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Green Synthesis, Characterization and Antibacterial Activity of Copper Nanoparticle from *Xanthium Strumarium L.* (Deha-Nikel) Plant Leaf Extract

By:

Temesgen Alem Tsegaw

April 2024

Wolkite, Ethiopia

SCHOOL OF GRADUATE STUDIES

Green Synthesis, Characterization and Antibacterial Activity of Copper Nanoparticle from *Xanthium Strumarium L.* (Deha-Nikel) Plant Leaf Extract

A Thesis Submitted to School of Graduate Studies in Partial Fulfillment of the Requirements for the Degree of Master in Chemistry

By:

Temesgen Alem Tsegaw

Major Advisor: Israel leka Lera (PhD)

Co-Advisor: Nigussie Alebachew (PhD)

April 2024

Wolkite, Ethiopia

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We hereby certify that we have read and evaluated this thesis entitled "**Green Synthesis, Characterization and Antibacterial Activity of Copper Nanoparticle from *Xanthium Strumarium L.* (Deha-Nikel) Plant Leaf Extract "prepared** under **our guidance** by Mr. **Temesgen Alem Tsegaw** we recommend that the Thesis shall be submitted as fulfilling the requirements for the award of a MSc**.** Degree in Chemistry

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LIST OF ABBREVIATION

Abs	Absorbance
CuNPs	Copper nanoparticles
PLE	Plant leaf extract
DNA	Deoxyribonucleic acid
Rpm	Revolutions per minute
EDX	Energy dispersive X-ray spectroscopy
FTIR	Fourier transform infrared spectroscopy
NPs	Nanoparticles
XRD	X-ray diffraction
SEM	Scanning electron microscopy
SPR	Surface Plasmon resonance
TEM	Transmission electron microscopy
UV-Vis	Ultraviolet-visible spectroscopy
ZOI	Zone of inhibition
MHA	Muller-Hinton agar
FWHM	Full width at half maximum

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ABSTRACT

The green nanotechnology is generating interest in researchers for the synthesis of nanoparticles in a simple, cost-effective and eco-friendly manner. This research is focused on the bio-synthesis of copper nanoparticles (CuNPs) using Xanthium strumarium L. plant extract. The biomolecules present in Xanthium strumarium L. plant extract act as self-reducing and stabilizing agents. The copper nanoparticles were synthesized by the addition of 100 mL of the aqueous plant leaf extract with 400 mL of copper nitrate solution (0.02M). The biosynthesized CuNPs were characterized by using UV-Vis analysis, Fourier Transform Infrared analysis (FTIR) and X-ray diffraction analysis (XRD) analysis. The maximum absorbance, λ_{max}, was found to be ***672 nm*** *for CuNPs due to surface Plasmon resonance. The presence of important functional groups associated with biomolecules is well characterized by Fourier Transform Infrared spectroscopy (FTIR). The X-ray diffraction pattern showed the formation of purely crystalline nature of CuNPs with face-centered cubic geometry and the average crystalline size is found to be* ***26.88 nm****. The antibacterial activity of the synthesized CuNPs and Xanthium strumarium L. plant leaf extract was established using both Gram-positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa) by using agar well diffusion method. The synthesized CuNPs have strong anti-bacterial activity against gram-negative bacteria as compared to gram-positive bacteria****.***

Keywords: *Green synthesis, Copper nanoparticles, Anti-bacterial activity, Xanthium strumarium L.*

CHAPTER ONE

INTRODUCTION

1.1.Background of the study

Copper is known as one of the elements used from ancient times by humans for treatment purposes, and recently is being used in many aspects of human life from health and hospitals to industry. In previous centuries, human beings have used fine and grinded copper for the treatment of diseases, and nowadays potential applications of metal nanoparticles in the era of Nano science and technology have generated an explosion in scientific interest in this class of materials over the last decade.

In recent years, nanotechnology combined with other branches of science is the main growing area of research.[1-3]. Nanotechnology refers to any technology that is implemented at the nanoscale and has actual applications. It is defined as the control or restructuring of matter at the atomic and molecular levels in the size range of about 1-100nm[4]. These molecules exhibit unique properties in contrast to particles of bulk materials; such as large surface -to- volume ratio, shape, small size etc. which eventually provide the NPs with strong surface activity, better catalytic function and easy interaction with other particles. All these are accountable for the multifunctional properties of NPs and their wide scale applications in various fields like drug delivery, dye degradation, wastewater management, molecular diagnosis, treating cancer cells and in therapeutic applications [5-7].

More specifically, the development of biosynthesized nanoparticles and their utility, applications are one of the important research projects in the concern of environmental chemistry. Green Synthesis, which provides benefit over conventional methods as it is cost-effective, eco-friendly, and easily scaled up for large-scale synthesis and the present method does not need high pressure, energy, temperature and toxic chemicals [8]. Green chemistry has been developed as an alternative to use of environmentally harmful processes and products due to the serious consequences that the world is facing [9].

The properties of these nanomaterials are critical for the technological revolution, which mainly depends on the methods of synthesis for potential applications such as bactericidal and antifungal

effects [10]. Nanoparticles are of great scientific interest as they bridge the gap between bulk materials and atomic or molecular structures[11].

Metallic nanoparticles are of great interest due to their excellent chemical, physical, medicinal and catalytic properties. Copper nanoparticles are one of the commonly used materials for their electrical, optical, catalytic, biomedical and antibacterial applications among various metal particles such as gold, silver, iron, palladium, zinc and quantum dots[12, 13]. It can give more yields in mild reaction conditions when compared to other traditional catalysts. Synthesis of CuNPs is cost-effective when compared to silver (Ag), gold (Au) and platinum (Pt) [14].

Green synthesis of CuNPs using nontoxic and inexpensive materials like curd, milk, and herbal extracts such as tamarind and lemon juice as capping agents was reported by Sastry et al.,[15]. CuNPs have attracted much attention from researchers due to their application in wound dressings and biocidal properties[16]. Due to these properties, CuNPs are used in processes such as gas sensors, catalytic processes, high-temperature superconductors, and solar cells[17].

Nowadays, bacterial infectious diseases caused by pathogenic microbes (bacteria, viruses, fungi, protozoa, parasites) are a serious burden in the world economy which kills millions of people around the world annually. Therefore, various research groups developed an antibiotic to treat bacterial infectious diseases due to their low cost and influential results. However, several studies have provided direct evidence that the widespread use of antibiotics has led to the emergence of multidrug-resistant bacterial strains. Abuse of antibiotics led to the development of super bacteria which resists nearly all antibiotics because of super bacteria-resistant genes (Tong et al. 2005; Hu and Xia 2006). Furthermore, most bacteria exist in the form of biofilms which in turn form barriers to resist antibiotics. Therefore, despite huge number of antimicrobial drugs and other modern antibacterial agents, infectious diseases due to bacteria is still a challenging issues .To overcome the resistance developed by microbes, nanoparticles-based treatment presents a very promising approach.

The use of plant extracts is increasing in usefulness and is conceived as an environmentally and economically friendly alternative for the synthesis of CuNPs. Extracts can be obtained from multiple parts of the plant; such as leaves, bark, seeds, peels, roots, coir, gum and fruits have been also reported[18].

Xanthium strumarium L. is a species of annual plants belong to the Asteraceae family and commonly known as "Cocklebur" or Deha-Nikel in Amharic. It may originate in Ethiopia and has been extensively spread out elsewhere. However, small quantities of this plant may be consumed, but seeds of this plant should not be eaten in large quantities because of their toxic chemical carboxyatratyloside. The plant's root and fruit, is used as traditional medicine. It is used for the diseases leucoderma, biliousness, and poisonous bites of insects, epilepsy, salivation and fever. This plant's leaf material has been found to have strong antimicrobial activity and there is no toxic. This plant has various useful biological activities and is found in literature[19, 20].

This work aims; for the green synthesis of *Xanthium strumarium L.* (Deha-Nikel in Amharic) leaf extract mediated copper nanoparticles, their characterization and antibacterial activity. The protocol used in this study is very simple, greener, and eco-friendly, and no other capping agent is required. The synthesis was carried out in an aqueous medium using Copper (II) nitrate trihydrate (Cu $(NO_3)_2.3H_2O$) as a copper ion source by a green method. The green synthesized CuNPs where characterized by UV-Vis, FTIR, and XRD instruments and their anti-bacterial activity was evaluated.

1.2. Statement of the problem

Different methods have been used for the synthesis of copper nanoparticles (CuNPs) which can be either physical, chemical or biological methods. Earlier methods used for the synthesis of CuNPs were toxic and hazardous chemicals were used for their synthesis. The risk of contamination is also higher. This enhances the growing need to develop environmentally-friendly processes through green synthesis and other biological methods. Thus, the use of environmentally-friendly processes, for the synthesis of CuNPs is known as green synthesis. Green synthesis is preferred over conventional synthesis because it is ecofriendly, cost-effective, single-step method of synthesis and it does not require for high pressure, energy, temperature, or toxic chemicals. The synthesis of copper nanoparticles using various plants materials and their extracts can be beneficial over other biological synthesis processes which involve the very

complex procedures of maintaining microbial cultures. The Synthesis of Some metallic nanoparticles (like Ag and Au) are expensive compared to cupper. The skin infection has increase for the people. CuNPs is not studied before from *Xanthium strumarium L*. plant leave extract.

Green synthesis of nanoparticles using plant extracts are among the main area of research and are used different applications. In this study, CuNPs have been prepared using leaves extract of *Xanthium Strumarium L*. (commonly known as Cocklebur or Deha-Nikel in Amharic) which is less toxic, eco-friendly, locally Easley available, safe to handle and cost effective as compared to other conventional synthesis methods and did not study before the antibacterial activity. The antibacterial activities of the synthesized CuNPs were tested. As far as we know, there has been no study on the anti-bacterial activities of copper nanoparticles synthesized using *Xanthium strumarium L*. plant leaf extracts

1.3.Objectives of the study

1.3.1. General Objective

The general objective of this study was to synthesize copper nanoparticles (CuNPs) from the leaves extract of *Xanthium Strumarium L*.**,** and characterize by different analytical instruments and evaluate its antibacterial activity.

1.3.2. Specific Objectives

- To synthesize CuNPs from leaf extracts of *Xanthium Strumarium L.*
- To characterized copper nanoparticles (CuNPs) by UV-Vis, FTIR, and XRD instruments.
- To evaluate the antibacterial activity of the synthesized CuNPs.

1.4.Significance of the study

The advantages of green synthesis of CuNPs by using plant parts are that the plants are easily available, inexpensive, environmentally friendly, stable, safe to handle and non-toxic and possess a large variety of active agents that can promote the reduction of copper ions. Green synthesis of nanoparticles using plants are significantly contributes to environmental sustainability throughout the production without causing harm to human health or the environment. It also reduces the risk of contamination. The most important point is the active agent contained in the plant parts which makes the reduction and stabilization possible. Supporting the use of indigenous medicine with scientific knowledge. Generally, the green synthesis of CuNPs from

the leaves extract of *Xanthium Strumarium L.* is beneficial for biomedicine and more particularly as antibacterial agent. The findings of this study will help to develop awareness of the social community about the dosage levels of this medicinal plant regarding to health effects. So green synthesized copper nanoparticles have highly preferable for biomedical industry, human health care, textiles, and bioremediation over chemically synthesized copper nanoparticles.

1.5.Delimitation (Scope) of the Study

The scope of this study covers:

Green synthesis of CuNPs from the aqueous leaves extract of *Xanthium Strumarium L.* was copper nitrate solution,

The leaves of *Xanthium Strumarium L.* were collected from the Wolkite University Gurage zone,

The synthesized CuNPs where characterized by UV-vis, FTIR, and XRD analysis,

The antibacterial activity of the synthesized nanoparticles was tested on the clinically isolated bacteria.

CHAPTER TWO

LITERATURE REVIEW

Nanotechnology is the upcoming and attractive area of research in the field of life science, chemical science, medical science and many more. It deals with the preparation of nanoparticles (NPs) in the range of 10^{-9}m and having dimensions of 1–100nm[21]. Nano particles acquire exclusive properties and find applications in various fields for which they are of great importance in the technological world [22-24]. The chemical surroundings, size and shape greatly influence the electronic, optical and catalytic properties of the nanoparticles [7, 25, and 26].

Nanotechnology proved that by producing smaller, faster, lighter and cheaper devices with greater functionality while using less raw materials and consuming less energy. Research on the materials that was synthesized by this technology is the initiation towards miniaturization that will play a vital role towards a sustainable usage of raw materials and energy[27]. Nanoparticles are of great importance due to their nanometer size and their high proportion of surface atoms, presenting high surface areas. At this size scale, the particles exhibit significantly different properties from their bulk counterparts. Their distinct physicochemical, electrical, catalytic, magnetic, optical, mechanical and biological properties have become the subject of intensive studies in recent years. Owing to their interesting properties, which are affected by their structural morphology, nanoparticles have been studied extensively[28].

Nanoparticles are prepared in many ways such as physical, chemical and green method. Green synthesis method has so many advantages compared to other methods and one of the best methods because of its cost-effective, simple, use of low energy, use of less toxic materials and eco-friendly [29, 30]. The copper nanoparticles are mostly found their applications in the field of medical, electronic devices, biosensors, and reagents in various reactions, lubricants, antibiotic, antimicrobial agents and many more.

Copper nanoparticles (Cu-NP) synthesis specifically has attracted more interest compared to other NPs' synthesis because of their useful properties achievable at much less cost than silver and gold [31]. Copper, like other noble metals, exhibits thermal and electrical conductivity, which makes it a candidate in electronic systems [32] and conductive inks [33]. Similarly, it has antimicrobial properties [34] and is readily available.

Since 1963, the fruits of Xanthium strumarium L. plant has been listed in the Pharmacopoeia of the People's Republic of China (CH. P), and currently over 60 formulas containing the fruits of X. strumarium have been applied for treating various diseases, including rhinitis, nasal sinusitis, headache, gastric ulcer, urticarial, rheumatism, bacterial and fungal infections, and arthritis[20, 35, 36]. So far, many studies have been devoted to the pharmacological and phytochemical studies of X. strumarium, and more than 170 chemical compounds have been isolated and identified from this plant, including sesquiterpene lactones, phenols, glycoside, alkaloids, fatty acid and others[37]. In addition, increasing evidence has indicated that X. strumarium possesses a wide spectrum of pharmacological activities including analgesic and anti-inflammatory, antioxidant, hypoglycemic, anti-cancer, antibacterial and antifungal, anti-trypanosomal, anti-tussive activities, and effects on nervous and digestive systems, as well as other effects[20].

