University of Kerbala

College of Science

Department of Chemistry

Green Synthesis of Copper Nanoparticles Using *Myrtus communis* Leaves Extract: Characterization and Applications

A Thesis

Submitted to the council of the college of science / university of Kerbala as partial fulfillment of the requirements for the Degree of Master in Chemistry Science

By

Ayat Kareem Daylee

Supervised by

Prof. Dr. Narjis Hadi Al-Saadi Assist. Prof. Dr. Mohammed A. Kadhem

2022 A.D 1443 A.H

Certification of Supervisor

We certify that this thesis "**Green Synthesis of Copper Nanoparticles Using *Myrtus communis* Leaves Extract: Characterization and Applications**" was prepared under our supervision at the Department of Chemistry, College of Science, University of Kerbela, as partial fulfillment of the requirements for the degree of Master of Chemistry.

Signature:

Name: **Dr. Narjis Hadi Al-Saadi**

Title: Professor

Address: University of Kerbala /College of Science / Department of Chemistry

Date: ٤ / ٧ / 2022

Signature

Name: **Dr. Mohammed A. Kadhem**

Title: Assist. Prof.

Address: University of Kerbala /College of Science / Department of Physics

Date: ٤ / ٧ / 2022

Report of the Head of Chemistry Department

According to the recommendations presented by the Supervisor and the Postgraduate Studies Director, I forward this thesis " **Green Synthesis of Copper Nanoparticles Using *Myrtus communis* Leaves Extract: Characterization and Applications** " for examination.

Signature: 

Name: **Prof. Dr. Luma Majeed Ahmed**

Head of Chemistry Department

Address: University of Kerbala /College of Science / Department of Chemistry.

Date: 4 / 7/2022

Examination Committee Certification

We, the examining committee, certify that we have read this thesis "**Green Synthesis of Copper Nanoparticles using *Myrtus communis* Leaves Extract: Characterization and Applications**" and examined the student (Ayat Kareem Daylee) in its contents and that in our opinion; it is adequate as a thesis for the degree of Master of Science in Chemistry.

Signature:
Name: **Dr. Nazar Ahmed Naji**
Title: Professor
Address: University of Tikrit, College of Science, Department of Chemistry.

Date: 26 / 5 / 2022

(Chairman)

Signature:
Name: **Dr. Thaer Mahdi Madlool**
Title: Assist. Prof.
Address: University of Kerbala, College of Science, Department of Chemistry.

Date: 26 / 5 / 2022

Signature:
Name: **Dr. Luma Majeed Ahmed**
Title: Professor
Address: University of Kerbala, College of Science, Department of Chemistry.

Date: 26 / 5 / 2022

(Member)

Signature:
Name: **Dr. Narjis Hadi Al-Saadi**
Title: Professor
Address: University of Kerbala, College of Science, Department of Chemistry.

(Member)

Signature:
Name: **Dr. Mohammed A. Kadhem**
Title: Assist. Prof.
Address: University of Kerbala, College of Science, Department of Physics.

Date: 26 / 5 / 2022 **(Member & Supervisor)**

Approved by the Council of the College of Science

Signature:

Name: **Dr. Jasem Hanoon Hashim Al-Awadi**

Title: Assistant Professor

Address: Dean of College of Science, University of Kerbala.

Date: 5 / 7 / 2022

Dedication

I wish to dedicate this thesis: -

To my late father. He taught me to persevere and prepared me to face challenges with faith and humility. He was a constant source for inspiration in my life. Although he is not here to give me strength and support, I always feel his presence that used to urge me to strive to achieve my goals in life.

To my mother who always had confidence in me and offered me encouragement and support in all my endeavors.

To the one who supported me and stood by me and the secret of my success in every step and bearing the difficulties of my studies…. My dear husband.

To the shining stars in the sky of our life …. My dear brothers and my beloved sister. To all of you I dedicated my humble efforts.

