GREEN SYNTHESIS OF BIMETALLIC Cu-Zn NANOFLAKES FROM LEAF EXTRACT OF *Magnolia champaca* AND STUDY OF ITS ANTI-MICROBIAL PROPERTIES

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BOARD OF EXAMINER AND CERTIFICATE OF APPROVAL

This dissertation is entitled "**GREEN SYNTHESIS OF BIMETALLIC Cu-Zn NANOFLAKES FROM LEAF EXTRACT OF *Magnolia champaca* AND STUDY OF ITS ANTI-MICROBIAL**" by Grishma Panthi, under supervision of Prof. Dr. Daman Raj Gautam, Department of Chemistry, Amrit Campus is hereby submitted for the partial fulfillment of the Master of Science (M.Sc.) Degree in Chemistry. This dissertation has been accepted for the award of a Master's degree.

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RECOMMENDATION LETTER

This is to certify that the dissertation entitled "GREEN SYNTHESIS OF BIMETALLIC Cu-Zn NANOFLAKES FROM LEAF EXTRACT OF *Magnolia champaca* AND STUDY OF ITS ANTI-MICROBIAL" has been carried out by Grishma Panthi as a partial fulfillment for the requirement of a Master's Degree in Chemistry under my supervision. During the research period, Grishma Panthi has performed the work sincerely and satisfactorily. To the best of my knowledge, this work has not been submitted to any other degree in this institute.

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DECLARATION

I, Grishma Panthi, hereby declare that the work presented in this thesis is original work done by me and not has been published or submitted elsewhere for the requirement of a degree program. Any literature, data or work done by others has been cited and given acknowledgement and listed in reference section.

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ABSTRACT

Nanotechnology has created huge impact in modern day pharmaceutical application. With such applicability, research on nanotechnology must be done with new creativity and ideas. Development in field is possible when number of research is carried out. So, this research is focused on green synthesis of nanoflakes both mono (CuO and ZnO) and bi (Cu-Zn alloy) metallic nanflakes using plant extract of *Magnolia champaca* (leaf) as reducing and capping agents, which inhibits aggregation and coagulation of metal nanoflakes. The formed bimetallic nanoflakes (CuZn) were light green in colour where as CuO was black and ZnO were white in colour. Synthesized nanoflakes were characterized using FTIR, UV, XRD, FESEM. Simultaneously, compared their anti-bacterial and antifungal properties to expand knowledge regarding which of them are more effective. Cu-Zn alloy nanoflakes shows high resistivity toward gram +ve bacteria while CuO nanoflakes shows resistivity against gram –ve and fungus and among them ZnO has lower resistivity. Phytochemical screening of *Magnolia champaca* leaf is done to know the biomolecules which involved in reduction.

Key words: *Magnolia champaca*, green synthesis, Cu-Zn nanoflakes, antibacterial, antifungal

LIST OF ACRONYMS

NPs: Nanoparticles

Nfs: Nanoflakes

UV: Ultra Violet Spectroscopy

FTIR: Fourier Transform Infrared Spectroscopy

ZOI: Zone of Inhibition

DPPH: 1,1-diphenyl 2-picryhydrazyl

Ppm: parts per million

EPR: Electron paramagnetic resonance

BSO: Breeding Seed Orchard

DPR: Department of Plant Resource

DFO: District Forest Office

CFUG: Community Forest User Groups

TISU: Tree Improvement and silviculture unit

Rpm: Rotation per minute

µg: micro gram

mL: mili litre

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CHAPTER I
INTRODUCTION

1.1 Nanochemistry and nanotechnology

Nanotechnology is a branch of science and technology that deals with materials that have a minimum one-dimensional size of 1–100 nm. The name "Nano" derives from the Greek word dwarf, which implies little. Nm is a billionth of a meter. A nanometer is roughly the length of 10 hydrogen atoms or 5 aligned silicon atoms (Goyal et al.,2018). It alters matter at the atomic and molecular levels while removing materials at the nanoscale to increase functionality (Tanvilian et al.,2019). Although nanotechnology has just recently attracted attention, it has long been known to have been in use. Because nanomaterials are more functional than bulk materials, they are widely used. Nanotechnology has a significant impact on the fields of science and technology.

History of Nanotechnology

Nanomaterials and nanotechnology have had a phenomenal rise in the twenty-first century. The fact that this field has existed for centuries means that it is not new. In the seventh century BC, the Assyrians created the well-known gold ruby glass, which is composed of a glass matrix with gold nanoparticles dispersed throughout. Kunkel in Leipzig invented it again in the seventeenth century. The Lycurgus cup and stained glass windows are two examples from the medieval and roman eras that have special optical qualities due to the presence of a few tens of parts per million (ppm) of gold and silver nanoparticles in the glass matrix. Faraday created a stable colloidal gold in 1857, but it was lost in World War II. Colloidal gold has been used for many years to identify illnesses and treat rheumatoid arthritis. Although there are some unusual examples of nanomaterials or nanotechnology that date back millennia, the enthusiasm around nanotechnology today is focused on the shrinking of gadgets like personal computers, laptops, smartphones, embedded CPUs, etc. At the American Physical Society's annual meeting in 1959, physics Nobel Laureate Richard Feynman spoke at California Institute of Technology with the title "There's Plenty of Room at the Bottom." Norio Taniguchi coined the word "nanotechnology" in 1974 to describe the exact and perfect tolerances needed for top-down approach cutting and finishing of materials (Goyal et al., 2018).

1.2 Classification of Nanomaterial

Classification of nanomaterials can be carried out in different basis. Some general babsis of classification of nanomaterials briefly presented below.

I. Classification on the basis of dimensions are given below.

Based on the dimension, nanomaterial can be classified into the following types.

a. Zero dimension nanomaterials: It is material having all dimension in nanoscale example: nanoparticles.
b. One dimension nanomaterials: It is material with one dimension out of nanoscale and other two in nanoscale example: nanotubes.
c. Two dimension nanomaterials: It is material with two dimension out of nanoscale and other one in nanoscale example: nano-sheets.
d. Three dimension nanomaterials: It is material with all dimension out of nanoscale example: lysosome.

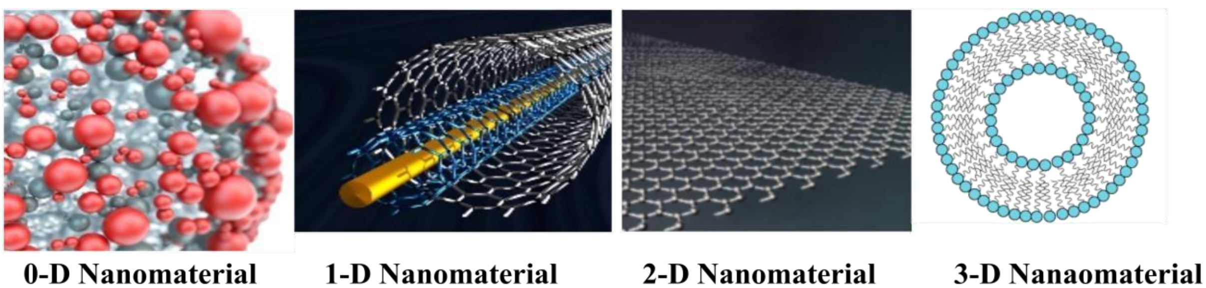

Fig 1: Nanomaterials of different dimension

1.3 Synthesis Approaches of Nanomaterial

Mostly there are two approach used for synthesis of nanomaterials.

1. **Top-down approach:** This method uses mechanical and chemical methods to primarily shrink bigger materials into nanoscale sizes (Goyal et al., 2018). It is a technique for creating extremely complex structures, however it has drawbacks such as uneven surface structure that may impact the properties of nanomaterials (Kanchi et al., 2018). Different types of synthesis techniques, such as mechanical alloying, equal channeling angular pressing, high

pressure torsion, accumulative roll bonding, nanolithography, and others, are included in top-down approaches.

2. **Bottom Up approach:** This method involves preparing nanomaterials from atoms, molecules, or clusters, or from smaller particles to the desired nanosize (nanomaterial and nanocomposite). It creates uniform, stable nanoparticles that can be functionalized with a capping agent to adjust particle size, shape, and composition (Kanchi et al., 2018). Physical vapor deposition, chemical vapor deposition, sprey conversion, sol-gel, electrospinning, and other bottom-up methods are included.

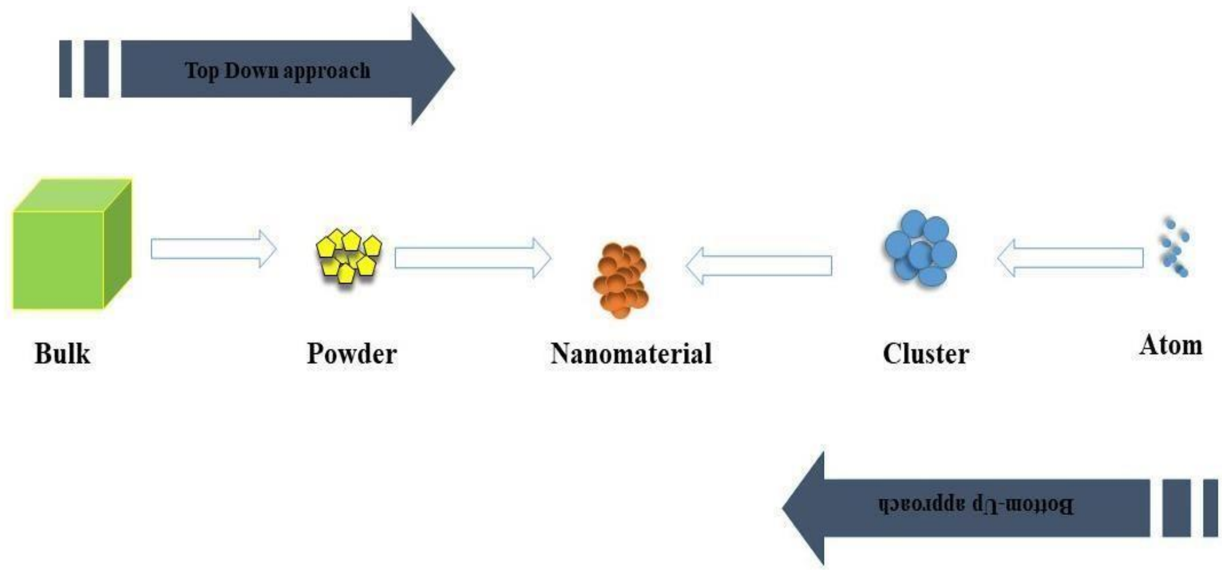

Fig 2: Schematic representation of Top-Down and Bottom Up synthesis of nanomaterials