2.1. Approaches for nanoparticles synthesis

Synthesis of nanomaterials can be achieved by using either the "top-down" or "bottom-up" approach. All the physical, chemical, and biological methods of synthesis of CuNPs follow one of these two approaches Fig. 1[38].

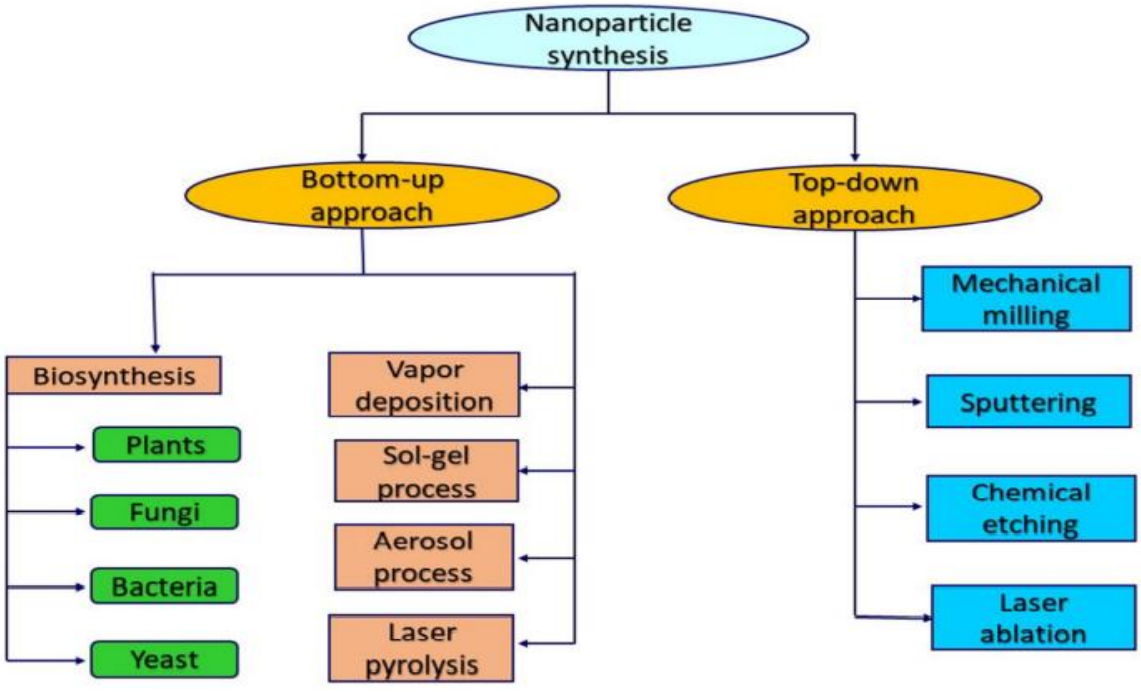


Figure 1: Nanoparticles synthesis via biological and physicochemical approaches.

2.1.1. Bottom-up approach

The bottom-up approach involves the generation of nanoparticles from small units like molecules and atoms or through the self-assembly of atoms into new nuclei, which further grow into a particle possessing nanoscopic dimensions and employing various chemical and biological methods Fig. 2.

2.1.2. Top-down Approach

In this approach, nanoparticles are formed by size reduction method that means suitable bulk material reduces to small units with the use of appropriate lithographic methods, for example crushing, spitting, and milling, Fig. 2[39].

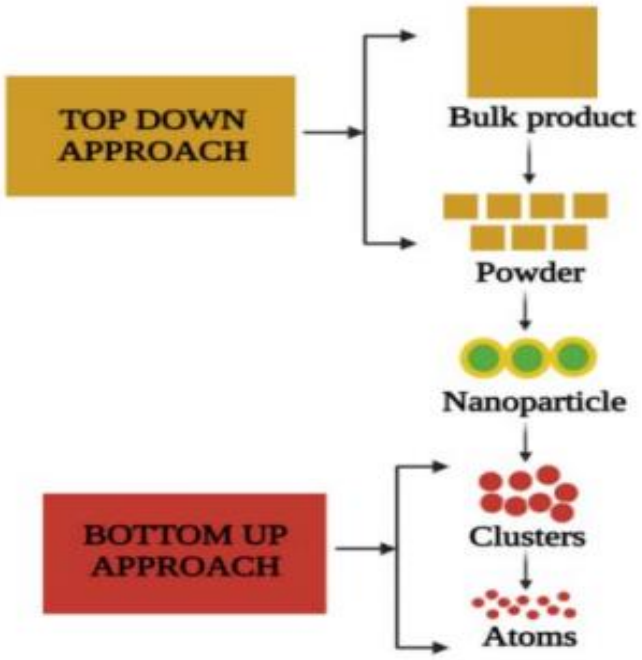


Figure 2: Approaches for the synthesis of nanoparticles

2.2.Methods of copper nanoparticles synthesis

A synthesis of copper nanoparticles by various methods. Copper nanoparticles were examined by various methods like chemical, physical and biological methods[40].

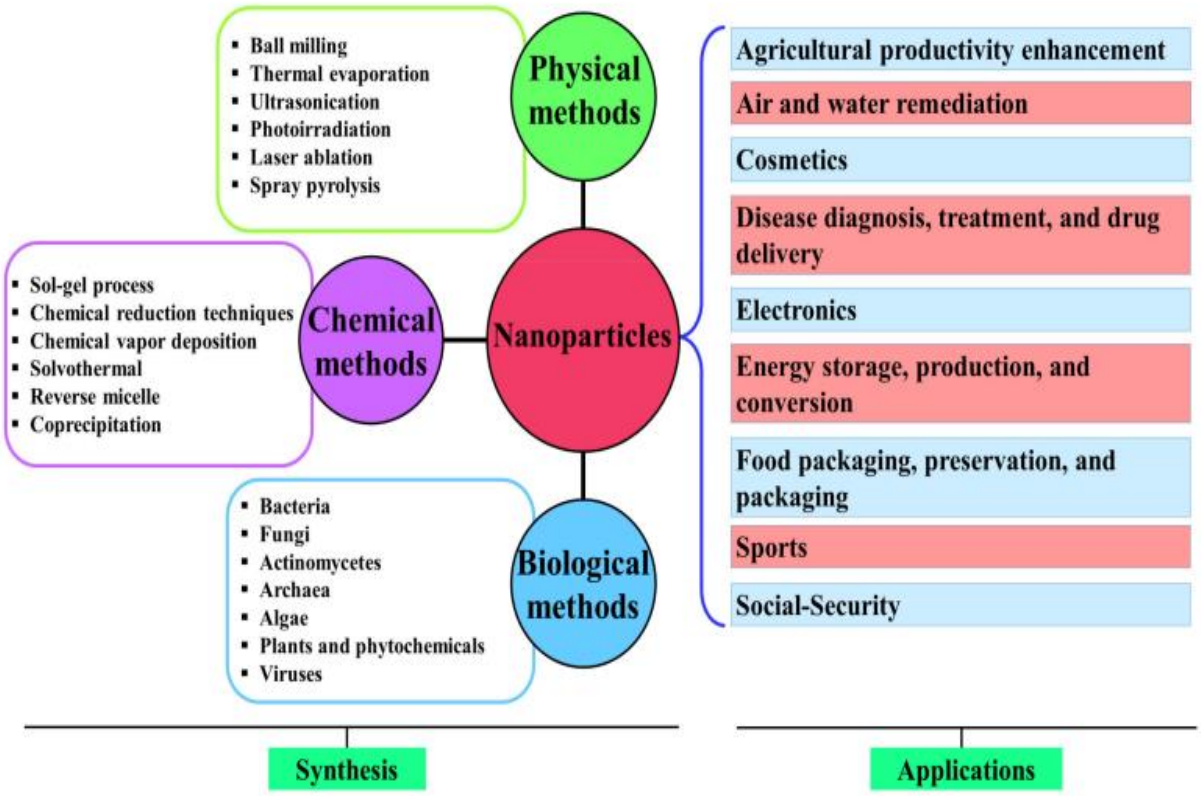


Figure 3: Schematic representation of various methods adopted for NP synthesis and its applications.

2.2.1. Physical methods

The most important physical synthesis methods are evaporation–condensation and laser ablation. Nanoparticles obtained through physical synthesis present no solvent contamination and have uniform distribution which is an improvement over chemical synthesis. Very small nanoparticles can be obtained using evaporation–condensation (6.2–21.5 nm and 1.23–1.88 nm) but the process requires a lot of energy to increase the operating temperature and it is time-consuming. The nanoparticles obtained with laser ablation have characteristics depended on the wavelength of the laser, the duration of its pulses, the laser fluence, the ablation time and the liquid medium. One study obtained nanospheres (20–50 nm) in water with femtosecond laser pulses at 800 nm[40]. Some disadvantages of physical obtaining methods are the requirement of expensive

equipment and high use of energy, thus making them less popular than chemical or green methods.

2.2.2. Chemical methods

Chemical methods are the most widely used to obtain copper nanoparticles. One major drawback is the use of toxic materials during the synthesis phase. Considering that nanoparticles are being used more frequently and have increasing human contact, it is essential to develop environmentally friendly processes. Various chemical methods are used to obtain nanoparticles, such as sonochemical reduction, hydrothermal synthesis, electrochemical and chemical reduction. The latter is the most commonly used one. It involves using hydrazine, ascorbic acid or sodium borohydride as a reducing agent. The chemical reduction method is often used to obtain CuNPs because it is simple, has high yield efficiency and requires limited equipment[41]. The major disadvantages this method is that reagents are toxic and the byproducts generated are not eco-friendly.

2.2.3. Biological methods

Green synthesis of metallic nanoparticles is widely used because of its harmless obtaining method. It uses molecules in plants and microorganisms (bacteria, fungi) as a reducing agent. It has the advantage of using more eco-friendly materials, being cheaper than chemical synthesis, simpler, more rapid and sustainable. It is preferable to use plant extracts to obtain nanoparticles rather than using microorganisms because of increased difficulty in preserving cell cultures[42]. Moreover, it reduces the complex process of maintaining cell cultures and it is also suitable for creating large scale synthesis of nanoparticles[43].

Considering the use of nanoparticles in medicine, there is an increased need to use an eco-friendly method of obtaining as they are regarded as the next step in battling diseases. Plant extracts act as a reducing and capping agent, give stability to the NPs, and help to increase the rate of reduction. Different plants contain different bioactive compounds which act as reducing and capping agents to reduce metal ions[44].

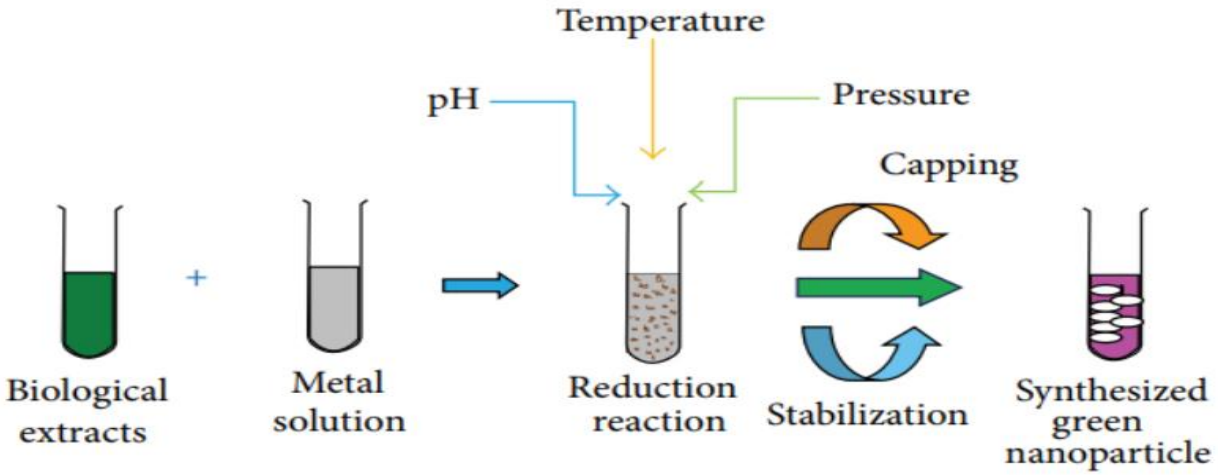


Figure 4: Biological synthesis of nanoparticles using green technology[45]

According to the experimental studies of **Murthy et al.,** CuNPs were successfully synthesized from the aqueous copper nitrate solution (Cu $(NO_3)_2 \cdot 3H_2O$) using *Hagenia Abyssinia* (kosso) leaves extract in a continuously heated and stirred mixture. The change in color from blue to light brownish visually after several minutes of reaction time as indicates the formation of CuNPs. The UV-visible absorbance spectrum recorded for g-Cu NPs exhibited λ_{max} of 403. This absorption band is basically due to surface Plasmon resonance of g-Cu NPs fig. 5[46].

