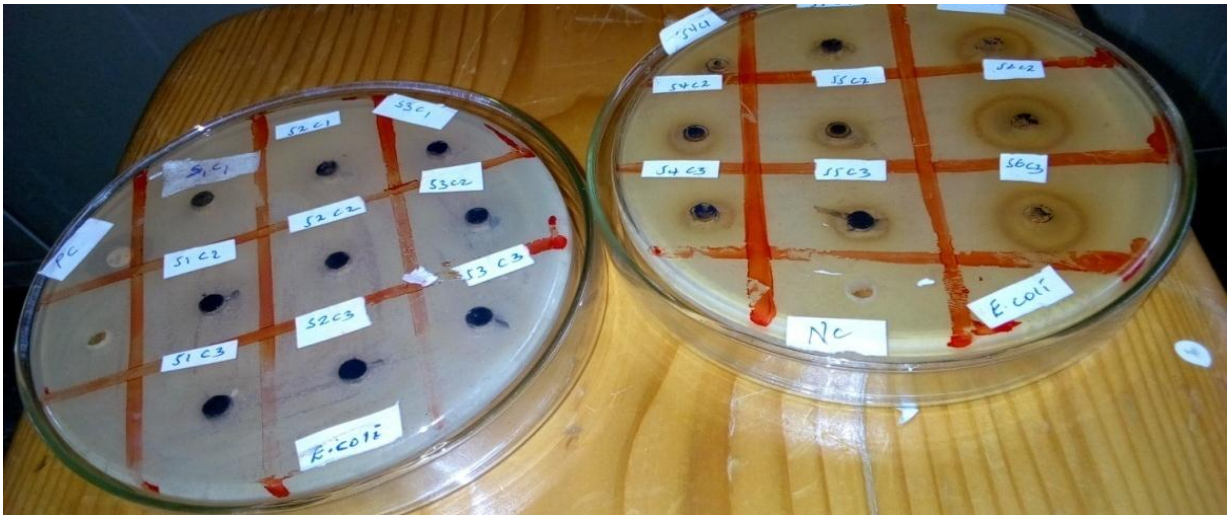



**Figure 19:** Zone of inhibition of CuO nanoparticles against Escherichia coli.

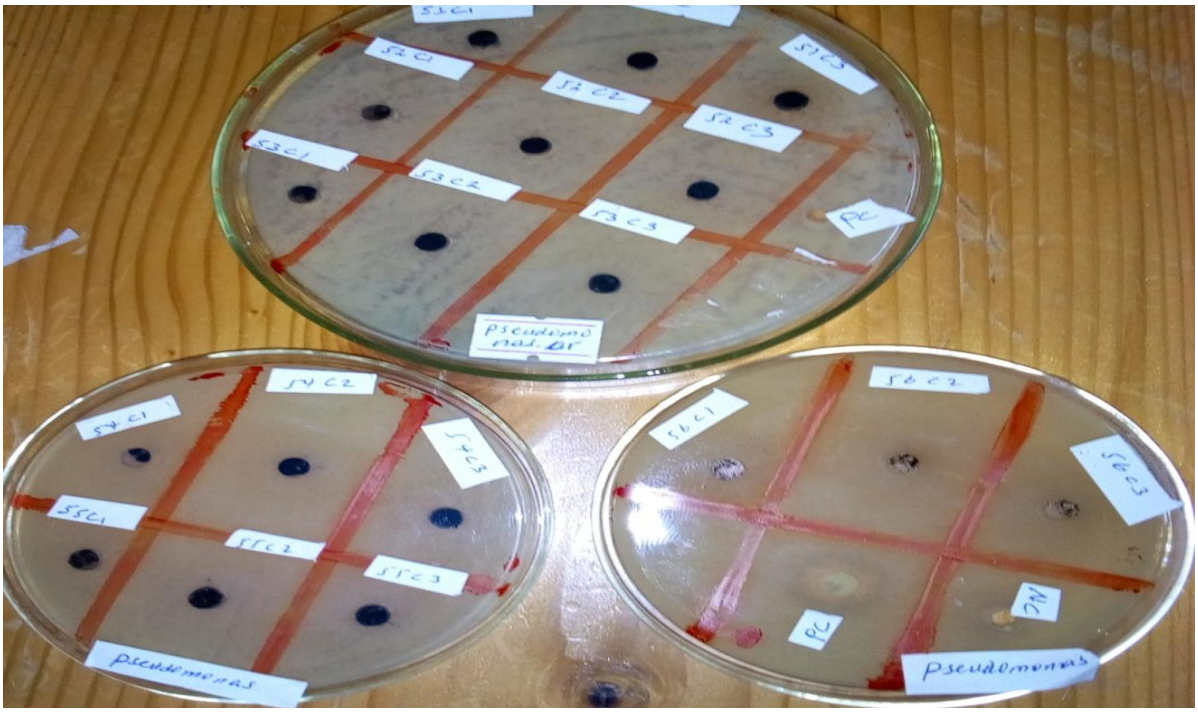



**Figure 20:** Zone of inhibition of CuO nanoparticles against Pseudomonas aeruginosa.

# 5. CONCLUSIONS

CuO NPs were successfully synthesized by the biological synthesis method using aqueous leaf extracts of *B. abyssinica*. The prepared copper oxide nanoparticles were characterized by using advanced techniques such as XRD and FTIR techniques. X-ray diffraction confirms the formation of crystalline monoclinic structure and the crystallite size (24.6 nm – 31.5 nm) of the synthesized CuO. The XRD analyses were confirmed that the ratio of the leave extract and precursor affects the grain size of synthesized nanoparticles. For ratio in which volume of precursor greater than volume of extract, the particle size was increased with increase in volume of precursor salt.The vibrational stretching around 530 $cm^{-1}$ was also supports the formation of CuO nanoparticles. The SEM image analyses showed that the ratio of the leave extract and precursor affects the particle size of synthesize nanoparticles. The EDX studies confirmed the major composition of the synthesized nanoparticles were copper and oxygen.

The synthesized CuO showed significant antibacterial activity against gram positive bacteria (*S.aureus* & *B. subtilis* ) and gram negative bacteria *Escherichia coli*. All the synthesized nanoparticles are not sensitive to gram-negative bacteria *Pseudomonas*. Compared with gram-positive bacteria; gram-negative bacteria are most resistant against antimicrobials because of their impenetrable cell wall. The study successfully demonstrates the convenient utilization of *B. abyssinica* to synthesize potentially antibacterial CuO nanoparticles. This process is an economical method for the preparation of copper oxide nanoparticles with respect to energy, time , simplicity and avoiding the toxic chemical using.

# 6. RECOMMENDATIONS

Based on the results obtained during the experimental work and observation made based on antibacterial application of the synthesized nanoparticles the following recommendations were suggested.

- In order to synthesis relatively low size copper oxide nanoparticle from *B.Abyyisinica* and Monohydrated copper (II) acetate further investigation will be required to use low concentration of the precursor salt with different ratios of plant extract.
- Further investigation will be required to use different calcinations temperature to synthesis copper oxide nanoparticle from *B.Abyyisinica* and Monohydrated copper (II) acetate.
- The synthesized CuO NPs in low size has effective antibacterial activity against gram-positive bacteria so further investigation will be made to determine minimum size CuO NPs which more effective as antibacterial against common drug resistance bacteria.

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