1.4 Green Synthesisof Nanotechnology

Green synthesis (biological precursor) has been a key component of nanotechnology among the several techniques for creating nanomaterial (Naiko et al., 2021). It enhances sustainability through its green method (Kanchi et al., 2018). In the recent years, harmful chemicals have been used in the chemical approach of making nanomaterials, but research has repeatedly demonstrated that the green method is preferable. According to a project study by the Woodrow Wilson International Center for Scholars, green chemistry encompasses waste reduction, safer chemical design, less hazardous material design, and safer process synthesis. Additionally, this vast scientific horizon envisions the reuse of old products, minimizes the use of chemical reagents, maximizes the economy of atoms, and employs catalysts. Green chemistry also entails reducing the possibility of accidents and achieving chemical process safety success (Kanchi et al., 2018).

Despite the fact that nanotechnology uses a variety of materials with different dimensions, nanoflakes, or two-dimensional material, have demonstrated unique and outstanding qualities over bulk materials, and their environmentally friendly synthesis method has garnered considerable attention. Utilizing bacteria, yeast, algae, fungi, and other microorganisms in addition to plant extract has demonstrated its capacity to manufacture safe and non-toxic nanoflakes. These organic ingredients function as capping and reducing agents. Metal is reduced into nanomaterial by protein and metabolites like flavonoid, phenol, alkaloid, etc.

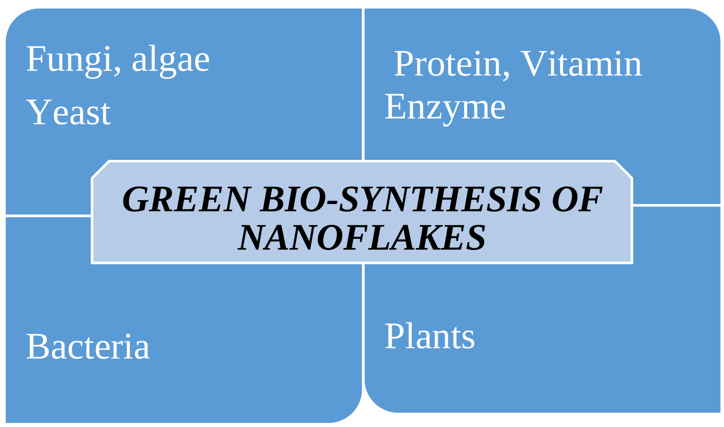

Fig 3: Different precaursors used in the green synthesis of nanomaterials.

Metals have been used since the beginning of human evolution; their importance can be understood by looking at just a few of their many uses. Certain metals are necessary for the body of a healthy human, such as zinc, which is essential for healthy immune function, immune system function, brain function, and enzymatic reactions (Ayhanci et al., 2008). Other metals including manganese, copper, and zinc function as cofactors in various superoxide dismutase enzymes to speed up the conversion of superoxide to hydrogen peroxide, a crucial signaling molecule for a number of biological processes (Kunikowska et al., 2002). Despite being extremely useful, they may display cytotoxicity when utilized for therapeutic and diagnostic purposes (Mody et al., 2010). Due to their expanding use in the biomedical area and ability to get over the limitations of metal ions and complexes, metal nanoparticles have drawn a lot of study interest.

1.5 Bimetallic Nanomaterial

Understanding how two metals interact to create an alloy nanomaterial is a complex process that makes for an attractive research topic (Landry et al., 2016). Compared to mono-metallic nanomaterial, they are more stable, more effective, and contain more reactive sites (Reshmy et al., 2021). One of these techniques for creating nanomaterials is known as green synthesis. Bimetallization even results in the development of nanomaterials with a smaller size than the corresponding individual metal, which has an impact on the ordering and miscibility of bimetallic structures (Behera 2020).

Nanomaterials with a high surface-to-volume ratio, as copperoxide NPs, have been thought to have more potent antibacterial effects (Khodashenas et al., 2014). Similarly, zinc oxide nanoflakes also possess high anti-microbial and dermatological property (Sangeetha et., al 2011). Both these nanoparticles exhibit surface plasmon resonance in UV visible region. Plasmon is the oscillation of electron atoms on a metal surface. Because of free vibrating electrons, metal is shiny. Electromagnetic waves are produced when the electrons interact with light that is incident on them. The energy that the oscillating electrons absorbed is subsequently reemitted as reflected light, and its wavelengths are measured as UV-visible spectra.

This study used a simultaneous reduction technique, in which the metal precursor solutions are combined and reduced concurrently to create bimetallic nanoflakes. It uses the same process as monometallic nanomaterial (Rao et al., 2000). It is simple method but difficulties arise to maintain uniformity without phase segregation. Therefore, reducing and stabilizing agent plays huge role to avoid segregation (Zhang et al., 2014). Choosing an appropriate reducing agent is crucial for the synthesis of nanomaterials since most reducing agents generally have low or moderate reducing capabilities at low pH but significant reducing powers at high pH (Zhang et al., 2011). Therefore, plant extract is good stabilizing and reducing agent as well as is sustainable. Copper oxide nanoflakes can be used as anti-microbial agent, in wound healing, drug delivery, waste water treatment, superconduction and so on (Chakraborty et al.,2022). Similarly, zinc oxide nanoflakes can be used as antibacterial agent, antioxidant, antifungal, gene delivery. Besides these, zinc oxide nanoflakes are being used in biomedical imaging, anticancer and biosensors.

1.6 Anti-Microbial Activity

A chemical that is antibacterial prevents microbial development. They can be divided into categories based on how they are used, such as anti-biotics for bacteria and anti-fungals for fungus. These anti-microbial substances ought to be poisonous to the microorganisms that are damaging to the host but not the host itself. Herbal plants have long been used to treat ailments in human history. The growth of the pharmaceutical industry has eliminated numerous human-devastating diseases. As a result, concerns about illnesses and human health have always existed each new technological creation. Many medications that operate as anti-microbial agents have been used for many years, however due to the excessive usage of certain of the drugs, certain bacteria and fungi have developed resistance to them. Researchers are now focusing on nanoflakes since they lack resistance to these bacteria and fungi. Nanoflakes that function as anti-microbial agents are therefore extremely important.

Antimicrobial properties are anticipated to be present in CuO and ZnO nanoflakes. The study of these nanoflakes as an antibacterial agent opens up new possibilities for the biomaterials industry. The transmembrane electrochemical potential was reduced as a result of the contact of CuO flakes with the bacterial cell membrane, which has an impact on the membrane's integrity. It was presumptively believed that metal flakes release the corresponding metal ions. Copper oxide nanoflakes and copper ions build up on bacteria's cell surfaces and create pits in the membrane, which allows cellular components to leak out of and inside of the cell, leading to cell death (Deryabin et al.,2013) (Shende et al., 2015). Reactive oxygen species (ROS) generation, which eventually leads to oxidative stress and cell death, is another significant factor in the strong antibacterial properties of nanoflakes (Rajeshkumar et al.,2021). Nanoflakes' small size and high surface area to volume ratio, which readily contact closely with microbial cell membranes, increase their antibacterial efficacy (Moses et al., 2014).

1.8 Plant Description

1.8.1 Classification

Kingdom: Plantae

Order: *Magnoliales*

Subkingdom: *Tracheobionta-Viridaeplantae*

Family: *Magnoliaceae*- Magnolia family

Division: *Magnoliophyte*

Subfamily: *Polemonioideae*

Class: *Magnoliopsida- Dicotyledons*

Subclass: *Magnoliidae*

Genus: *Magnolia*

Species: *Champaca*

(a) **(b)**

Fig 4: Plant Diagram (a) magnolia tree (b) leaf portion

Evergreen *Magnolia Champaca* trees can reach heights of 164 feet (50 m). *Champaca* is a common name for it. Crown of the tree is umbelliform and thin. It has fragrant flowers that range in color from cream to yellow-orange. Large green leaves on trees range in length from 20 to 35 cm and are spirally arranged with wavy borders. There are roughly 200 blooming plant species in the Magnolia genus (Sarker et al., 2003). The leaves are simple, petiolate, alternating, and have enormous stipules that initially encircle the stem before falling off as the leaf expands and leaving a distinctive scar around the node (Sarker et al., 2003). The stem is solid, woody, woody-branched, and aerial. 18 to 21 m long, straight, cylindrical bole with a closely tapered crown made up of ascending branches. It is a medium-growing tree that can reach heights of 33 meters or more and girths of 2.4 to 3.7 meters (Negi et al., 1987). The outside bark is smooth, grey to greyish-white, whereas the inner bark is fibrous, yellow to brown, and only approximately two centimeters thick. Depending on the region, the flower's hue varies. Large, fragrant, golden flowers develop individually at the base of each leaf. After four to five years of age, the trees begin to bloom (Oyen et al., 1999). Flowers on short, axillary brachy blast, big, tepals 6–21, in 3-6 typically sub-equal whorls, white to yellow; stamens many; anthers with a short to noticeably extended connective; gynoecium stipulates; with spirally organized, free or connate carpels holding many ovules. The tree bears fruit and blooms all year long. (Orwa et al., 2009).