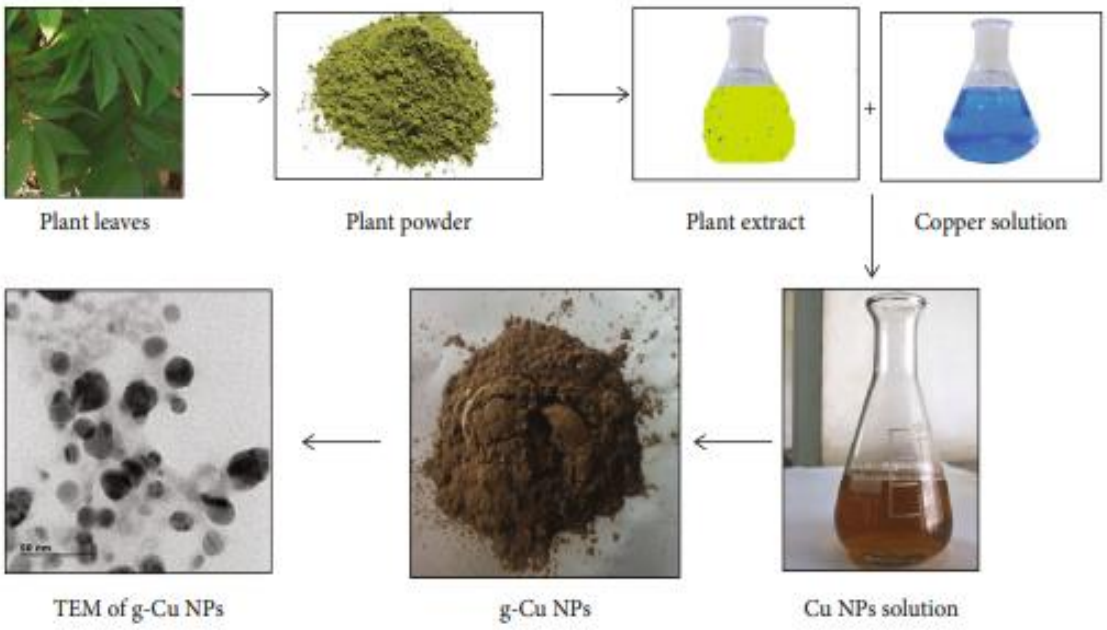


Figure 5: The scheme of synthesis of g-Cu NPs from *Hagenia Abyssinia* leaves extract.

Table 1: Different precursors, plants leave extract for synthesis and characterization techniques for CuNPs.

Sr. No.	Precursor	Plants/plant extract	Size (nm)	Shape	characterization techniques used	Ref
1	Copper Sulfate ($CuSO_4$)	*Capparis zeylanica*	50 –100	Cubical	UV-Vis, FTIR, XRD, SEM, EDX & TEM	[47]
2	Copper (II) sulfate pentahydrate $CuSO_4.5H_2O$	*Datura meta L.*	15 –20	Spherical	UV-visible, Particle size analyzer (PSA), TEM, EDX and FTIR	[48]
3	Copper Sulfate ($CuSO_4$)	*Albizialebbeck*	100	Spherical	UV-Vis, SEM,TEM, EDS &XRD	[49]
4	Copper Sulfate ($CuSO_4$)	*Euphorbia esula.L*	20 –110	Spherical	UV-vis, XRD, FTIR& TEM	[50]
5	Cupric chloride dehydrate ($CuCl_2.2H_2O$)	*Pistachio*	9	Spherical	XRD, FTIR and SEM	[51]
6	Cupric chloride dehydrate ($CuCl_2.2H_2O$)	*Malvasylvestris*	14	Spherical	XRD, FTIR and SEM	[52]
7	Copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$)	*Magnolia Kobus L.*	37 –110	Spherical	UV-visible, ICP, EDS, XPS, and HRTEM	[53]
8	Cupric chloride dehydrate ($CuCl_2.2H_2O$)	*Ginkgo biloba Linn*	15 –20	Spherical	UV-visible spectroscopy, TEM, EDS and FTIR	[54]
9	Copper Sulfate ($CuSO_4$)	*Gymnemasylvestre*	65 –302	Spherical	UV-visible, FTIR and SEM	[55]
10	Cupric chloride dihydrate ($CuCl_2.2H_2O$)	*Otostegia persica*	40	Spherical	UV-visible spectroscopy, XRD, FTIR and TEM	[56]
11	Cupric chloride	*Thymus vulgaris L*	30	FCC	UV-visible, XRD,	[57]

	dihydrate ($CuCl_2.2H_2O$)				FTIR, TEM, EDS, TGA and DTA	
12	Copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$)	*Ocimum bacilicum*	40-60	Spherical	UV-visible spectroscopy, FTIR and HRTEM	[58]
13	Copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$)	*Asparagus adscendens*	10-15	Spherical	UV-visible spectroscopy, FTIR and HRTEM	[58]
14	Copper Sulfate ($CuSO_4$)	*Eucalyptus sp. L.*	38.62	----	UV-Vis, FTIR, XRD&SEM	[59]
15	Copper Nitrate($CuNO_3$)	*H.rosasinensis*		Spherical	UV-visible spectroscopy, FT-IR, and TEM	[60]
16	Copper Chloride($CuCl_2$)	*Papaya*	20	Spherical	UV-visible, Absorption Spectrometer, XRD, FTIR, SEM and TEM	[61]
17	Copper (II)sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$)	*O.sanctum*	77	Spherical	XRD and FTIR	[62]
18	Cupric chloridedihydrate ($CuCl_2 \cdot 2H_2O$)	*PsidiumguajavaL.*	13.13±0.19	-----	UV-visible spectroscopy	[60]
19	Copper (II) Sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$)	*Artabotrys odoratissimus*	135	---	Particle size analysis(PSA)	[63]
20	Copper Sulfate($CuSO_4$)	*NeriumOleander*	---	---	UV-visible spectroscopy and FTIR	[64]

2.3. Mechanism of synthesis of copper nanoparticles

In the biosynthesis of CuNPs, extracts from biological sources may act as both reducing and capping agents. Combinations of biomolecules included in these extracts, such as proteins, amino acids, vitamins, carbohydrates, glycosides, alkaloids, polyphones, terpenoids, ascorbic acid, oxalic acid and polysaccharides, reduce Cu^{+} ions in an environmentally favorable but chemically complex. Copper ions were bound on the surface of proteins in extract via electrostatic interactions, which served as a reduction process[65, 66](fig. 6) [39].

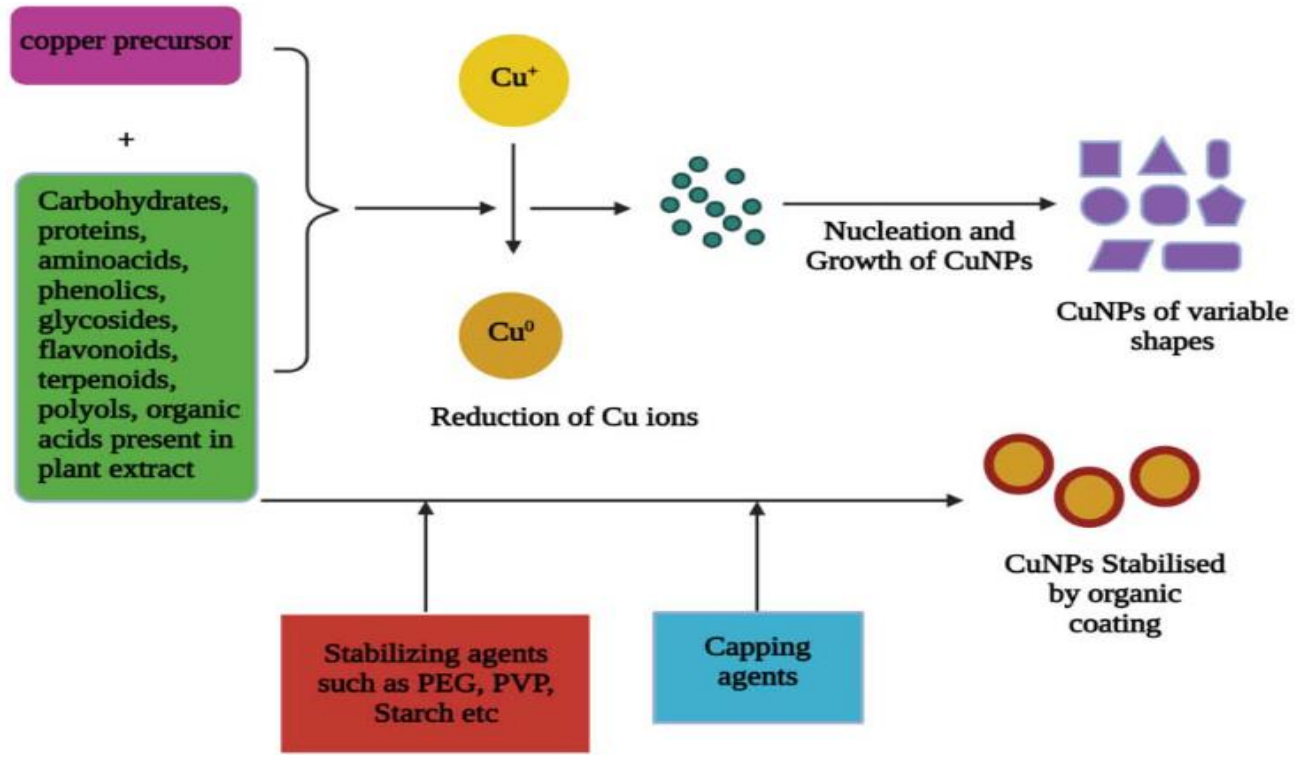
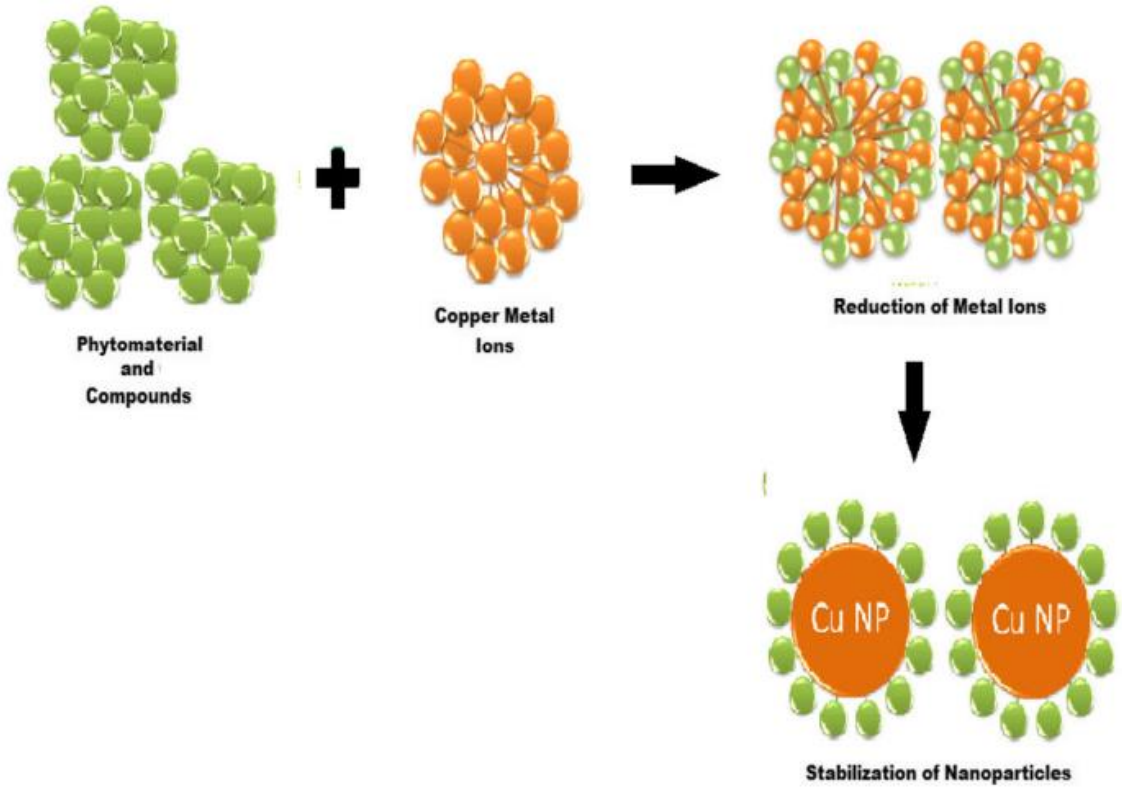


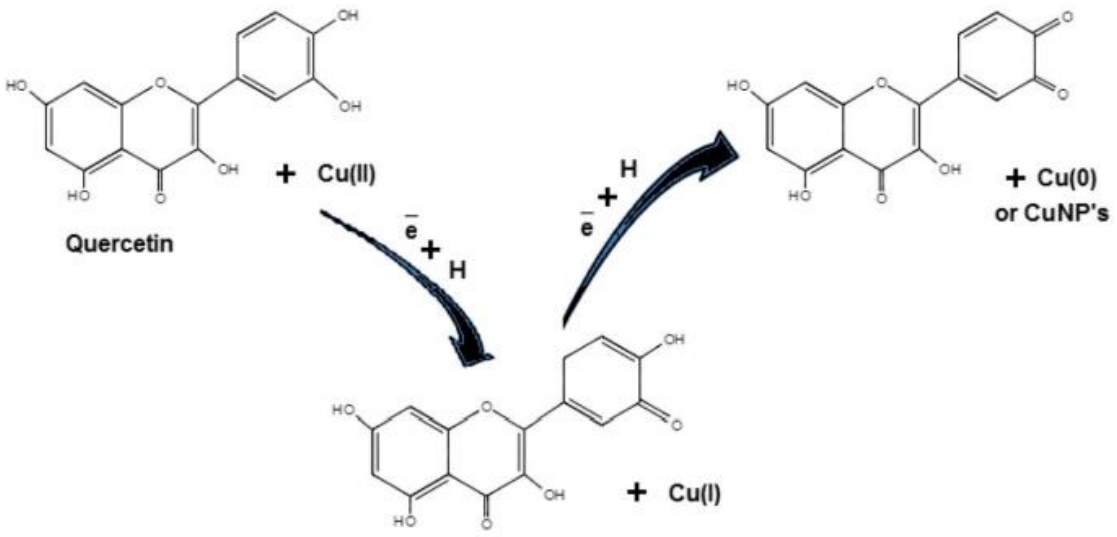
Figure 6: Probable mechanism for the synthesis of CuNPs

Phytochemicals have a main role in first reducing the metal ions and then stabilizing the metal's nuclei in the form of nanoparticles as shown in Scheme. 1. The interaction of phytochemicals with metal ions and the concentration of these phytochemicals control the shape and size of CuNPs. Flavonoids contain polyphenolic compounds, e.g., quercetin, catechins, flavanones, isoflavones, santin, penduletin, alizarin, pinocembrin, anthocyanins, flavones, tannins, and saponins, which are present in different plants such as Ginkgo biloba [54]. Various functional groups present in the flavonoids are responsible for the reduction of the copper ion. It has been assumed that a reactive hydrogen atom in the flavonoids may be released during the tautomeric alterations of the enol form to the keto form which can reduce copper ions to form copper nuclei

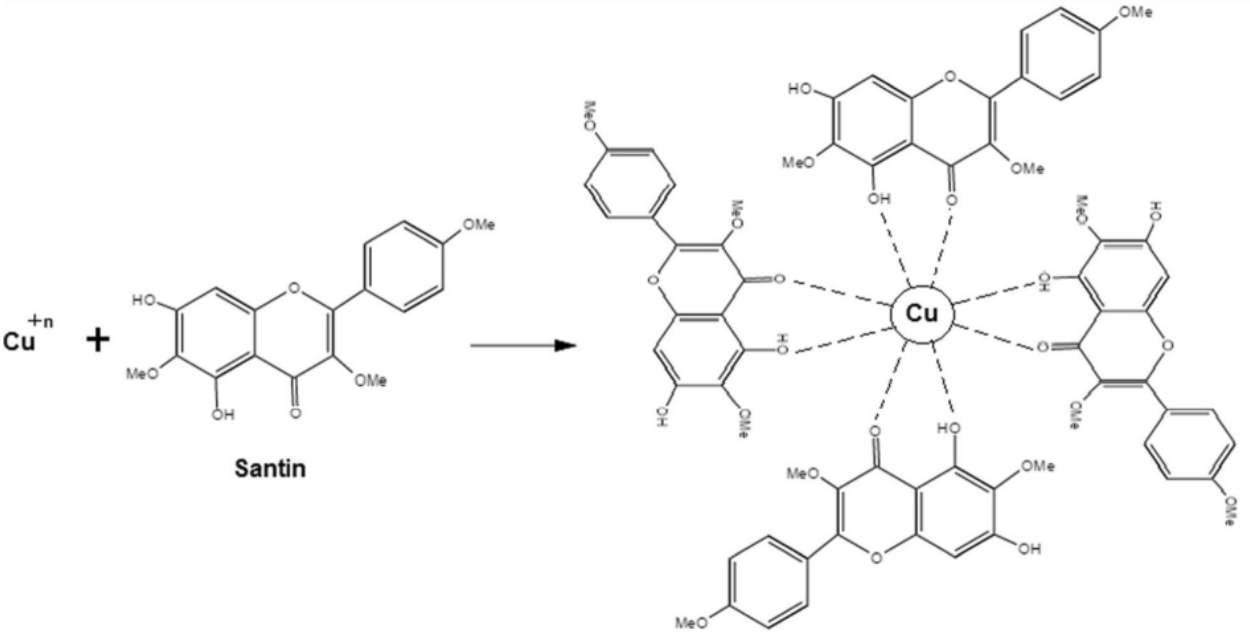
or CuNPs. For example, it is assumed that in the case of Ginkgo biloba plant extracts, it is the transformation of quercetin (flavonoid) which plays a main role in the reduction of copper metal ions into copper nuclei or CuNPs due to the change of enol form to keto form as shown in Scheme. 2. Some flavonoids have an ability to chelate the CuNPs with their π electrons and carbonyl groups. Quercetin and santin are flavonoids with strong chelating activity due to the presence of two functional groups involving the hydroxyls and carbonyls. These groups chelate with copper nanoparticles by following the previous mechanism and also explain the ability of adsorption of santin (flavonoid) on the surface of CuNPs as shown in Scheme. 3.



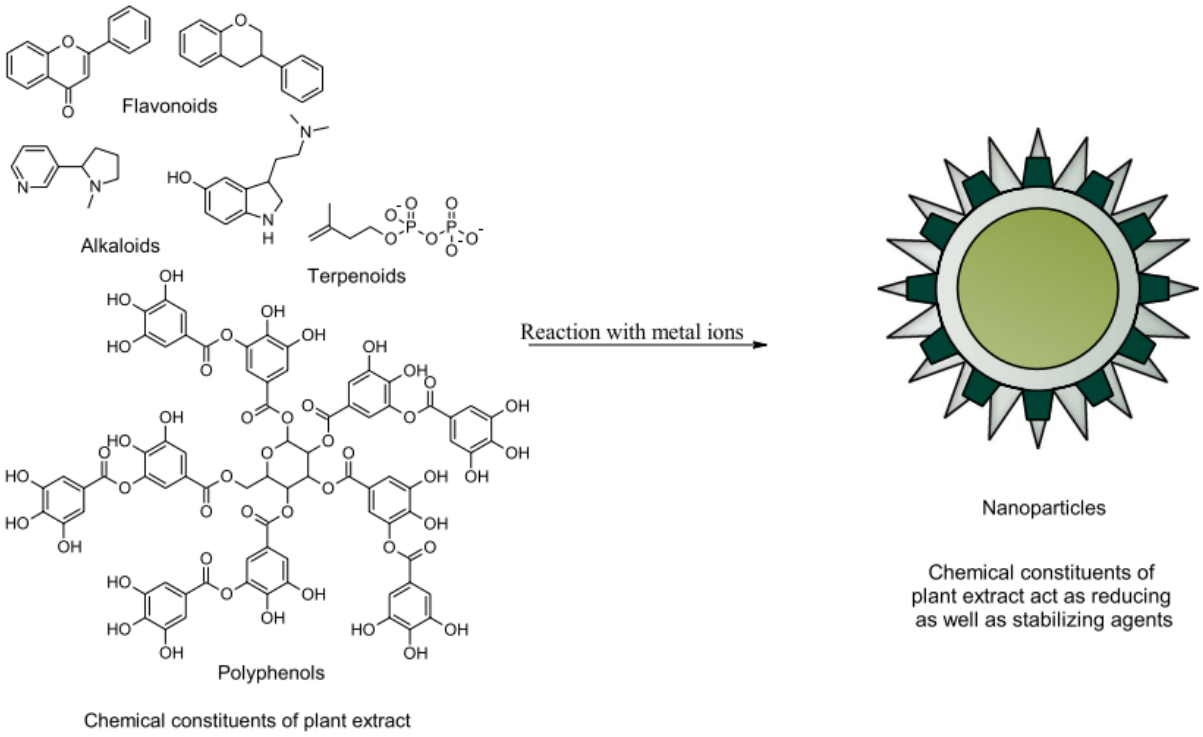
Scheme 1: A protocol for reducing the metal ions and then stabilizing the metal's nuclei[18]



Scheme 2: Reduction of copper ions by quercetin[18].



Scheme 3: Stabilization of copper nanoparticles by santin[18]



Scheme 4: Possible chemical phytochemicals of plant extract associated with metal nanoparticles synthesis[67]

2.4. Plant-mediated Green Synthesis of CuNPs

The main advantage of the green synthesis of CuNPs is that they are easily available, safe to handle and possess a broad variability of metabolites. In the light of IR spectroscopic research, the primary phytochemicals responsible have been identified as terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids. The main water-soluble phytochemicals like quinines, flavones and organic acids were responsible for immediate reduction. Redial tautomerization occurs in anthraquinone compounds, resulting in the formation of nanoparticles. The stability of the green synthesized CuNPs is enhanced and thereby it increases the rate of reaction of CuNPs by preventing the formation of agglomerates[68, 69]. The part of the plants such as leaf, fruit, flower, bark, root and stem along with the precursor copper salts such as copper acetate, copper nitrate, copper sulphate and copper chloride were processed as per the time and temperature is given in fig. 7. The extracts of plants have been efficiently applied for this purpose. Synthesis of CuNPs has been successful with extracts of various parts of plant species that include *Eucalyptus* leaf[70], *Camellia sinensis* leaf[71], *Ocimum sanctum* leaf[72], *Punica granatum* peel [73], *Citrus medica Linn*. (Idilimbu) juice [74], *Ziziphus spina-christi* (L.) *Willd* fruit[75],

Eclipta prostrata leaf [76], *Ginkgo biloba Linn* leaf[54], *Plantago asiatica* leaf[77], *black tea* leaf[78], *Terminalia catappa* leaf [79], *Azadirachta indica* leaf [80] and *Piper retrofractum* fruit[81].

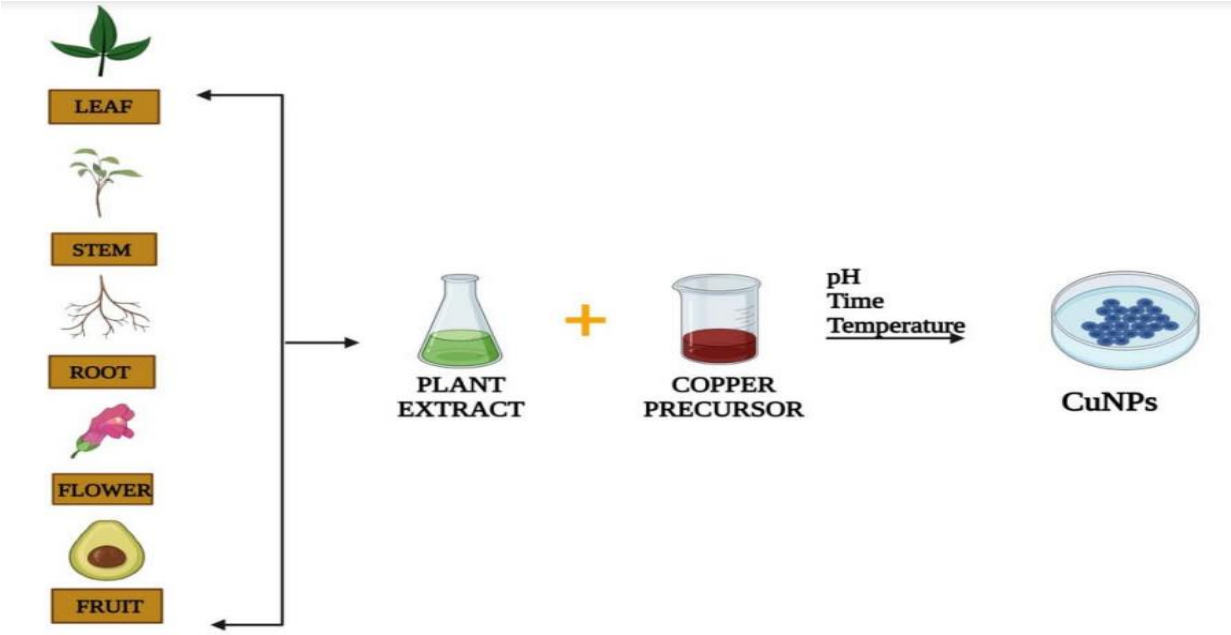


Figure 7: Plant mediated synthesis of copper nanoparticle**s [39]**

2.4.1. Xanthium strumarium L. (Common Cocklebur)

In Ethiopia, the plant is used traditionally for the treatment of human skin infections[82]. A number of studies have been conducted to explain the putative traditional medicinal uses of *X. strumarium*. A methanol extract was shown to have in vitro antibacterial and antifungal activities [83]. *Xanthium strumarium* can be used as a medicinal plant. A yellow dye can be made from its leaves. However, these uses cannot compensate for its overall negative impacts[84].

2.4.2. Physical characteristics of Xanthium strumarium L. (Common Cocklebur)

Xanthium strumarium L. (Cocklebur) (Asteraceae), is an herbaceous annual or a short-lived perennial plant of worldwide distribution in North America, Brazil, China, Malaysia, and hotter parts of India. The plant is erect, up to 2.5 m tall, and presents blotched purple stems. Its leaves are dark green on the upper surface, similar in shape to grape leaves, 3-18 cm long by 3-18 cm wide on long and roughly textured with minute bristles. The flowers are yellowish green, inconspicuous, both male and female, occurring in leaf axils towards the end of the branches. In addition, the flowers develop into hard woody burrs (fruits). The fruit is called a bur,

its ovoid (oval shaped) and it measures about 1.2-2 cm long, green, turning yellow and then brown in stalked auxiliary clusters. Each bur has two stouts, curved or straight horns and is covered with hooked spines. X. strumarium is extremely competitive with other crops. It has long been considered one of the worst weeds in soybean plantations [84-86].

Figure 8: *Xanthium strumarium L.*.

2.5. Characterization of CuNPs synthesized from plants

It is necessary to characterize the nanoparticles in order to understand the control of synthesis and their uses in wide applications. Various techniques are available for synthesis of copper nanoparticles. Microscopic techniques such as UV-Vis's spectroscopy, X-ray Diffractometer (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) are commonly used to characterize the nanoparticles. Some of the important physical properties like size and shape of particle, size distribution, crystallinity and morphology of nanoparticles were investigated using these characterization techniques [81]

2.5.1. UV–visible spectroscopy analysis

The synthesis of the copper nanoparticles was immediately followed by UV -Vis's spectroscopy analysis. The spectrum was recorded over a range of 200-900nm. UV-Vis spectral analysis of CuNPs from *Fortunella margarita (kumquat)* plant leave extract shows in Figure 9. The peak

observed at 679 nm, which exhibits a SPR property within 9 min. The red-colored bands indicate the presence of metallic copper, hence providing evidence for the formation of CuNPs. The peak is also intense which shows that the particles are small in size and are easily dispersed in the solvent[94].Based on other experimental work, CuNPs was synthesized from *Capparis zeylanica* leaves using 1mM copper sulphate solution. The result obtained from UV-Visible spectroscopy analysis of the sample is presented in Fig 9b. It is the most important method of analysis to detect the Surface Plasmon Resonance property of CuNPs. The CuNPs formation was confirmed from the peak at 531 nm[47].