The flowers have anti-ulcer, anti-diabetic and anti-inflammatory properties and used in the ailment of ulcer, skin diseases and wounds. It is also famed for its aromatic volatile oil from flowers, which is used in the perfume industry. *Champaca* flowers are one of the herbal substances used in indigenous system of medicine and found as an herbal substance commercially (Prabakaran et al., 2014). Leaf extract is toxic to the rice fungus, *Pyriculariaoryzae*. Fatty oils extracted from the seeds show antibacterial activity against *Bacillus pumilus, B.subtilis, Salmonella typhosa, S. paratyphi, Micrococcus pyogenesvar albus* and *Staphylococcus aureus* (Shrestha et al., 2019).

1.8.2 Distribution: From the Himalayas eastward to Japan and southeastward across the Malay Archipelago to New Guiana and New Britain, 80% of the species are found in temperate and tropical Southeast Asia. America, from temperate southeast North America through tropical America to Brazil, makes up the remaining 20% of the world's population. The three genera Magnolia, Talauma, and Liriodendron, which also occur in Asia and have independently discontinuous distributions, include all of the American species (Sarker et al., 2003).

1.8.3 *Magnolia champaca* in Nepal

Magnolia champaca is frequently referred to as "chap" in Nepal. It typically grows between 450 and 1500 meters above sea level and prefers regions with more rainfall, including those east of the Arun River and along mid-hills. It can be found along the banks of the Kaligandaki River in the western part of the moister zone, mixed together with *Castanopsis indica* and *Schima wallichi*.

Out of 25 designated vulnerable and vanishing species, the International Board for Plant Genetic Resources seminar held in Kathmandu on September 23–25, 1981, listed *Magnolia champaca* as one of the threatened and vanishing tree species. The Nepali government has developed a breeding seed orchard (BSO) of 1.4 hector in Kathmandu and a seed stand of 10 hector in Palpa district (TISU 2013). The Department of Plant Resources had built a special garden for the preservation of *Magnoliacea* (DPR 2018).

Magnolia champaca, known as Champ, was categorized as a prohibited species for felling, transportation, and commercial export under the Forest Act of 1993 and the Regulation of 1995. However, the Nepal Gazette of November 5, 2007 removed *Magnolia champaca* off the list of prohibited plants. Champ was the third-most (498.78 cft) timber sold in Community Forest User Groups (CFUGs), according to the District Forest Office (DFO) Lamjung's annual progress report, and the highest on private properties, at 2768.56 cft (DFO Lamjung 2016) (Shrestha et.,al 2019).

1.8.4 Application of Magnolia champaca

Magnolia chamapaca can be used for the following applications.

a. Anti-inflammatory
b. Corrosion inhibitor
c. Anti-diabetic
d. Antiseptic

1.9 Toxicity of Nanomaterials

The use of nanotechnology has enhanced the realm of science and technology. However, issues regarding the toxicity of nanomaterials and their limitations come up. Nanomaterials are prevalent in air, water, soil, plants, human bodies, and animals due to their microscopic size, and we may not be aware of their toxicity. Through the processes of manufacture, shipping, handling, use, waste disposal, and recycling, nanomaterials may come into touch. The size, content, and surface area of nanoparticles all affect their toxicity. The human body can be exposed through ingestion, inhalation, and skin contact. If it is present for a longer period of time, it may result in birth defects, Parkinson's and Alzheimer's disease, bronchitis, lung and liver cancer, asthma, and other conditions (Goyal et al.,2018). It is necessary to conduct additional research on the toxicity of nanomaterials to prevent technology from destroying habitat and humans.

CHAPTER II
RESEARCH OBJECTIVES

2.1 General Objectives

- Synthesis of Cu and Zn bimetallic nanoflakes from *Magnolia champaca* and determination of their anti-bacterial, anti-fungal property.

2.2 Specific objectives

- Synthesis of CuO and ZnO nanoflakes and Cu-Zn bimetallic nanoflakes.
- Characterization of CuO, ZnO and Cu-Zn bimetallic nanoflakes.
- To analyze active compound of *Magnolia champaca* by phytochemical analysis
- Determination of anti-bacterial and anti- fungal properties of CuO, ZnO, and Cu-Zn nanoflakes.

CHAPTER III
LITERATURE REVIEW

About 200 species of magnolia are found worldwide, so, it has wide range of applicability. However, literature conducted on different parts of *Magnolia champaca* plants has been found on synthesis of nanoparticles.

Hasan et al., (2020) studied the antioxidant and antibacterial properties of stem-bark extract (chloroform, n- hexane, ethyl acetate and aqueous extract) of *Magnolia champaca* and observed high antioxidant property (Hasan et al., 2020). Kumar et al. (2013) investigated the effectiveness of a flower extract from *Magnolia Champaca* as a corrosion inhibitor for mild steel in an acidic solution utilizing the weight loss method, potentio-dynamic polarization, and EIS measurements. The extracts worked well as inhibitors in a solution of 0.5 M H_2SO_4. It was discovered that extract concentration increased the effectiveness of inhibition. It was suggested that the inhibitory behavior could be explained by the adsorption of components in plant extract on the surface of the metal (Kumar et al., 2013). Besides these, *Magnolia champaca* was used for the Synthesis of Nanoparticle by green synthesis method. In order to reduce the toxicity of the copper oxide nanoparticles, Jayakodi et al. (2020) used a floral extract of Magnolia champaca as a reducing agent. The outcome demonstrated that copper oxide nanoparticles are non-toxic substances with strong antioxidant properties (Jayakodi et al., 2020). Using Magnolia champaca plant extract as a reducing agent, Verma et al. (2020) created iron nanoparticles that were used to remove nitrate from drinking water (Verma et al., 2020).

CuO nanoflakes were created and their anti-bacterial properties were examined by Pandiyarajan et al. in 2013. CuO nanoparticles were created using the sol-gel technique at ambient temperature. Studies using X-ray diffraction revealed that the particles are monoclinic (crystalline). Images obtained using scanning electron microscopy (SEM) demonstrate with clarity that the produced particles have a flake-like structure. *Shigella flexneri*, *Staphylococcus aureus*, *Staphylococcus epidermidis, Salmonella typhimurium, Bacillus subtilis, Escherichia coli, Vibrio cholera, Pseudomonas aeruginosa*, and *Aeromonas liquefaciens* bacterial strains were examined for their antibacterial characteristics. *S. flexneri* and *B. subtilis* among these bacterial strains were more sensitive to copper oxide nanoparticles than the less sensitive *S. typhimurium* strain and the positive control (Penicillin G). Results revealed that sensitivity was strongly influenced by CuO nanoflakes' concentrations (Pandiyarajan et., al 2013).

By employing an extract from the *Opuntia humifusa* fruit, Chennimalai et al. (2021) created zinc oxide nanoparticles and examined their antibacterial properties. The change of the structural, optical, and electrical properties of the ZnO nanoparticles was greatly aided by the fruit extract from *Opuntia humifusa*. Additionally, the ZnO nanocomposite demonstrated superior antibacterial action against dangerous bacterial pathogens that the Advisory Committee on Dangerous Pathogens (ACDP) had designated as such as *Staphylococcus aureus, Bacillus cereus, Proteus, pseudomonas*, and *Escherichia coli*. The findings indicate that green ZnO nanoparticle manufacturing could be employed successfully in a variety of medical, industrial, agricultural, and food safety applications (Chennimalai et., al 2021). Zinc oxide nanoparticles were created by Jayanchandran et al. in 2021 utilizing *Cayratia pedata* leaf extract and used to immobilize the enzyme glucose oxidase. When glucose oxidase was immobilized with the green produced ZnO nanoparticles, a relative activity of 60% was attained (Jayanchandran et al., 2021). In the study of green synthesis and characterization of biocompatible zinc oxide nanoparticles and evaluation of its antibacterial potential carried out by Ramesh et al., (2021). It was discovered that zinc oxide had potent antibacterial properties against *Proteus mirabilis*, *Bacillus subtilis*, *Klebsiella pneumonia*, and *Bacillus subtilis*. As a result, it has many uses and can be applied to the surface coating of food packaging to prevent bacterial contamination. (Ramesh et al., 2021).

Chakraborty et al., 2020 green synthesis of zno nanoparticles using *Averrhoe carrambola* fruit extract for the photo-degradation of congo red dye. The green synthesized ZnO nanoparticles were successfully characterized in this study, and its photocatalytic activity was investigated. The generation of nano-sized ZnO flakes with a size range of about 20 nm was confirmed by UV-Visible and FTIR spectroscopy, SEM, TEM, and XRD investigation. Even at greater dye concentrations, ZnO nanoparticles demonstrated outstanding catalytic activity, proving the material's effectiveness as a photo-catalyst for dye removal from wastewater (Chakraborty et al., 2020).

Cheirmadurai et al., (2014) synthesized copper nanoparticles and fabricated a conducting nanobiocomposite films using as-prepared Cu nanoparticles and collagen fibers discarded from leather industry. This study showed the potential of produced nanoparticles and nanocomposite for different electronic devices. The developed nano-biocomposite film was shown to be able to conduct electricity when inserted between batteries and illuminate a light emitting diode (LED) lamp. The conductivity value of Cu nanocomposite was found to be better than those reported for collagen or chitosan based conducting composites. It demonstrates the potential for several electronic applications (Cheirmadurai et al., 2014).

Wang et al., (2021) synthesized copper nanoparticles using green coffee bean and examine their applications for efficient reduction of organic dyes. Cu NPs were produced with an average particle size of 5-8 nm. When sodium borohydride (NaBH4) was utilized as the reducing agent in the reduction of amido black 10B (AB-10B), methylene blue (MB), and xylenol orange (XO), it was discovered that Cu NPs are efficient catalysts (Wang et al., 2021). Turakhia et al., (2020) synthesized copper oxide nanoparticles and used in development of antibacterial textiles. Using electron microscopy and a universal testing equipment, the hydrophobicity and mechanical characteristics of the fabric were assessed after the nanoparticles had been dispersed throughout it. The tensile strength of treated cotton fabric was higher than that of untreated cotton fabric (32 MPa) whereas the hydrophobicity of copper nanoparticle-coated cotton fabric was moderate. Additionally, the cotton fabric coated with CuONPs showed enhanced antimicrobial activity even after 30 cycles of washing, demonstrating that it has a higher potential to be used as a medical textile to prevent cross-infection in a clinical environment (Turakhia et al., 2020).

Noman et al., (2019) synthesized copper-zinc bimetallic nanoparticles from secondary metabolite of *Aspergillus iizukaegrown* in pumpkin medium and Cu/Zn NPs were used for inhibiting growth of *E. coli* and *S. aureus* in grey water. By using Atomic Force Microscopy (AFM), Energy Dispersive X-Ray Spectroscopy (EDS), and Field Emission Scanning Electron Microscopy (FESEM) to analyze untreated and treated bacterial cells, it was discovered that the Cu/Zn NPs had damaged the cell wall structure. Additionally, the Cu/Zn NPs caused the bacterial cell wall's amino and carbohydrate structures to degrade, according to Raman Spectroscopy. As a result, CuZn nanoparticles can be used as a potent pathogen bacterial inhibitor in grey water (Noman et al., 2019).

Merugu et al., (2021) synthesized (green) bimetallic Ag/Cu and Cu/Zn nanoparticles using toddy palm. Ag/Cu bimetallic nanoparticles were brown when they were first made, whereas Cu/Zn nanoparticles were green. As-produced nanoparticles were examined and found to be 80 and 100 nm in size, respectively. The Ehrlich ascites carcinoma (EAC) cell lines and nasopharyngeal cancer (KB) cells were both successfully eradicated by the bimetallic nanoparticles. *Alcaligenes faecalis*, *Staphylococcus aureus*, *Citrobacter freundii*, *Klebsiella pneumonia*, and *Clostridium perfringens* showed good antibacterial action (Merugu et al., 2021). Khatak et al., (2021) prepared monometallic Zinc and bimetallic Cu-Zn nanoparticles using stem extracts of *Cissus quadrangularis* (Haddjod). The herb has strong anti-inflammatory and antioxidant properties. The presence of bioactive chemicals in the plant that have detectable antibacterial activity against bacteria was validated by pathogenic germs. The visible transformation of the colloidal solution's color from green to a light cream tint indicated that zinc oxide nanoparticles had been synthesized. An optical absorption band peak at 393 nm was produced by UV-spectral analysis in the range of (300-600 nm), which was used to further confirm the synthesis. Bimetallic copper-zinc nanoparticles were validated by a visible color change of the solution from green to blue green, while XRD examination revealed an average particle size of 32 nm. The experiment also showed that nanoparticles are more effective against gram-negative *Escherichia coli* than *Staphylococcus aureus* (Khatak et al., 2021).

Minal et al., (2016) synthesized Cu-Zn and Ag-Cu Bimetallic Nanoparticles from the aqueous leaf extract of *Ocimum sanctum* and used it as Larvicide to Control Malaria Parasite Vector and compared to Copper-Zinc bimetallic nanoparticles, it has been found that the combination of Silver-Copper bimetallic nanoparticles effectively kills the third instar of Anopheles mosquito larvae. Despite similar molar concentrations of the precursor ingredient, bimetallic nanoparticles' efficacy results outperformed those of monometallic nanoparticles. (Minal et al., 2016). Mazhar et al., (2021) synthesized bimetallic of Zn-Cu nanoparticles from leaf extract of Citrus limon and Evaluated its antibiofilm activity against *E. coli*. Antimicrobial as well as antibiofilm activity of synthesized bimetallic nanoparticles was checked against *E. coli*. Upon synthesis, the color of the particles changed from blue to green, and they were identified as being of the triclinic primitive type, with an average particle size of 27.76 nm as shown in PXRD. Characteristic peaks of functional groups were revealed via FTIR analysis. Successful doping and particle grain size were confirmed by SEM-EDX. Pink rods in the gram staining of bacteria isolated from samples indicated gram-negative *bacilli*. *E. coli* was discovered in samples by biochemical analysis. With

MIC of synthetic nanoparticles 0.5 mg/mL and crystal violet assay assuring antibiofilm characteristics of Zn-Cu, characteristic zones of inhibition in the range of 12-18 mm establish good antibacterial properties. The study's findings may help with the development of nanotechnologybased treatments for infections that produce biofilms (Mazhar et al., 2021). There have only been few studies regarding green synthesis of bimetallic nanomaterial. Here, it shows requirement of more research regarding bimetallic Cu-Zn nanomaterial.

CHAPTER IV
MATERIALS and METHODS

4.1 Materials

4.1.1 Collection of Plant Material

Leaves of *Magnolia champaca* were collected from Lalitpur, Nepal in month February 2022. All the chemicals were purchased from local vendor inside Kathmandu. All the chemicals were of analytical grade.

4.1.2 Equipment

a. Digital weighing balance
b. Spectrophotometer (Labrotonics-2802)
c. Sieve number 250 µ
d. Hot air oven,
e. Auto Deluxe Digital pH meter, Labrotonics-10, India.
f. cuvettes, pipettes, water bath, beakers, conical flasks, test tubes, dryer, measuring cylinder, volumetric flasks.

4.1.3 Solvents and chemicals

a. Chemicals used were analytical as well as laboratory grade.
b. Cupric Sulfate (Molecular mass – 249.68, Qualigen)
c. Zinc Nitrate (Molecular mass – 297.48, (96%) Qualigen)

4.2 Methods

4.2.1 Preparation of *Magnolia champaca* leafs extract

Leaf extracts was prepared by taking approximately 50 g leaves. These were thoroughly washed with distilled water, dried, and made fine powder using mixer. 15 g of powdered leaves were taken with 300 mL of distilled water in a beaker and boiled at 80 °C for 1 hour which is then vacuumfiltered using filter paper to obtain the extract. About 100 mL of extract was obtained from the filtration.

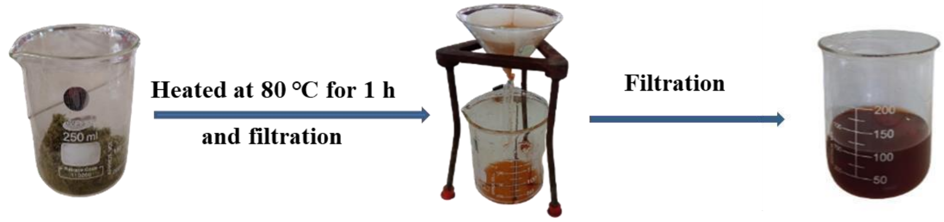

Fig 5: Preparation of plant extract

4.2.2 Phytochemical Analysis

Phytochemical Analysis helps to determine the bioactive compounds present in plant extract of *Magnolia champaca*. Presence of the bioactive compound was confirmed by the observing the color reactions using specific reagent for specific bioactive compounds. The procedure is given in Appendix.

4.3 Reagent preparation

4.3.1 Preparation of copper sulphate solution

5.2 g of copper sulphate pentahydrate was dissolved in 250 mL distilled water to prepare 0.083 M copper sulphate solution.

4.3.2 Preparation of zinc nitrate solution

About 6.5 g of zinc nitrate hexahydrate was dissolve in 250 mL distilled water to prepare 0.087 M Zinc nitrate solution.

4.3.3 Synthesis of CuO, ZnO and Cu-Zn nanoflakes

About 50 mL of copper sulphate pentahydrate solution was taken in a beaker and 30 mL of plant extract solution was poured into it. Then, it was stirred by small magnet keeping the temperature constant at 50 °C for 4 hours. When orange color changes to dark brown color then heating was stopped (Oli et al., 2018).

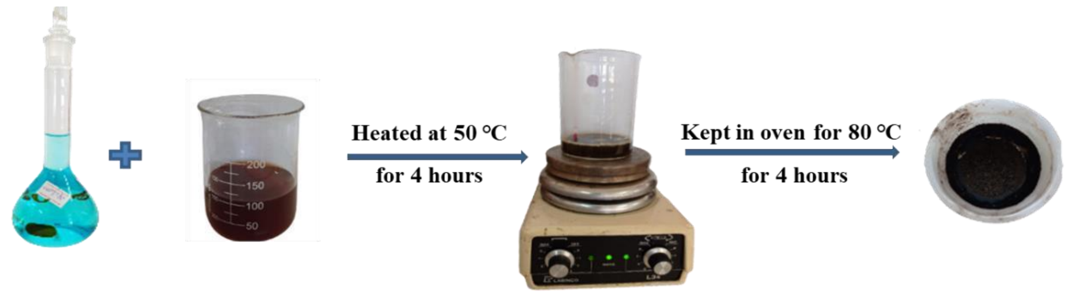

Fig 6: Preparation of copper oxide nanoflakes

About 50 mL zinc nitrate hexahydrate solution was taken in a beaker and 20 mL of plant extract was poured into it. It was also stirred by small magnet keeping the temperature constant at 65 °C for 3 hours and color changes from brown to the light orange color until precipitation was seen. Modified techniques from Chennimalai et al., 2021 and Jayachandran et al., 2021 were used for this synthesis.

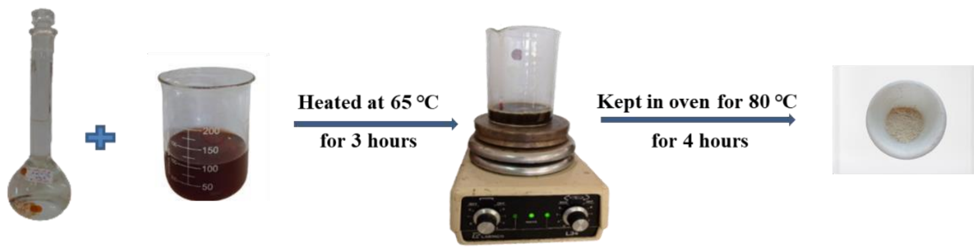

Fig 7: Preparation of Zinc oxide nanoflakes

About 20 mL each solution of copper sulphate pentahydrate and zinc nitrate hexahydrate was taken then, 90 mL distilled water added along with 10 mL plant extract and boiled in water bath at 70 °C for about 3 hours. Then, green color precipitation was obtained. Modified methods from Minal et al., 2016 and Meguru et al., 2020 were applied for synthesis of bimetallic nanoparticles.