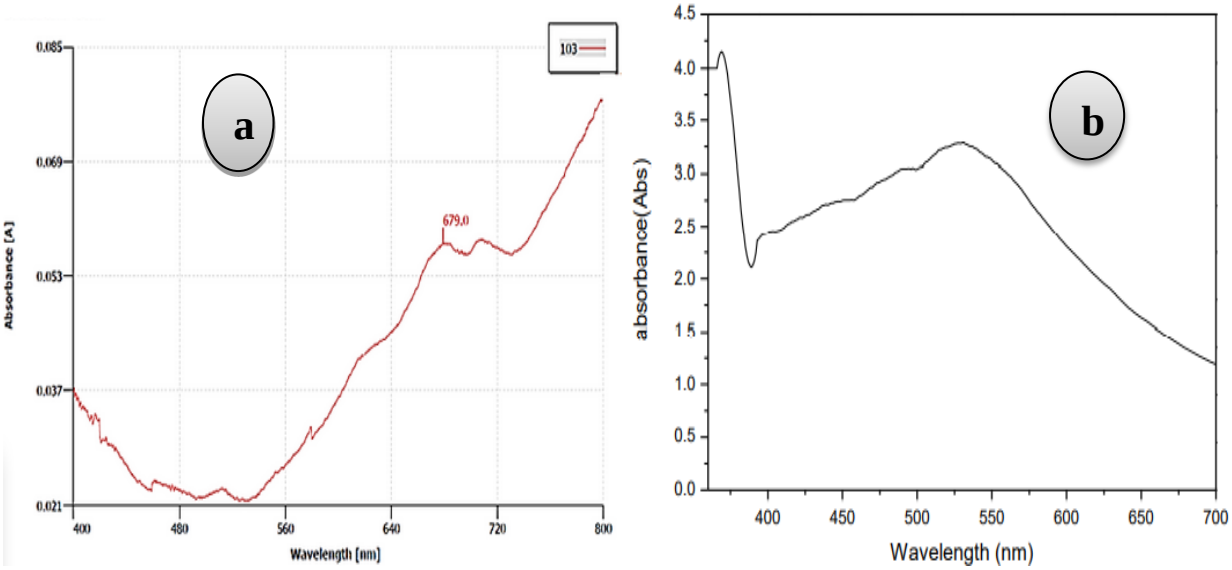


Figure 9: UV-Vis's spectra of CuNPs synthesized from the extract of (a) *Fortunella margarita* leaves and (b) *Capparis zeylanica* leaves.

2.5.2. Fourier-Transform Infrared (FT-IR) spectroscopy analysis

Fourier transform infrared spectroscopy gives data on functional groups present in compounds in the extract that interact with metal ions. The FTIR spectra of copper nanoparticles from *Ocimum sanctum* extract are shown in figure 14: The following peaks were observed in spectrum and the band at 3407·06 cm^{-1} is assigned to the O-H stretching of H-bonded alcohol and phenols. The band at 2926·89cm^{-1} attributed to O-H stretching of carboxylic acids. The band at 1628·67cm^{-1} corresponds to the NO_2 stretching of Nitro compound and presence of amide I and II, which arises due to the carbonyl stretch and -N-H- stretch vibration in the amide linkages of the proteins. The band at 1423.14 cm^{-1} is related to the C-C stretching of aromatic ring structure, scissoring and bending of alkanes. The band at 1081.91 cm^{-1} is related to the C-O stretching of alcohol, ether, esters, carboxylic acid. The peak at 601.86, 437.73, 437.91 cm^{-1} indicated

fingerprint region is complicated by the large number of different vibrations that occur here. FTIR spectrum of copper nanoparticles suggested that copper nanoparticles were surrounded by different organic molecules such as terpenoids, alcohols, ketones, aldehydes and carboxylic acid (fig. 10a)[72]. The FTIR spectra of CuNPs from *Capparis zeylanica* leaves extract also indicated in Figure 14b, absorption peaks are observed at 3427, 2918, 2091, 1602, 1394, 1258, 1059, 795 and 599 cm^{-1}. The peaks at 2918, 1602, and 1059 cm^{-1} indicate the C-H asymmetric stretching, C=O aromatic vibrations, and C=C stretching, respectively. The peak at 2091cm^{-1} confirms the carboxylate ions. The peak positions at 1394 and 1258 cm^{-1} correspond to the organic and aromatic molecule derivatives like phenolic, alkaloids, flavonoids, tannins, aldehydes, and ketones present in the plant extract the peaks at 795 and 599 cm^{-1} identified as the aromatic ring of amino acids. The peak observed at 3427 cm^{-1} may be assigned to OH or NH stretching of phenolic compound. The involvement of phytochemicals in the formation of Cu NPs is confirmed with the FTIR analysis. Hence we can conclude these phytochemicals play a significant role in synthesizing Cu NPs[95].

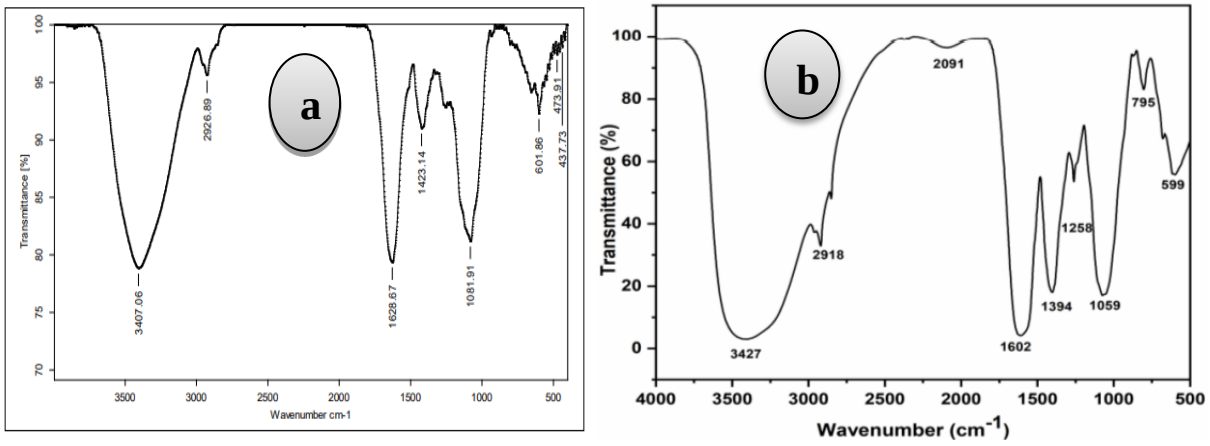


Figure 10: FTIR spectra of biosynthesized copper nanoparticles by (a) *Ocimum sanctum and (b) Capparis zeylanica* leaves extract.

2.5.3. X-ray Diffraction (XRD) analysis

The X-ray diffraction (XRD) system is used to analyses the structure of crystalline materials. The energetic X-rays can penetrate deep into the materials and provide information. If the NPs are not crystalline (without clearly defined shape), this technique can't be used to identify sample structure. It's is based on Scherer's equation that determines the size of crystallites in the form of powder. The equation is as follows:

$$D = \frac{K\lambda}{\beta \cos \theta} \qquad \text{..... (Eq.1)}$$

where D is the mean nanoparticle diameter, λ is 1.5418 Å (wavelength of the radiation source), K is the Scherer's constant or shape factor with a value of 0.9, **θ** is the Bragg angle, and β1/2 is the width of peak at half height[96, 97].

According to **Roy et al., 2016**, the XRD diffraction pattern of CuNPs from *Heliconia psittacorum*leaves extract is presented in Figure 15. The peaks observed in the pattern at 2θ values = 43.34°, 50.48°, and 74.26° corresponds to (111), (200), and (220) lattice planes of face centered cubic structure of Cu NPs, and the diffraction data were in good agreement with JCPDS cardfile no. 04-0836 see fig. 11a[98]. On the hand, X-ray diffraction pattern of CuNPs the lemon extract and CuSO4 solution is shown in Figure 7. The figure shows sharp peaks at diffraction angles 43.5°, 50.6°, and 74.0° which are equivalent to the reflections revealed by different literatures. It depicts the presence of (111), (200) and (220) planes indicating formation of face centered cubic (fcc) crystals of CuNPs which follows Joint Committee of Powder Diffraction Standards (File No.089- 2838). Various other small peaks observed at 26°, 31° and 36° may be due to formation of oxidized copper because sample was oven dried at less than 80°C. The average crystallite size was estimated to be 17.7 nm see fig.11b[99].

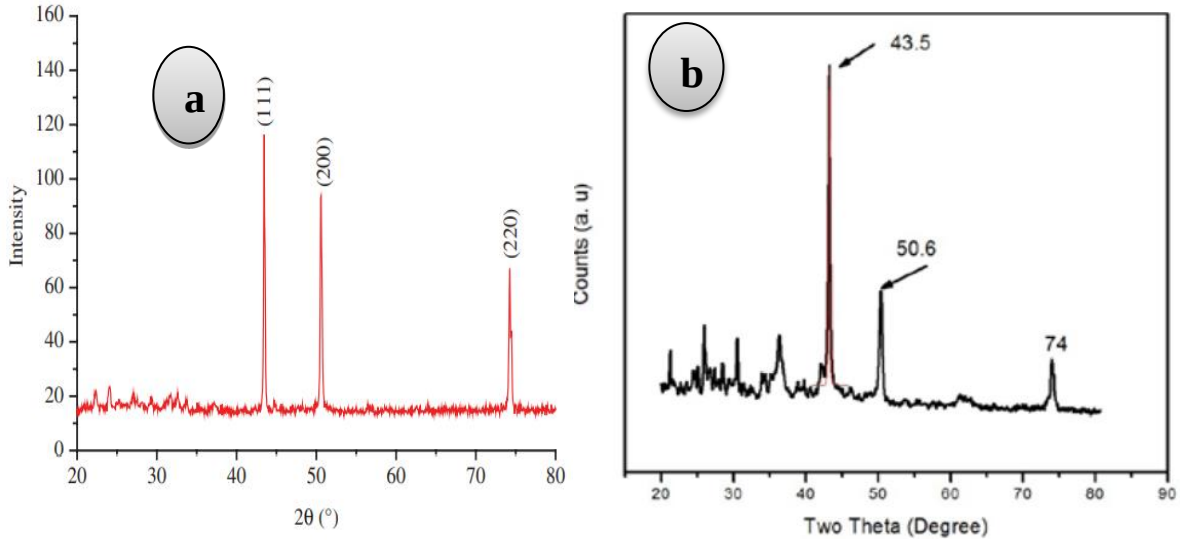


Figure 11: XRD diffraction pattern of CuNPs from (a) *Heliconia psittacorum*and (b) *lemon extract* leaves extract.

2.6. Biological applications

Copper nanoparticles are most commonly used in the emerging interdisciplinary field of Nano biotechnology and in biomedical technology. CuNPs have extensive applications in various fields due to their constant renewable surface, nontoxic and low cost of preparation[18, 60]. This review suggests that CuNPs can act as antioxidant, anticancer, antibacterial, antifungal, anti-diabetic, and anti-nociceptive and wound healing agents (fig. 12).

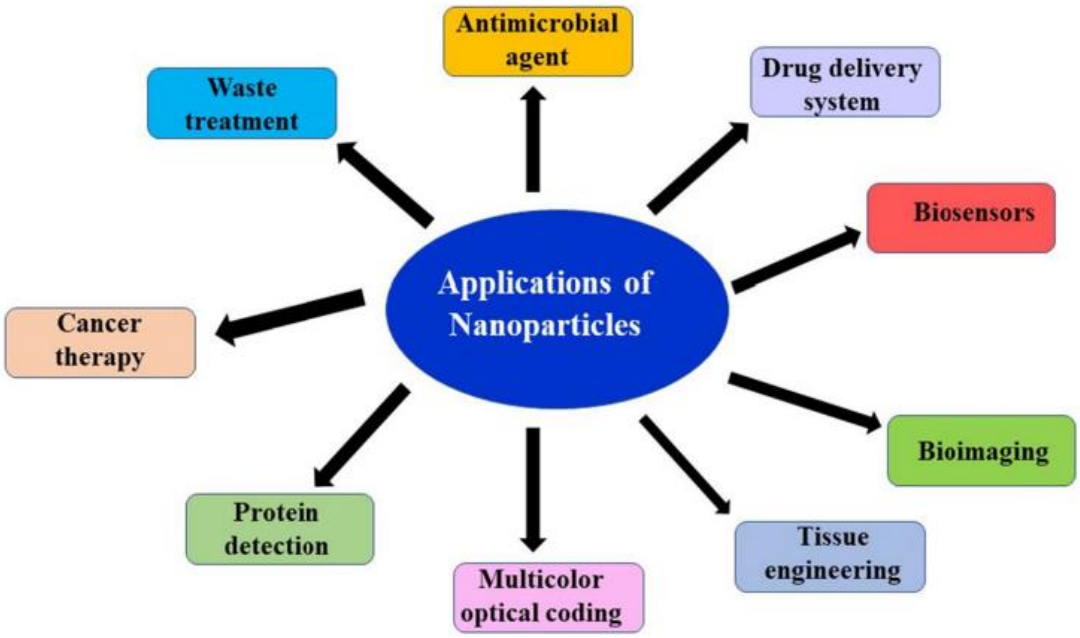


Figure 12: Applications of green synthesized nanoparticles in environmental and biomedical fields.

2.7. Antibacterial Applications of copper nanoparticles

Since ancient times among various antimicrobial agents, copper compounds have been commonly used in agriculture as herbicides, algaecides, fungicides, and pesticides as well as in animal husbandry as a disinfectant. CuNPs is known for its antimicrobial properties and has been used for years in the medical field for antimicrobial applications. Copper NPs have been found to have a wide range of antimicrobial activity against pathogens. The mode of action is in different manner such as inactivating the enzymes, generating hydrogen peroxide, membrane damage and binding with DNA molecules of the microbes thus interrupting the double stranded structure are a few to be mentioned. The antimicrobial activity of copper NPs recommended their possible application in food preservation field especially preventing microbial contamination to fresh cut,

ready to eat fruits and vegetables. Copper NPs are also used in food processing as well as packing[101].