Then all the nanoparticles solution was heated in oven to dry them at 80 °C for 4 hr and washed with ethanol to remove impurities. Solution of ethanol with nanoparticles was centrifuged at 3000 rmp for 20 minutes. It was then again heated in muffle furnace for 2 hr at 400 °C. nanoflakes were then collected.

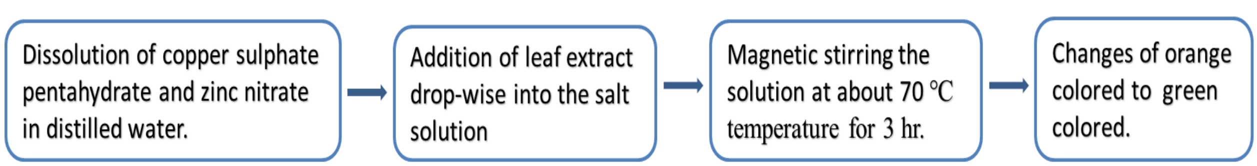

Fig 8: Flowsheet diagram of preparation of Cu-Zn nanoflakes

4.4 Characterization

Structural characterization was carried out using different instruments like FT-IR, XRD, UV-spectroscopy and FESEM. The antibacterial test was performed for gram-positive, gram-negative and fungus. The phytochemical test was based on the visual color change. The change in color of precursor solution to dark brown color, light orange and light green color for CuO, ZnO, and CuZn nanoflakes, respectively.

UV-visible spectroscopy was used to determine the formation of copper oxide, zinc oxide, and copper-zinc bimetallic nanoparticles. UV-visible spectrophotometer (LABTRONIC, Model LT2802) having double beam wavelength from 200 to 800 nm was run through the sample solution using cuvette and observed the amount of light absorbed by the sample. It was carried out in Amrit campus research laboratory.

Fourier transform infrared (FTIR) spectroscopy was carried out in Amrit campus research laboratory. It was used to identify the functional group present in the sample as a reducing and stabilizing agent. The FTIR spectra were recorded with a FTIR spectrophotometer between the wavelength range 500-4000 cm^{-1}.

X-Ray diffraction (XRD) method was used to study the crystalline nature of as-synthesized materials. The crystal structure of the prepared CuO, ZnO and Cu-Zn was studied by X-ray diffraction (XRD). It was carried out in National Academy of Science and Technology.

Surface morphology and topography of the as-synthesized nanoflakes was observed using field emission scanning electron microscopy (FE-SEM, SUPRA40VP) under different magnification.

4.5 Anti-microbial Test

Anti-bacterial and anti-fungal test was determined using Agar well diffusion method for CuO, ZnO and Cu-Zn nanoflakes. Inhibition of bacterial and fungal growth were tested by Zone of inhibition.

First Agar surface was inoculated by spreading a volume of microbial inoculum over the entire agar surface, it was incubated in bacteriological incubator for 12 hours at 37 ℃. Next day, the agar plate was then, separated with sign pen for different nanoparticles and filter paper was kept in the disk plate on four side. 5µL of Standard Kanamycin was loaded into respective section with help of micropipette. Kanamycin was used as standard for gram positive (*Bacillus subtilis*), gram negative (*Escherichia coli*) and fungus (*Candida albicans*), respectively. Small pinch of nanoparticles was kept in respective section of plate. Then kept in incubator for 15 minutes and each plate was then observed for the zone of inhibition (ZOI) produced by antibacterial and antifungal activity. ZOI was measured by the use of scale.

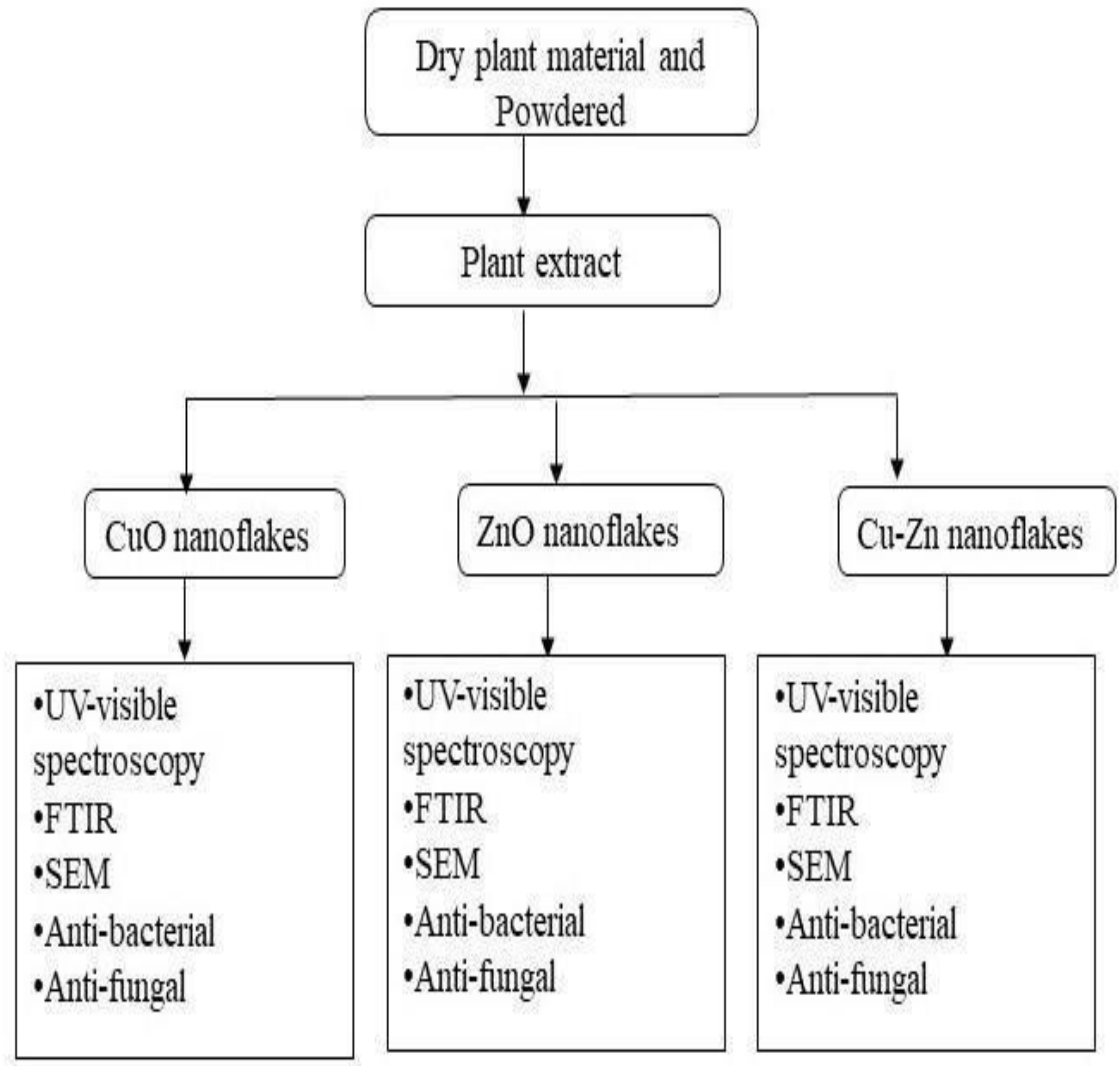

Fig 9: Schematic diagram for green synthesis of Mono-metallic and Bi- metallic nanoflakes

CHAPTER V
RESULTS AND DISCUSSION

5.1 Phytochemical Analysis

Phytochemical analysis of plant extract of *Magnolia champaca* was done according to standard procedure. The results are tabulated below.

Table 1: Phytochemical analysis of plant extract of *Magnolia champaca*

S.N	Class of Compounds	*Magnolia champaca* extract
1	Alkaloids	Absent
2	Flavonoids	Absent
3	Polyphenols	Present
4	Terpenoids	Present
5	Quinones	Present

The above table shows that there is presence of bio-active compounds in plant extract of *Magnolia champaca*. There is absence of Alkaloids and flavonoids and presence of polyphenols, terpenoids and quinones. These bio-active compound present in plant extracts acts as reducing as well as stabilizing agent in formation of nanoparticles. While, study done by Mahas et al., 2017 shows that *M. champaca* leaves and stem extracts contain most of the phytochemicals tested. The phytoconstituents are tannins, glycosides, steroids, flavonoids, and reducing sugars but polysaccharides were absent in both leaf and steam. Saponins were absent in all leaf extract. Similarly, Mullaicharam et al., 2011 reported the presence of alkaloids, flavonoids, glycosides, tannins and sterols in the leaf alcoholic extract, Whereas the leaf aqueous extract was devoid of alkaloids, carbohydrates, glycosides, and sterols.

5.2 Visual Observation of nanoflakes

Initially, formation of Nfs was characterized by visual observation of the obtained nano-powder after drying the solutions. Upon observation, the visual color change of a mixture of copper salt precursor and plant extract from light brown to dark brown indicates the formation of copper oxide nanoflakes. Similar observation was found by Jayakodi et., al 2020 having copper oxide nanomaterial as dark brown. Rajeshkumar et., al 2021 also observed the copper nanoparticles as dark brown through calcination.

Similarly, when color of zinc salt with plant extract changes from light brown to orange and finally to white powder upon heating and after drying, it indicates the formation of zinc oxide nanoflakes. There also have been other studies done where visual observations of white powdered showed the synthesis of zinc oxide nanoflakes. Fakhari et., al 2019 also observed solid product with a light yellow color and after drying overnight in an oven at 60°C white color powder material was obtained as zinc oxide nanomaterial. Similarly, Devi et., al 2014 also observed yellowish white powder as zinc oxide nanomaterial.

Likewise, formation of Cu-Zn bimetallic nanoflakes was indicated when light green powder was obtained after the solution of copper salt and zinc salt got changed from light brown to the green color. It is further characterized by UV- visible spectroscopy. Merugu et., al 2020 synthesized Ag/Cu and Cu/Zn bimetallic nanoparticles using toddy palm where obtained Cu-Zn nanomaterial were green in colour. Minal et., al 2016 also found the colour of Cu-Zn solution (nanomaterials) as greenish in colour. Hence, visual observation can also be considered to confirm the synthesis of required nanomaterial.

Fig 10: Powder of copper oxide, zinc oxide and copper-zinc nanoflakes

5.3 UV-visible spectroscopy

For characterization, small amount of solution containing CuO Nfs, ZnO Nfs and Cu-Zn Nfs was taken in cuvette and placed in the cavity of the UV-visible spectroscopy. The result obtained from UV-visible spectroscopy of the biosynthesized CuO Nfs, ZnO Nfs and Cu-Zn Nfs is shown in 10 (a), (b), and (c).

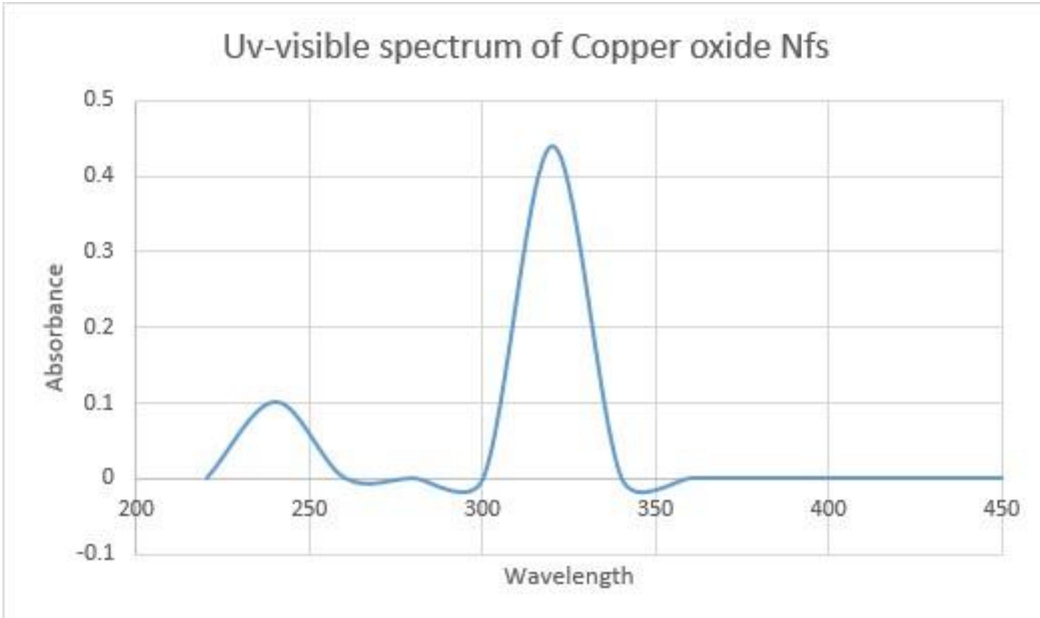

Fig 11: UV-visible spectrum of (a) copper oxide Nfs

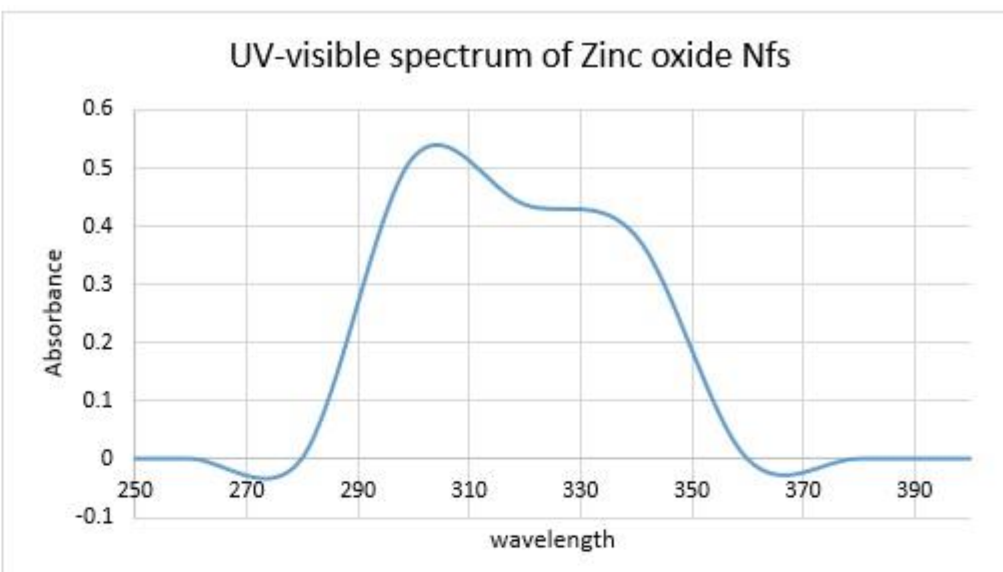

Fig 11: UV-visible spectrum of (b) zinc Nfs

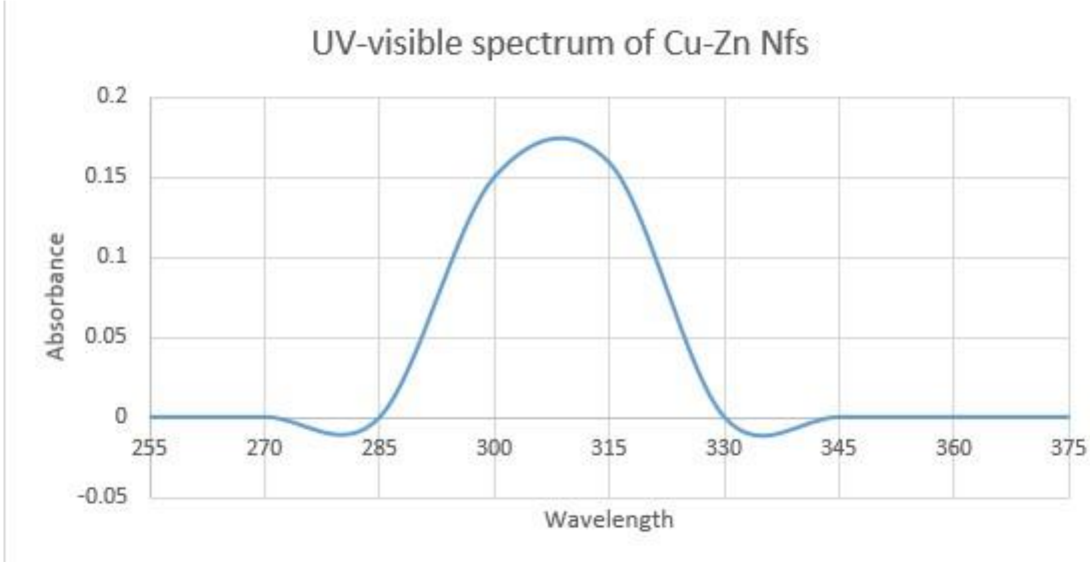

Fig 11: UV-visible spectrum of (c) Cu-Zn Nfs

The UV-visible spectrum of copper oxide nanoflakes shows peaks at 320 nm. As mention by other different research Rajeshkumar et al., 2021 synthesis copper oxide nanoparticles whose UV spectroscopy was found to be 275nm and likewise Rehana et al., 2017 synthesize copper oxide with surface plasmon resonance absorption band at 220–235 nm in the UV–vis spectra. But research done by Amer et al., 2021 to synthesize copper nanoparticles, its UV- visible spectra was found to be 579nm. So, it can conclude that UV- visible spectra obtained less than 400nm must be copper oxide nanomaterial as obtained in above UV- visible spectra.

Similarly, UV-visible spectrum of zinc oxide nanoflakes shows peaks at 300 nm. Other studies done under this topic also state that zinc oxide nanoflakes (Chennimalai et al., 2021) obtained has UV- visible spectrum of 378nm and Jayachandran et al., 2021 also synthesized zinc nanoparticles whose UV-visible spectrum was obtained as 100- 400 nm.

Peaks at 310 nm can be seen in the Cu-Zn nanoflakes' UV-visible spectra. Studies done by Merugu et al., 2020 on synthesis of bimetallic Cu-Zn nanoparticles also shows its UV-visible spectra peaks on 338nm. Likewise, Minal et al. (2016) measured the wavelength of the UV-visible spectrum of Cu-Zn nanoparticles to be 401nm.

5.4 XRD analysis

XRD pattern of Cu-Zn bimetallic nanoflakes exhibit diffraction pattern at 2θ values 25.43° (003), 31.41° (100), 35.57° (002), 56.40° (021) as shown in the fig12. These data are in quite agreement with those reported by Mazhar et al. (Mazhar et al, 2021). The copper oxide flakes showed some peaks at 18.34°, 32.63°, 43.8° and so on. Similarly, Jayakodi et al., 2020 determine the XRD of copper oxide showing peaks at 32.05, 35.24, 37.16, 48.83, 53.02, 58.89, 61.30, 65.12, 67.19, 72.54 and 75.33. However, the XRD peaks at 31.76°, 34.28°, 36.06°, 47.46°, 56.42°, 62.73° and 67.37° could be related to the zinc oxide nanoflakes. The aerial oxidation of zinc may lead to the formation of zinc oxide nanoparticle. The crystal structure of as-synthesized Nfs was studied in terms of xray diffraction pattern. The size of the nanoparticle can determine using Debye Scherer formula.