2.7.1. Mechanism of the bactericidal effect of copper nanoparticles

The antibacterial mechanism of copper nanoparticles has been attributed to the fact that Cu^{2+} ions eluted from nanoparticles are absorbed by bacteria when the nanoparticles concentration is high enough. Copper ions are absorbed onto the bacterial cell surface, imparting damage to the cell membrane by solidifying protein structure or altering enzyme function[102, 103]. Bacterial cells are immobilized and become inactivated by the presence of copper nanoparticles in the growth medium, which results in hampering of their replication process, with subsequent cell death [104]. Reportedly, recycling redox reactions between Cu^{2+} and Cu^{1+} are possible at the surface of E. coli cells, generating hydrogen peroxide, causing damage to the cytoplasmic membrane [105]. Under physiological conditions, the intracellular enzyme activity of bacteria treated with copper nanoparticles is believed to increase, suggesting that permeability of the cell membrane also increased, with bacteria suffering injury as a result. From these results, it is believed that binding of copper ions to the bacterial cell surface plays an important role in bactericidal activity[106]. Copper has the potential to disrupt cell function in multiple ways, since several mechanisms acting simultaneously may reduce the ability of microorganisms to develop resistance against copper [107]. These investigated mechanisms provide insight into the complicated antimicrobial action of copper nanoparticles.

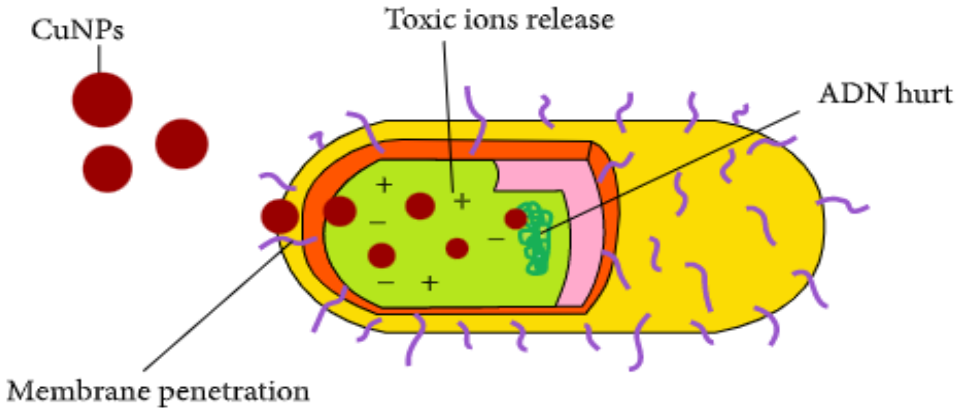


Figure 13: Possible Cu NPs action mechanism in a bacterium membrane[10].

CHAPTER THREE

MATERIALS AND METHODS

3.1. Materials

3.1.1. Chemicals and reagents

The following chemicals, reagents, plant leaf samples were used for this research work:

- Copper (II) nitrate trihydrate (Cu $(NO_3)_2.3H_2O$, 99.5%), ethanol, Distilled water, Muller agar solution, dimethyl sulfoxide (DMSO), Chloroform, Ferric chloride ($FeCl_3$, 99%), Sulfuric acid (H_2SO_4, 98%), Hydrochloric acid (HCl, 37%), Sodium hydroxide (NaOH, 98 %) and *Xanthium strumarium L*. plant leaves.

3.1.2. Apparatus and Instruments

The following equipment's and analytical instruments were used for this research work:

- Conical flasks, Volumetric flasks, Beakers, Hot-plate, Analytical balance, Magnetic stirrer, Spatula, Pipette with bulb, Mortar and pestle, Mechanical shaker, Aluminum foil, Test tubes, Oven, Centrifuge, Whatman No.1 filter paper, Filter funnel, Autoclave, Micro Pipette with tips and Refrigerator.

3.2. Methods

3.2.1. Description of the Study Area

The sample was collected from the major planting areas in Ethiopia. Wolkite is the capital town of Gurage zone district, and also 158 km from Addis Ababa on the main road of Jimma and 430 km through former southern nation's nationalities and peoples, regional state (SNNPRS) Hawass. This means that it could be reach from Addis Ababa to or via Hawass. It also Gubre sub-city is located in Estern Gurage zone district 14km western direction of Wolkite town. This area was selected to represent the area where *Xanthium strumarium L*. Plant is dominantly planted and used as traditional medicine.

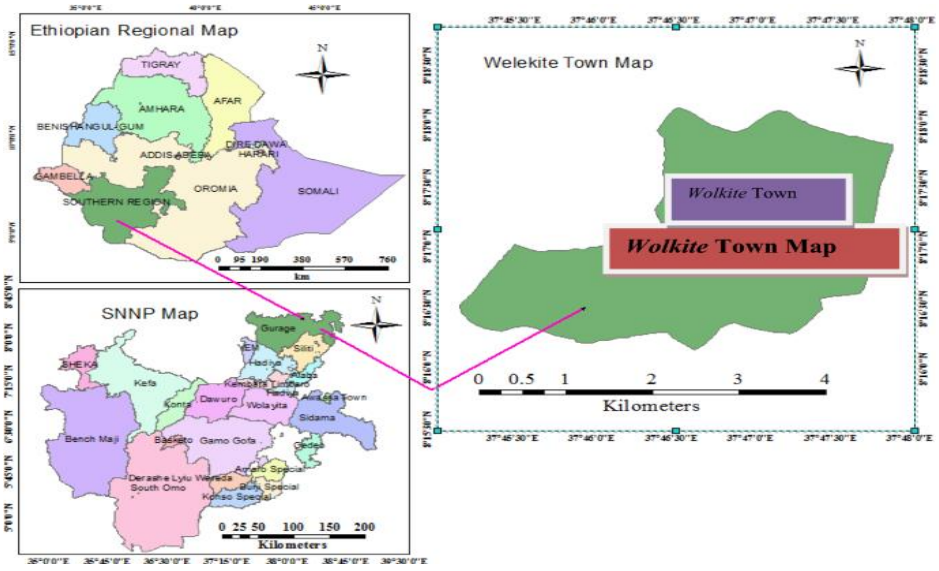


Figure 14: Map of study area.

Source: Geographical information system version 10.1.

3.2.2. Collection of *Xanthium strumarium L.* plant leaves

Xanthium strumarium L. plant leaves were collected from its natural habitat in November **4, 2024** at Wolkite, located in Eastern Gurage zone district 14km western direction of Wolkite town.

3.2.3. Preparation of *Xanthium strumarium L.* leaf extract

The preparation of leave extracts was done with slight modification of the previous method[46]. The leaves of *Xanthium strumarium L.* was surface cleaned and washed repeatedly with tap water followed by distilled water to remove dust particles and then allowed to dry under shadow for 10 days to remove moisture contents from the leaves. The dried leaves were ground using a grinding machine to get a fine powder of the sample. The extraction was carried out by taking 20 g of the powdered leaves of *Xanthium strumarium L.*in a 500 ml of conical flask containing 400 ml of distilled water. The flask was later covered with aluminum foil, to prevent the effect of light. After that, the mixture was Shaked using a mechanical shaker for 90 minutes and allowed to warm at 50° C for 1 hour on a hot-plat with magnetic stirrer, then it was allowed to cool down to room temperature overnight. The prepared solution was filtered through Whatman No.1 filter paper to get clear solution. The filtrate was stored at 4° C for future experiments[46].

3.2.4. Qualitative Photochemical analysis of *Xanthium strumarium L.* Leaves extract

The *Xanthium strumarium L.* leaves extract was tested for the presence of bioactive compounds by using following standard methods[108].

3.2.4.1. Test for phenols and tannins

1ml of *Xanthium strumarium L.* leaves extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

3.2.4.2. Test for glycosides

Liebermann's test

Xanthium strumarium L. leaves extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added. A cooler change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

3.2.4.3. Test for proteins

a) Millon's test

Xanthium strumarium L. leaves extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

3.2.4.4. Test for flavonoids

i. Alkaline reagent test

3 ml of plant leaves extract was mixed with 2ml of 2% solution of NaOH. An intense yellow color was formed which turned colorless on addition of few drops of diluted acid which indicated the presence of flavonoids.

3.2.4.5. Test for saponins

2ml of plant leaves extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

3.2.4.6. Test for steroid

5ml of plant extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. A red color produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing plant extract with 2ml of chloroform. Then 2ml of each of

concentrated H_2SO_4 and acetic acid was poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

3.2.4.7. Test for alkaloids

1 ml of HCl was added to 3 ml of each extract in a test tube. The mixture was heated for 20 minutes, cooled and filtered. 2 drops of Wagner's reagent were added to 1 ml of the filtrate and observed for reddish brown precipitate.

3.2.4.8. Test for terpenoids

Xanthium strumarium L. leaves extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids.

3.2.5. Green synthesis of copper nanoparticles

It was performed as per previous method with slight modifications[46]. A 0.02 M aqueous copper nitrate solution (Cu $(NO_3)_2.3H_2O$) was prepared and stored in brown bottles. A 100 ml of *Xanthium strumarium L.* plant leaf extract was mixed with 400 ml of 0.02 M copper nitrate solution (1: 4) slowly dropwise with constant stirring in a 500 ml of conical flask. The mixture was incubated at room temperature for 24 hrs. A color change from light blue to dark green was observed after the reaction conditions and suggesting for formation of copper nanoparticles. The reduction of Cu^{2+} was confirmed from the UV–Visible spectrum of the reaction solution. After that, the copper nanoparticles were separated out from the mixture by high-speed centrifugation at 10,000 rpm for 15 min. The obtained CuNPs was washed by distilled water and ethanol to remove any impurities. Finally, the NPs was allowed to dry and stored at room temperature for further characterizations.

3.2.6. Characterization of copper nanoparticles

For characterization of synthesized copper nanoparticles, different techniques were used. The green synthesis of CuNPs and the reduction of copper ions was analyzed for SPR by using double beam UV-vis spectrophotometer (Agilent technologies, Cary 60 UV-Vis; USA) in the wavelength range of 200–800 nm. Fourier-transform infrared (FTIR) analysis was measured by using FTIR spectrometer (Jasco FT/IR-6600typeA, Japan). FTIR spectra of the synthesized CuNPs were obtained in the range of 4,000–400 cm^{-1} by crushing a small amount of finely powdered CuNPs with KBr pellets. The FTIR analysis was utilized for determining the organic

functional groups linked to the surface of copper nanoparticles responsible for the reduction, stabilizing and capping agents. X-ray diffraction (XRD) analysis was also carried out to reveal the crystallographic nature of biosynthesized CuNPs by using advanced X-ray Diffractometer (Shimadzu Corporation (Japan); XRD-7000 X-ray Diffractometer)with Cu-Kα radiation of wavelength 1.5406 Å and scanning angle 2θ from 10° to 80°.

3.2.7. Antibacterial studies of copper nanoparticles

The antibacterial activities of biosynthesized CuNPs was performed as per the previous method with slight modification[100]. The antimicrobial activity of pathogens was established using agar well diffusion methods. The bactericidal effect of copper nanoparticles has been attributed to their high surface to volume ratio and small size which allows them to interact very closely with microbial membranes. The antimicrobial study of CuNPs was carried out using one gram-positive bacteria (*Staphylococcus aureus*) and three gram-negative pathogenic bacteria (*Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) clinically isolated pathogenic bacteria. The media used was Mueller-Hinton Agar (MHA) and it was prepared according to the manufacturer's instruction, where 35g of powder media was mixed with one litter of distilled water and enclosed in a container and autoclaved at 121 °C for 15 minutes. The media were later dispensed into sterile Petri dishes. After the media was solidified, 100 µl of the working stock culture was spread with sterile cotton swab and wells were made in each petri dish by a stainless-steel cork borer. Then different concentration of CuNPs and the plant leave extract (50, 100, 150 and 200 µl) was prepared separately from 20 mg/ml stock solution. Wells were filled with the prepared solutions of CuNPs, plant leave extract solutions, DMSO (negative control), ciprofloxacin (positive control) and kept in refrigerator for 15 minutes until it diffuses. The petri dishes were incubated at 37 °C for 24 h. Finally, zone of inhibition of green synthesized CuNPs and the plant leaf extract with different concentrations against the pathogenic bacteria was measured in millimeter.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Phytochemical Analysis of the Plant Leaf Extracts

Qualitative phytochemical screening analysis was done on *Xanthium strumarium L.* leaf extract to determine the presence of some phytochemicals in the leaves of this medicinal plant. The presence and/or absence of useful bioactive compounds such as phenols, tannins, glycoside, proteins, flavonoids, saponins, steroid, alkaloids and terpenoids in *Xanthium strumarium L.* leaf extracts were revealed by the confirmatory test, involving color changes, precipitate formation and other confirmations. The phytochemical characteristics of the leaf extract of *Xanthium strumarium L.* investigated are summarized in table-1 below. The results revealed that the presence of bioactive compounds like phenols, tannins, flavonoids, Alkaloids, saponins, proteins and glycoside in the leaves of *Xanthium strumarium L.*, while terpenoids and steroids were absent in this plant leaf[108]

Table 2: Results on Phytochemical screening of *Xanthium strumarium L*. Plant leaf extract[108].

S/No.	Bioactive phytochemicals	Test/Reagents	Result
1	Phenols/tannins	Ferric chloride test	+
2	Proteins	Millon's reagent test	+
3	Flavonoids	Alkaline reagent test	+
4	Saponins	Frothing test	+
5	steroid	Salkowski test	-
6	alkaloids	Mayer's And Wagner's reagents	+
7	Terpenoids	Salkowski test	-
8	Glycoside	Liebermann's test	+

Note: + = Present and − = Absent

4.2. Color Change Observation on the Synthesis of CuNPs

The copper nanoparticles (CuNPs) were successfully synthesized using 1:4 volume ratio of *Xanthium strumarium L.* plant leaf extract to copper nitrate solution ($Cu(NO_3)_2.3H_2O$) as a reducing and capping agent, copper nitrate as a precursor. It was observed that solution of copper nitrate (light blue) turned dark-green on addition of leaves extract, this indicating the reduction

of Cu^{2+} to metallic copper of zerovalent (Cu^0). This change in color of the reaction mixture was taken as a primary evidence for the formation of copper nanoparticles[46]. The synthesized CuNPs (Figure 15) were later subjected to various characterization methods.