The grain size (D) = $\frac{0.9\,\lambda}{\beta\cos\theta}$

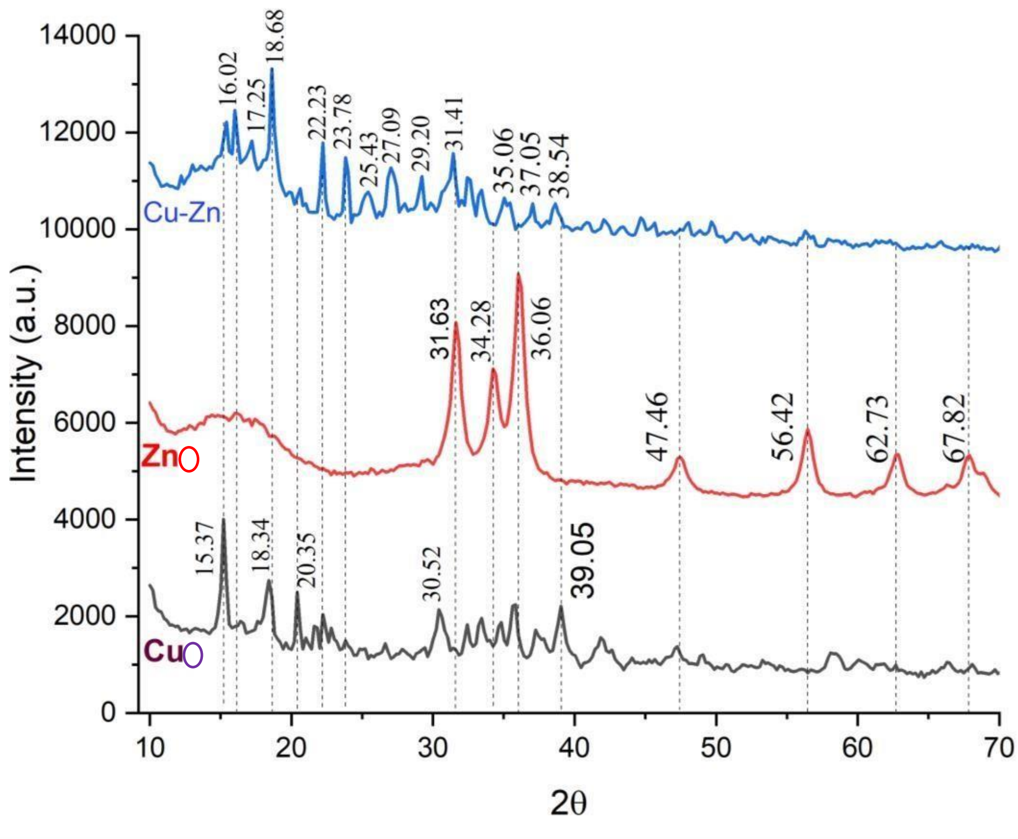

Fig 12: XRD pattern of Cu, Zn and Cu-Zn Nfs

5.5 Fourier Transform Infrared Spectroscopy (FTIR)

The result of FTIR spectrum of Cu, ZnO, Cu-Zn synthesized by using leaf extract of *Magnolia champaca* is shown in Fig12 (a), (b), (c). The figure shows the presence of various kinds of biomolecules associated with synthesized nanoparticles.

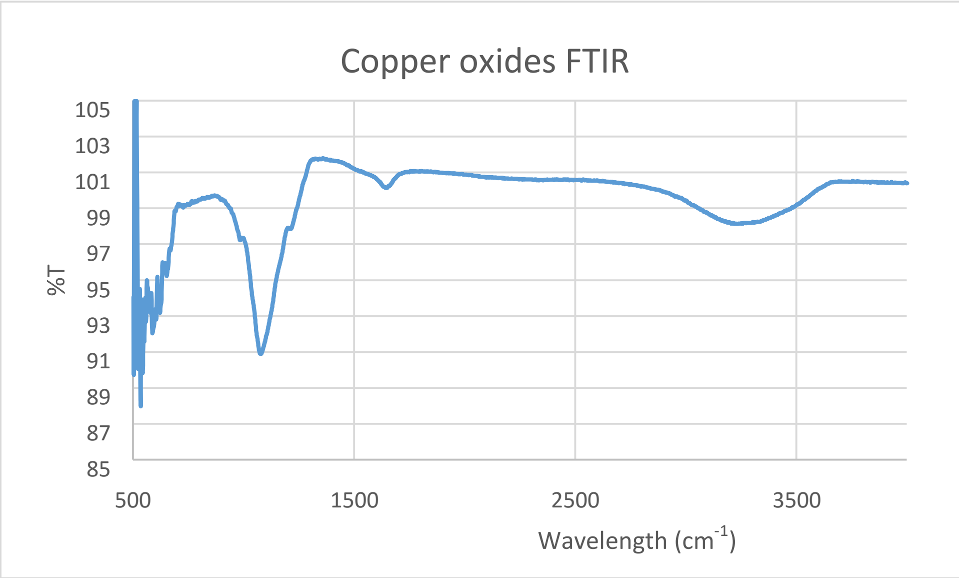

Fig 13(a): FTIR of Copper Oxide Nanoflakes

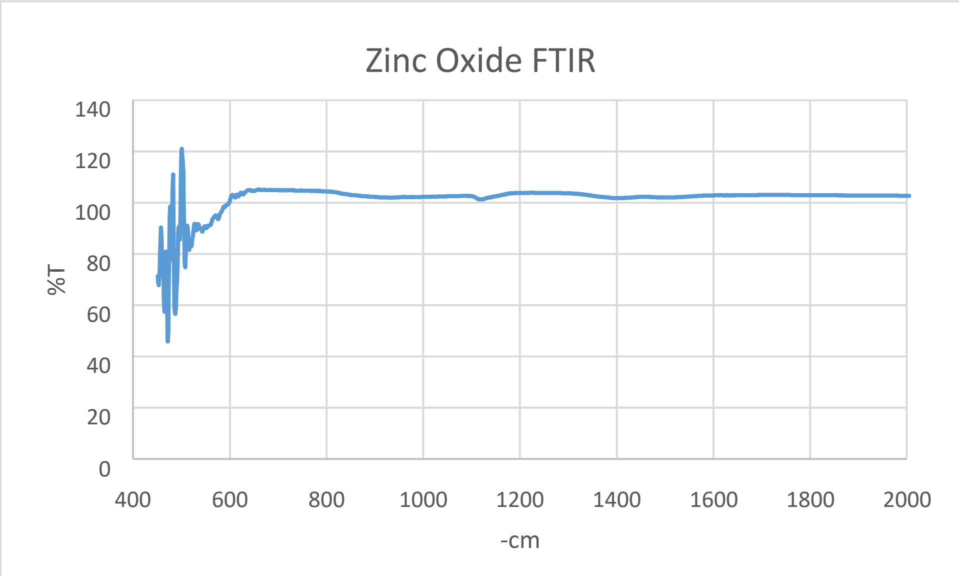

Fig 13(b): FTIR of Zinc Oxide Nanoflakes

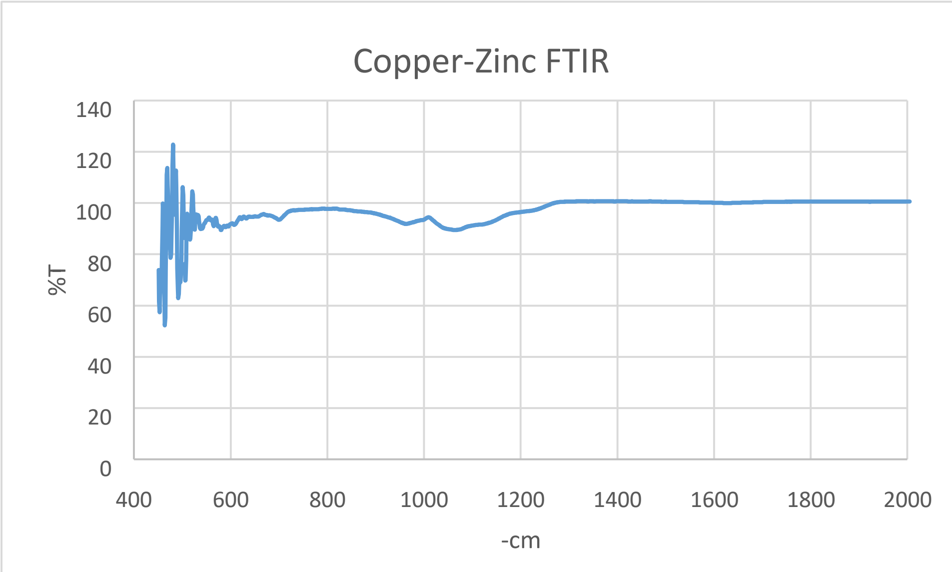

Fig 13(c): FTIR of Copper-Zinc Nanoflakes

FTIR analysis was done to identify the possible biomolecules for stabilizing and capping agents. From the copper oxide, Zinc oxide and Copper-Zinc nanoparticles, peaks observed was 3233 cm^{1} for hydroxyl group, 1627 cm^{-1} for aromatic ring, 1083 cm^{-1} for C-O stretching, 1000 cm^{-1} for out of plane bend C-H plane (Silverstain et al., 1969). These are the functional groups that which binds the nanomaterial and prevent agglomeration.

5.6 FESEM image

The FESEM gives the morphology of obtained nanomaterial. The FESEM image of CuO nanoflakes, ZnO nanoflakes and Cu-Zn nanoflakes are given in the figure:14. The image resembles flakes or rods in parts. Although its thickness is in the nano-range, its flat dimension is in the micrometer range. The nanoparticles are an additional component of the zinc-oxide nanoflakes. Additionally, the copper-zinc composites clearly display nanometer-thick flakes and a few nanogranules. Flake formation has a larger surface area and is similar to a two-dimensional image.