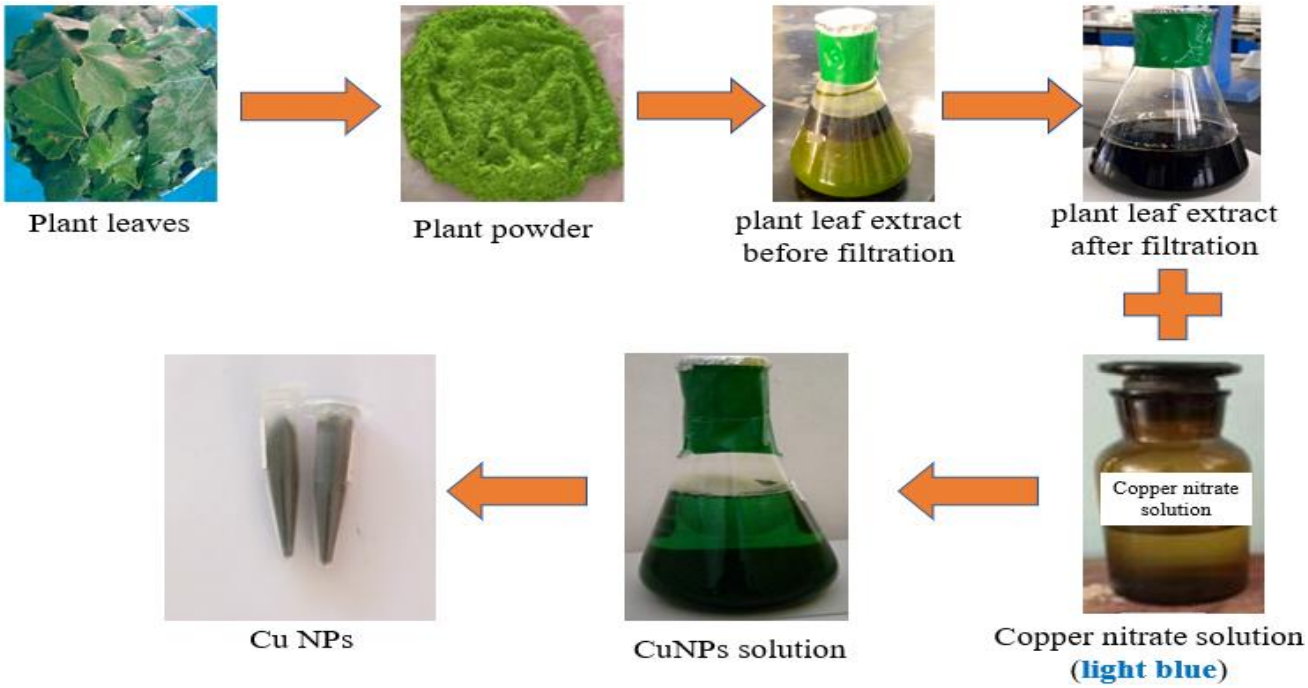


Figure 15: Green synthesis of CuNPs from Xanthium strumarium L. plant leave extract after incubation at room temperature for 24 hrs. And colour change observation.

4.3. Characterization of Synthesized copper nanoparticles

4.3.1. UV-Visible Spectroscopy Analysis

The process of the bio reduction of copper ions to CuNPs was applied in this spectroscopy study by UV-vis spectroscopy. UV-vis spectroscopy might be used to detect the size and shape-controlled NPs in aqueous suspensions. The formation of CuNPs was monitored using UV–Vis's by measuring the absorbance in the scanning range of 200– 800 nm. After mixing the solution of copper nitrate ($Cu(NO_3)_2.3H_2O$) with aqueous leaf extract, the reaction mixture changed its color rapidly. The synthesized CuNPs were dark green in aqueous solution due to excitation of electrons and changes in electronic energy levels, reflecting the reduction of Cu^{2+} in to metallic copper of zerovalent (Cu^0)[109].The UV-visible absorbance spectrum recorded for CuNPs exhibited λ_{max} of 672 nm. The UV-visible absorbance spectrum recorded for *Xanthium strumarium L*. plant leave extracts exhibited λ_{max} of 685 nm due to the polyphenolic compounds present in the *Xanthium strumarium L.* leaf extract[110, 111] .Therefore, this spectrum strongly

supports the presence of phenolic as indicated in literatures[111]. The studies of this result correlates to previously reported by **Amjad et al., (2021)**[94]. Various reports have established that the SPR band of copper nanoparticles were observed between 620 to 710 nm [112]. The peak value was found to be gradually decreased with increase in particle size.

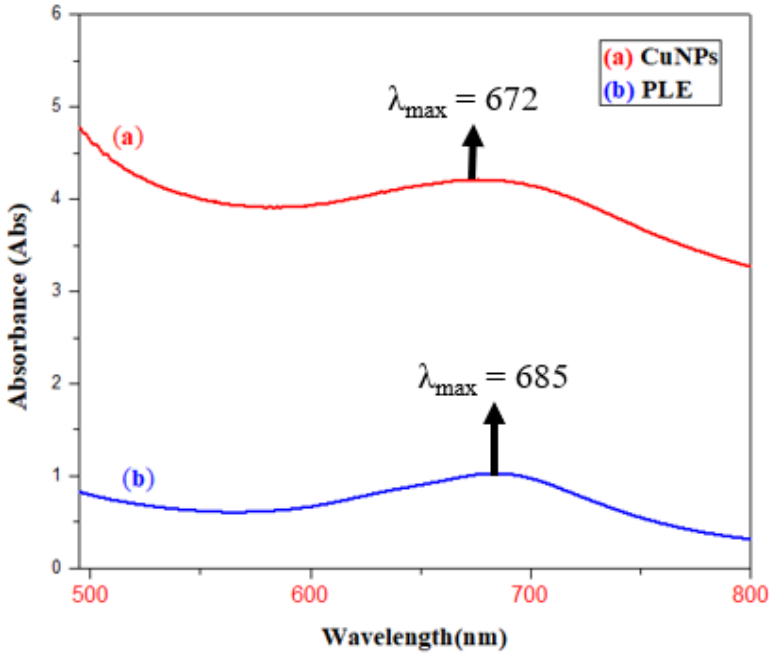


Figure 16: UV-visible spectra of biosynthesized CuNPs using *Xanthium strumarium L.* plant leave extract with $Cu(NO_3)_2.3H_2O$ and *Xanthium strumarium L.* plant leave extracts.

4.3.2. Fourier-Transform Infrared (FTIR) Analysis

The analysis of FTIR spectra gives an idea about biomolecules bearing different functional groups which are present in underlying system of *Xanthium strumarium L.* leaf extract containing copper nanoparticles. To identify the capped biomolecules with CuNPs, The FTIR analysis was used to identify the capping, reducing and stabilizing capacity of the leaf extract. FTIR absorption spectra of green synthesized CuNPs and *Xanthium strumarium L.* leaf extract was studied in the range of 400-4000 cm^{-1}.The FTIR analysis was done for both *Xanthium strumarium L.* plant leaf extract and green synthesized CuNPs. The major spectra show bands at 3432, 2929, 2117, 1636, 1412, 1276, 1053 and 666 cm^{-1}.A broad band at 3432 cm^{-1} is due to the O-H group of the overlapping of the stretching vibration and attributed for water and phenolic group and N-H stretching vibration of amines group present in *Xanthium strumarium L.* leaf extract. The intense peaks shown at 2929 cm^{-1} corresponding to asymmetric C-H stretching

modes[113]. The peak value at 2117 cm^{-1} is shows the presence of C≡C stretching of alkyne group[58]. The stretching mode of the carbonyl group (C=O) was observed at 1636 cm^{-1}, suggesting the presence of carboxylic, aldehydes, esters, or ketones-containing compounds arises from flavonoids and tannins. The bands at 1412 cm^{-1} were assigned to show N-H stretch vibration in the amide linkage. The intense band at 1053 cm^{-1} could be assigned to the C-N stretching vibrations of aliphatic amines and also it shows the presence of C-O stretching of alcohols. Bands at 1276 cm^{-1} are attributed to the C-O group of phenolic compounds. The peak value at 666 cm^{-1} shows the presence of an alkyne C-H bend[114]. Whereas the green synthesized CuNPs from *Xanthium strumarium L*. leave showed the major and strongest vibration modes at around 591, 1044, 1263, 1387, 1620, 2116, 2923, and 3419 cm^{-1} as located. A band peak around 3419 cm^{-1} corresponds to -OH stretching of the phenolic group and N-H stretching vibrations of amino groups present in the CuNPs. The peak at 2923 cm^{-1} corresponds to C-H stretching modes. The peak at 1620 cm^{-1} is indicating to C=O stretching. The peak value at 2116 cm^{-1} shows the presence of C≡C stretching of the alkyne group. An intense peak around 1387 cm^{-1} shows N-H stretch vibration in the amide linkage and bands at 1263 cm^{-1} is due to the C-O group of phenolic compounds. The intense band at 1044 cm^{-1} could be assigned to the C-N stretching vibrations of aliphatic amines also it shows the presence of C-O stretching of alcohols. An intense peak around 591 cm^{-1} corresponds to bending modes of vibrations of the C–H bond. The presence of these functional groups indicated the possible involvement of reductive groups on the surfaces of the CuNPs[115]. From the FTIR study, it may be concluded that the amine groups along with the aromatic compounds (like phenol, etc.) probably capped and stabilized the copper nanoparticles during production in the reacting medium[116]. Interestingly, the band showed a shift to a lower frequency, which suggests a contribution to the reduction and capping process of the biosynthesized CuNPs. As evidenced by FTIR spectra, selection of *Xanthium strumarium L.* plant was an excellent option for the reduction of copper ions into CuNPs. The studies of this result correlates to previously reported by **Saddik et al., (2020)**[117].

Table 3: Band shift in FTIR spectrum of *Xanthium strumarium L.* plant leaf extract and Green synthesized CuNPs.

Bands	PLE (cm^{-1})	CuNPs (cm^{-1})	Functional groups	
1.	3432	3419	-OH stretching of phenolic group and N-H stretching vibrations of amino groups	The band showed a shift to a lower frequency (PLE to CuNPs), which suggests a contribution to the reduction and capping process of the biosynthesized CuNPs.
2.	2929	2923	C-H stretching modes	
3.	2117	2116	C≡C stretching of alkyne group	
4.	1636	1620	C=O stretching	
5.	1412	1387	N-H stretch vibration in the amide linkage	
6.	1276	1263	C-O group of phenolic compounds	
7.	1053	1044	C-O stretching of alcohols C-N stretching vibrations of aliphatic amines	
8.	666	591	bending modes of vibrations of the C–H bond	

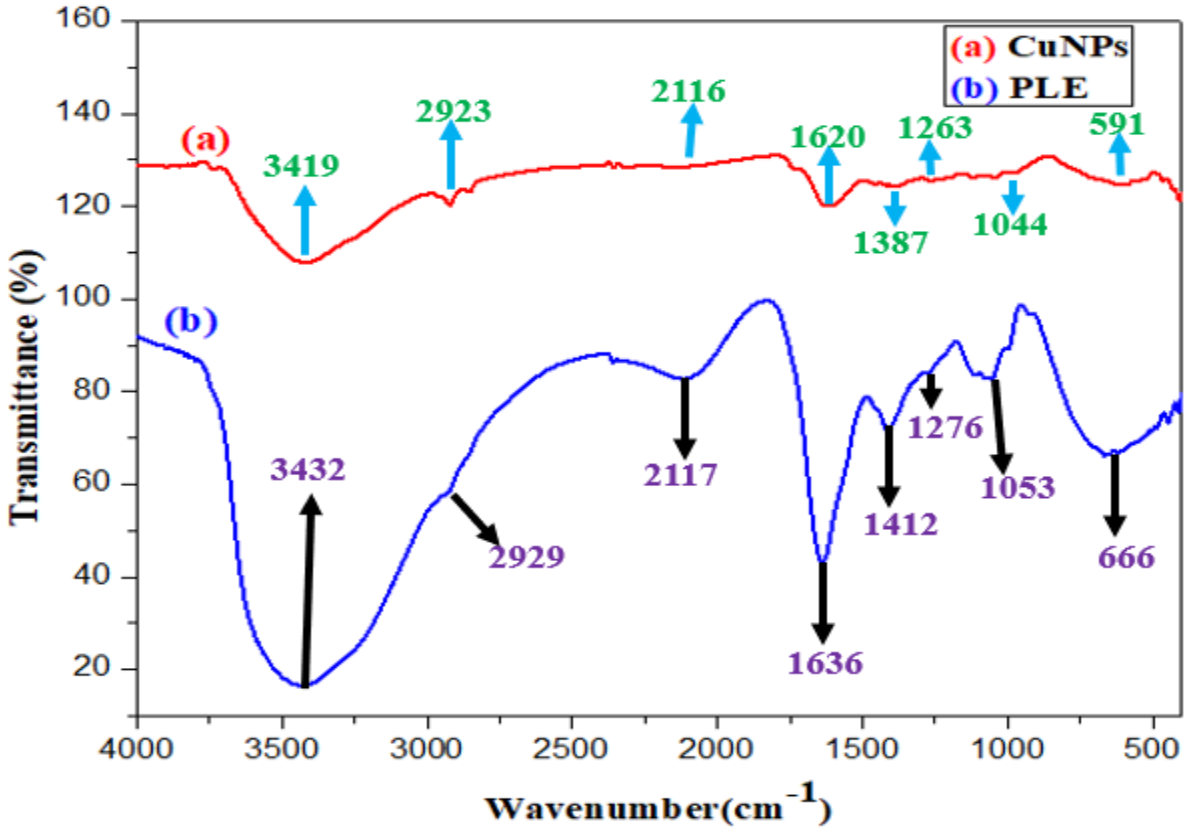


Figure 17: Comparative FT-IR Spectrum of *Xanthium strumarium L.* plant leave extract and CuNPs.

4.3.3. X-ray Diffraction (XRD) Pattern Analysis

The crystalline characteristic of synthesized CuNPs powder was analyzed by XRD. The XRD pattern was recorded in the range from 10° to 80°. The XRD pattern of green synthesized copper nanoparticles (CuNPs) from *Xanthium strumarium L.* plant leaves extract was confirmed by the characteristic peaks observed in the XRD pattern as clearly described in Figure 24. In this study of the XRD pattern, three prominent diffraction peaks were observed at 2θ = 43.24°, 50.46° and 74.12° which correspond to (111), (200) and (220), Bragg's reflections of the face-centered cubic (fcc) structure of metallic copper, respectively as correlates to the study reported by **Gondwal & Joshi nee Pant, (2018), Amjad, et al. (2021),** and **Roy et al., (2016)** [94, 98, 118]. Other small peaks observed at 22.34°, 37.66° and 44.64° may be due to the formation of any impurity. These planes confirmed the crystalline nature of the green synthesized CuNPs. The highest peak intensity of the (111) plane with narrow FWHM illustrates the good crystalline nature of synthesized CuNPs as observed from the XRD images. The resulting peaks and their corresponding Bragg's reflections strongly agree with the Joint Committee on Powder

Diffraction Standards (JCPDS, file no. 96–500-0217). The average crystallite sizes of the particles were calculated by using Debye-Scherer's equation.

$$D = \frac{K\lambda}{\beta \cos \theta}$$

Where, **D** is the crystalline size of NPs, **K** is the Scherrer constant with a value from 0.9 to 1. **λ** is the wavelength of the X-ray source (0.15406 nm) used in XRD, **β** is the full width at half maximum (FWHM) of the diffraction peak and **θ** is the Bragg's angle in degrees. According to Debye Scherrer equation the average crystalline size (D) of the synthesized copper nanoparticles is found to be 26.88 nm.

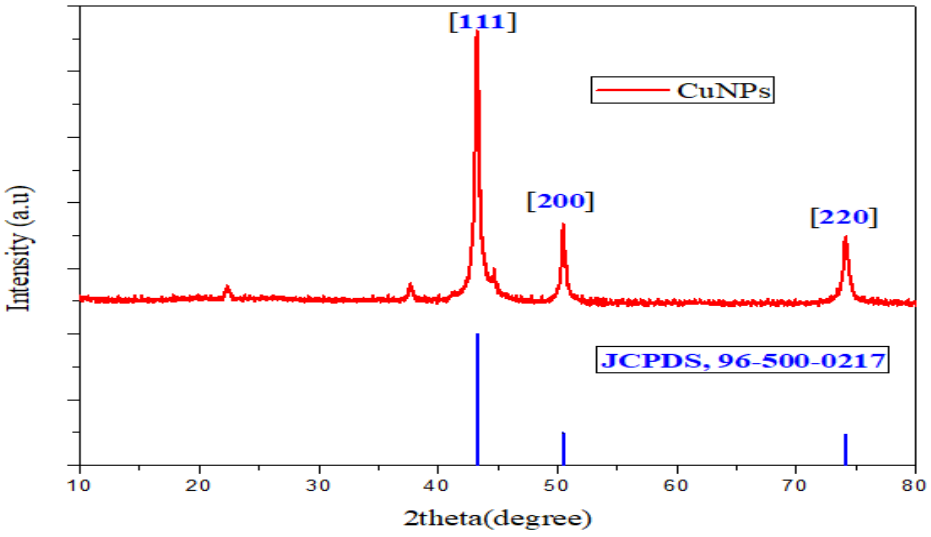


Figure 18: XRD pattern of green synthesized CuNPs from *Xanthium strumarium L*. plant leaf extract.

4.4. Anti-bacterial Studies of Green Synthesized CuNPs

Green synthesized copper nanoparticles from *Xanthium strumarium L*. leaf extract and the leaf extract alone as a comparison were studied for their antibacterial activity against clinically isolated one Gram-positive bacteria (*Staphylococcus aureus*) and three Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and by following standard agar well diffusion method, and ZOI was recorded.

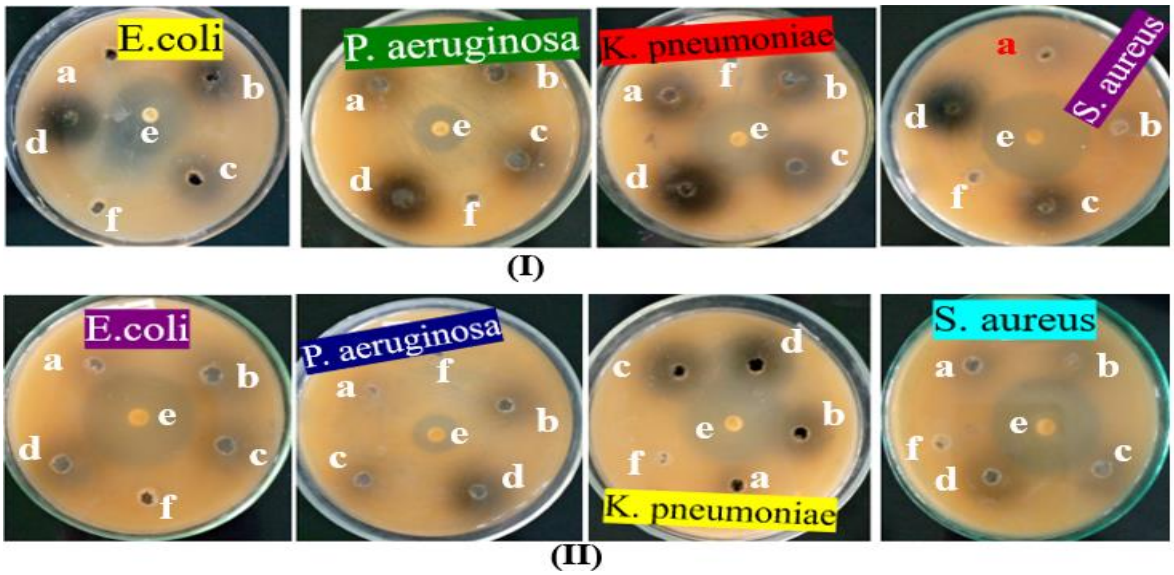


Figure 19: (I) Antibacterial activity of green synthesized CuNPs and (II) Xanthium strumarium L. leaf extract alone; (a = 50 µl, b = 100 µl, c = 150 µl, d = 200 µl, e = ciprofloxacin (+ve control), and f = DMSO (-ve control)).

Table 4: Anti-bacterial activity of Xanthium strumarium L. plant leaf extract and synthesized CuNPs against clinically isolated human pathogenic bacteria.

S.No.	Name of organisms		Zone of inhibition(mm)				Ciprofloxacin (+ve-control)	DMSO (-ve-control)
			50µl	100µl	150µl	200µl		
1	Staphylococcus aureus	PLE	5±1	5±1	10±0	13±1	33	0
		CuNPs	8±1	11±0	16±1	20±1	33	0
2	Escherichia coli	PLE	3±1	8±1	11±1	15±1	31	0
		CuNPs	5±1	14±1	19±1	22±1	28	0
3	Pseudomonas aeruginosa	PLE	4±1	10±1	10±1	14±1	20	0
		CuNPs	11±1	15±1	19±1	21±1	23	0
4	Klebsiella pneumoniae	PLE	6±1	11±1	13±1	16±1	30	0
		CuNPs	16±1	18±1	21±1	24±1	30	0

PLE; Plant leaf extract

Antibacterial activity was shown by an inhibition zone which was characterized by a black zone between the wells (containing samples) and a certain distance. Formation of inhibition zones around the wells showed bacterial sensitivity to antibacterial and antibiotic drugs (which are used as positive controls). The positive control used in the well was a ciprofloxacin and functioned as

a control of the test solution by comparing the diameter of the inhibition zone formed. On the contrary, DMSO as negative control was used to determine the effect of solvents in the test solution on the growth bacteria. The zone of inhibition on all bacterial strains was measured after 24 h of incubation at 37 °C.

As clearly described by Figure 19 and Table 4, the synthesized copper nanoparticles and *Xanthium strumarium L*. plant leaf extract showed anti-bacterial activity against all bacterial strains tested. However, it was more effective against gram-negative *Klebsiella pneumoniae* bacteria in both the synthesized nanoparticles and the plant crude extract as the concentration increased the antibacterial activity also increased. The antibacterial activity of green synthesized copper nanoparticles against gram-negative (*Klebsiella pneumoniae*) bacteria was maximum with an inhibition zone of 24±1 mm concerning the highest concentration (200 µl) (Table 4). Whereas the anti-bacterial activity of *Xanthium strumarium L*. plant leaf extract alone against gram-negative *Klebsiella pneumoniae* bacteria in terms of maximum ZOI was recorded as 16±1 mm with the highest concentration of 200 µl (Table 4). Based on this result, we conclude that the green synthesized CuNPs had the highest antibacterial activity against gram-negative bacteria strains especially *Klebsiella pneumoniae* as compared to *Xanthium strumarium L*. Plant leaf extract. From the antibacterial test, ZOI of CuNPs of different concentrations is found greater for Gram-negative bacteria than Gram-positive bacteria due to differences in the thickness of peptidoglycan layers[100, 119]

The higher inhibition zone (ZOI)of Gram-negative bacteria by synthesis of CuNPs could be partially explained by the facilitated influx of smaller-sized nanoparticles into the cell wall of Gram-negative bacteria that consists of a single outer membrane layer and a single peptidoglycan layer as compared to the cell wall of Gram-positive bacteria with several peptidoglycan layers[120, 121]. In addition, CuNPs have been speculated to attach to Gram-negative bacteria cell walls due to electrostatic interaction, or the copper ions facilitate rapid DNA degradation and reduction of bacterial respiration[122]. In some Gram-negative strains, copper ions alter the conformation of the associated reductases and electron transferase, leading to inhibition of membrane cytochrome [123].

Table 5 Comparison of CuNPs from anti-bacterial activities in different plant leaf extract

No,	Plant extract used	CuNP (Size of particle)	Shape of CuNP	Name of organisms	Zone of inhibition(mm)	+ve-control	-ve-control	References
1	Capparis zeylanica plant leave	50 –100	Cubical	E-coli	11±1mm	-	-	[47]
				Pseudomonas aeruginosa	10±1mm			
				Staphylococcus aureus	10±1mm			
2	Datura plant Extract	15 –20	Spherical	E-coli	8mm	Chloramphinicol	-	[48]
				B.subtilis	8mm			
				S.aureus	5mm			
				B.megaterium	7mm			
3	*Albiziale bbeck*	100	Spherical	S. aureus	17mm	Chloramphenicol.	-	[49]
				B.subtilis	21mm			
				E. coli	19mm			
				S.bacillus	18mm			
4	Malvas ylvestris leaf extract	14 nm	spherical	Shigella and	15 mm	-	-	[52]
				Listeria bacteria	18 mm			
5	*NeriumOleander*	-	-	Escherichia coli,	(10mm).	Gentamycin		[64]
				Staphylococcus aureus	(21mm)			
				Klebsiella pneumoniae	18mm			
				Salmonella typhi	20mm			
				Bacillus subtilis.	15mm			

	Peel extract of Punica granatum	5 to 20 nm.	Spherical	P. aeruginosa	18.67 ± 1.53mm	Strepto mycin	-	[73]
				S. enterica	9.67 ± 1.53mm			
				E. aerogenes	9 ± 1mm			
				M. luteus	0.33 ± 1.5mm			
6.	Ocimum sanctum leaf extract.	25 nm	rod	Rhizoctonia solani	10±0.81mm	-	-	[72]
				Xanthomonas axonopodis pv. Citri	13.5±1.29 mm			
				Xanthomonas axonopodis pv. Punicae	17.25±0.95mm			
7	X.Strumarium L. plant leaf extract	26.88	-	Staphylococcus aureus	20±1mm	Ciproflo xacin	DMSO	My work
				Escherichia coli	22±1mm			
				Pseudomonas aeruginosa	21±1mm			
				Klebsiella pneumoniae	24±1			

Comparing the anti-bacterial activity of various bacteria with published sister journals is an interesting comparison from the above table. Overall the performance of copper nanoparticle from xanthium strumarium leaf extract in anti-bacterial activity is competitive when compared to the previously reported copper nanoparticle, but without considering concentration, reaction temperature, bacterial type even positive and negative control full and direct comparison of this nanoparticle is quite difficult. This comparison gives us the confidence that my finding have higher anti-bacterial activity both gram-positive and gram-negative bacteria

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1.Conclusion

From the above results we can able to conclude that, *Xanthium strumarium L*. plant leaf extract can synthesize copper nanoparticles in an easy, less toxic, eco-friendly and cost-effective manner. In this study very less amount of chemicals where used for the synthesis of copper nanoparticles and hence it is a green technology. The green synthesized CuNPs were characterized by different spectroscopic techniques. The functional group present in the leaf extract was confirmed by FTIR analysis. The bioactive compounds like phenols, tannins, flavonoids, Alkaloids, saponins, proteins and glycoside are presence in the leaves of *Xanthium strumarium L.* These functional groups were mainly responsible for the reduction of copper metal ions (Cu^{2+}) into CuNPs. Surface Plasmon resonance with an intense peak of CuNPs and *Xanthium strumarium L.* plant leave extract was observed at 672 and 685 nm by UV-Vis spectrophotometric measurements respectively. The crystalline structure with FCC geometry and three prominent peaks of the green synthesized CuNPs was determined by XRD analysis and an average crystal size 26.88 nm. The green synthesized CuNPs and plant leave extract exhibited excellent antibacterial activity against both Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). But, the synthesized CuNPs showed strong inhibition in gram-negative bacteria compared to the same tested pathogenic bacteria strain of the plant leaf extract. The antibacterial activity was also tested for different concentrations of the Cu NPs and found increased activity with the increase of concentration.

5.2.Recommendation

The synthesis of copper nanoparticles from *Xanthium strumarium L*. plant parts by green route is simple, low cost and has several advantages such as reduce toxicity, good yield, eco-friendly, and the synthesized nanoparticles have many applications in different fields of study. Therefore, I suggest the following recommendations for further investigations. To get the morphological features and exact particle size of the green synthesized CuNPs, scanning electron microscopy (SEM) analysis and the shape and size of the synthesized CuNPs, Transmission Electron Microscopy (TEM) analysis should be performed. The synthesized copper nanoparticles applied on other bacterial strains which cannot be included in this study.

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7. APPENDICS

Figure 20: The image was captured from the study area during sample collection.

Figure 21: Plant leaf powder mass measurement using analytical balance during experimental work and washing by using distil water.

Figure 22: Filtration (Filtering the macerated aqueous PLE to get pure and clear PLE)

Figure 23: crushing and drying (during synthesis of CuNPs from *Xanthium strumarium L*. plant extract).