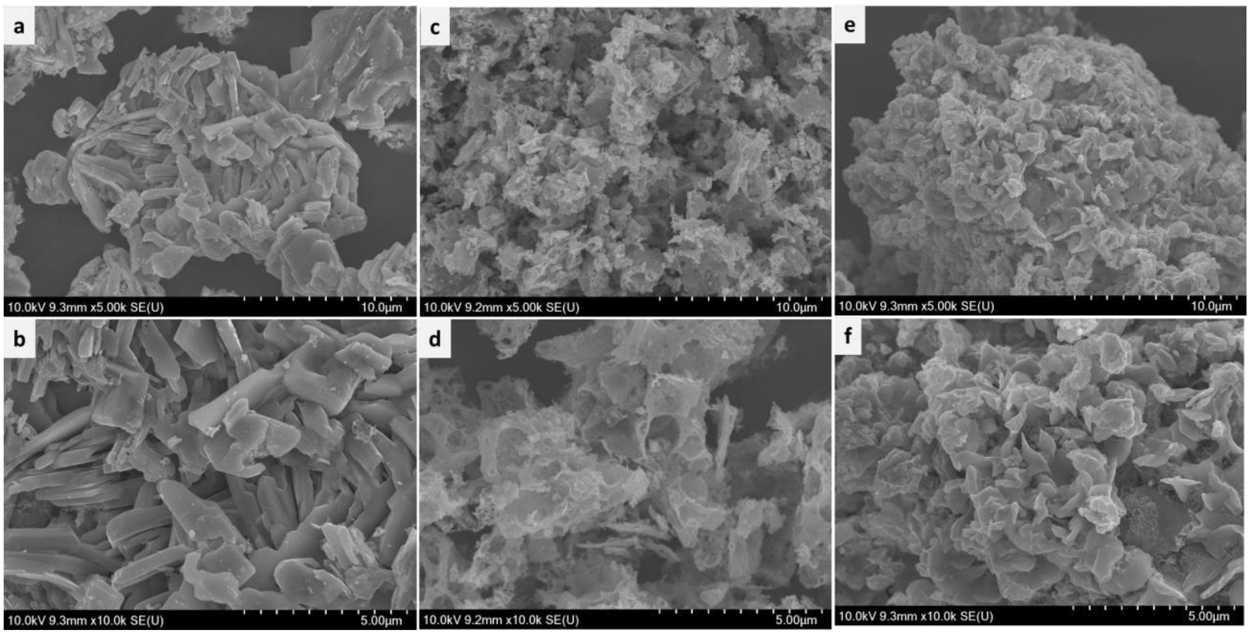

Fig14: FESEM image of (a and b) Cu nanoflakes, (c and d) zinc oxide nanoflakes and (e and f) Cu-Zn composite nanoflakes. Image a, c and e are in × 5 k and images b, d and f are ×10 k magnification.

5.5 Antimicrobial Activity

The diameter of zone of inhibition (ZOI) produced by the CuO, ZnO, and Cu-Zn nanoparticles on particular bacteria and fungus was measured for the estimation of antimicrobial activity. The area around the anti-microbial disc where there is no growth of the microorganism is called zone of inhibition (ZOI) i.e helps in determination of anti-microbial property. The result obtained are given in table below:

Table 2: Antimicrobial analysis of different nanoparticles

S.N	Microorganism	Zone of Inhibition (ZOI) in cm			
		Cu	ZnO	Cu-Zn	Kanamycin (standard)
1	Bascillus subtilis (gram +ve)	1.4	0.7	1.45	1
2	Escherichia. coli (gram –ve)	1.55	0.6	1.25	1
3	Candida albicans	1.5	0.6	1.2	1.05

The results obtained from the antimicrobial test on above table shows that Cu-Zn bimetallic nanoflakes have more antibacterial effect than the monometallic nanoflakes. It shows higher resistivity against the gram +ve bacteria i.e *Bascillus subtilis*. while comparing bimetallic nanoflakes antimicrobial activity against the gram –ve and fungus (*Candida albicans*), monometallic nanoflakes i.e Cuo shows more anti-microbial effects. But Zinc oxide nanoflakes has resulted low resistivity against all of the microbial activity. There is significant inhibitory activity against both gram-positive and gram-negative bacteria as well as fungus by bimetallic nanoflakes of Cu-Zn. Therefore, bimetallic nanoflakes may have huge significance in the medicinal filed.

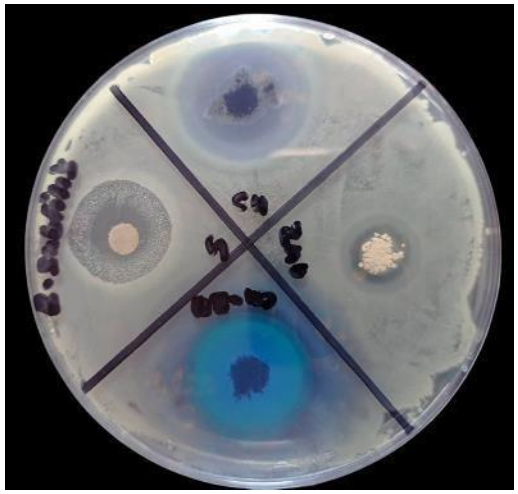

Fig 15: Antimicrobial activity of nanoflakes against *Bacillus subtilis*

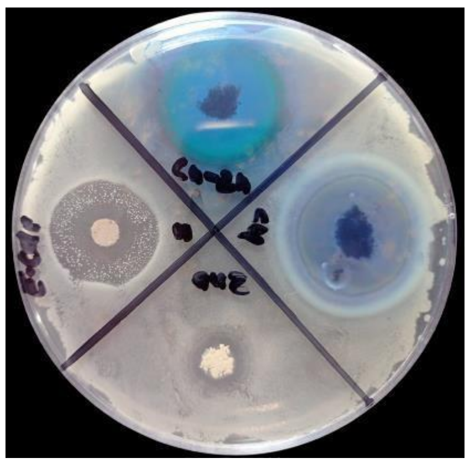

Fig 16: Antimicrobial activity of nanoflakes against *Escherichia coli*

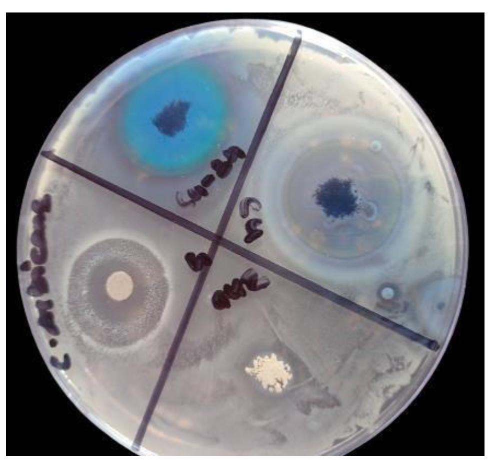

Fig 17: Antimicrobial activity of nanoflakes against *Candida albicans*

There has not been much studies regarding the mono-metallic and bimetallic nanomaterial antimicrobial properties and their comparsion but individually their microbial properties have been researched. Mazhar et.,al 2021 synthesize bimetallic nanoparticles by leaf Extract of Citrus limon and Evaluation of its Antibiofilm Activity Against E. coli which concluded that bimetallic nanoparticles has good microbial properties. Similarly, meregu et., al 2020 also studied bimetallic nanoparticles of Cu-Zn anti- bacterial activity, the nanoparticles were investigated for their antibacterial activity against *Alcaligenes faecalis, Staphylococcus aureus, Citrobacter freundii, Klebsiella pneumoniae* and *Clostridium perfringens* showing Cu-Zn nanoparticles as good antibacterial agent.

CHAPTER VI
CONCLUSIONS

6.1 Conclusion

Copper-zinc nanoflakes were successfully synthesized using *Magnolia champaca* leaf extract on the copper and zinc precursor by green synthess route. The result shows the aquous extracts of leaves of *Magnolia champaca* acts as a good reducing and capping agent. It also proves that green methods are more reliable and less toxic than chemical methods. The phytochemical assay of aqueous solution of plant extract was determined and shows the presence various bio-active compounds helps in reduction and stabilization.

On the other hand, Synthesis of nanoflakes were confirmed by the visual observation, UV-Vis, XRD, FTIR and FESEM. FESEM image showed the flakes like structure with thickness of nanometer dimension. Similarly, microbial tests of nanoflakes shows that bimetallic Nfs have better properties than monometallic Nfs as it has higher resistivity againsts gram +ve bacteria while CuO shows higher resistivity against both fungus (*Candida albicans*) and grem –ve bacteria than Zinc-oxide Nfs with significants ZOI value. CuO NPs shows more secondary metabolites i.e functional groups than other Nfs. Hence, bimetallic may have properties than cannot be overlooked and beneficial than monometallic NPs.

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APPENDIX

(A) Phytochemical Analysis Protocol

1. **Test for Alkaloids**

(i). Meyer's Test: Few drops of Meyer's reagent was added to the plant extract in test tube. Formation of pale yellow precipitate indicate the presence of alkaloids.

(ii). Dragendroff's Test: Few drops of Dragendroff's reagent was added to the plant extract in test tube. Formation of orange red precipitation indicate the presence of alkaloids.

2. **Test for Terpenoids:** To about 1mL of extract, 2mL of chloroform ($CHCl_3$) and 3mL of concentrated sulphuric acid (H_2SO_4) were added carefully. Formations of reddish brown coloration at the interface indicate the presence of terpenoids.
3. **Tests for Flavonoids:** 5mL of dilute ammonia, 2mL of extract and Conc. H_2SO_4 was added sidewise. Formation of yellow precipitation indicates the presence of Flavonoids.
4. **Tests for Polyphenols:** To about 2mL extract was taken in test tube followed by addition of 3 drops of 5% of $FeCl_3$. The appearance of greenish blue coloration indicates the presence of polyphenols.
5. **Test for Quinones:** To about 2mL of extract, 1mL freshly prepared ferrous sulphate ($FeSO_4$) solution and few crystals of ammonium thiyocynates (NH_4SCN) were added. Then the solution was treated with Conc. H_2SO_4 drop by drop. The appearance of persistent deep red coloration indicates the presence of quinones.