Acknowledgements

Praise be to Allah, the Lord of the worlds, and the prayer and peace upon the messenger, mercy to the worlds, our prophet Mohammad, and to his household. In the beginning, I would like to thank the dean of the College of Science, University of Kerbala. I, also want to thank the head of the department of Chemistry (Dr. Luma Majeed Ahmed), and the staff in the Department of Chemistry for their help.

Words cannot express my sincere gratitude and appreciation to the supervisor Professor Dr. Narjis Hadi Al-Saadi and Dr. Mohammed A. Kadhem for their assistance, great interest, kindness, and supportive advice in this work. I am very grateful to her for her continuous encouragement and support during the completion of the work. Calling on God Almighty to give her long health and more scientific prosperity.

To my angel in life, to the meaning of love, compassion, and dedication to the smile of life. To the secret of existence and to those whose pray was the secret of my success (My dear parents, my husband).

I would like to thank those who have great credit for encouraging me and their presence gave me strength. My thanks go to all the professors and staff of the Department of Chemistry.

I am grateful to those who were friendly and distinguished by loyalty. Also special thanks are due to Mr. Hussein Mubarak and Dr. Nibal Muteer for their assistance.

Summary

The biological synthesis of nanoparticles is being carried out by different organisms such as plants, bacteria, fungi, seaweeds, and microalgae. Plants extracts are considered one of the safest methods in green chemistry for synthesizing nanoparticles.

This study was designed to synthesize copper nanoparticles from safety substance (plant) and evaluate their biochemical and biological activity.

Different techniques were used to characterize copper nanoparticles. Ultraviolet-Visible absorption spectroscopy (UV-Vis) was used to examine the Surface Plasmon Resonance (SPR) of the nanoparticles in the range from 300 to 700 nm. The influential functional groups that can bio-reduce the copper ion Cu^{2+} were identified using Fourier Transform Infrared (FT-IR) spectroscopy. The crystal structure of copper nanoparticles was determined using X-ray diffraction (XRD), as evidenced by the peaks at 2θ values of 43.35, 50.50, and 74.21°. In transmission electron microscopy (TEM), the particles have spherical morphology with an average diameter of 35–75 nm, while scanning electron microscopy (SEM) reveals the sphere-like shape of Copper nanoparticles. The atomic force microscopy (AFM) analysis showed the size and the surface properties of biosynthesized nanoparticles and revealed an average size of 55 nm.

synthesized Copper nanoparticles in this study were examined as antioxidants and catalysis. The results showed that Copper nanoparrticles had superior radical scavenging activity when compared to an extract from *Myrtus communis* leaves. It is common to use 4-nitrophenol (4-NP) as a model reaction to evaluate synthesized nanomaterials' catalytic properties, results show that CuNPs have better catalytic activity than extract at higher concentrations.

In this study, the efficacy of both CuNPs and extract against bacteria and their anti-inflammatory activity were tested. Antibacterial activity of the (CuNPs) and *M. communis* extract was evaluated against Gram-negative bacteria *(Klebsiella pneumonia and Pseudomonas aeruginosa)* and Gram-positive bacteria *(Staphylococcus aureus and Lactobacillus salivarins)*. *In vitro*, the anti-inflammatory activity was evaluated for both extract and CuNPs using (albumin denaturation assay, membrane stabilization assay, and proteinase inhibitory activity) at different concentrations. The results of CuNPs exhibited a strong anti-inflammatory activity compared with standard drug (Aspirin).

In conclusion, *M. communis* is considered a good source for reducing copper salt into copper nanoparticles and the last have antioxidant and anti-inflammatory activity, besides they can be used as a catalyst.

List of Contents

Subject No.	Subject	page
	Summary	I
	List of contents	III
	List of Tables	VII
	List of Figures	VIII
	List of Abbreviations	XI
	Chapter One: Introduction and Literature Review	
1-1	Introduction	1
1-2	Literature Review	3
1-2-1	*Myrtus communis Lin.*	3
1-2-2	The Constituents of Plants	4
1-2-3	Biological Activity of *Myrtus communis*	7
1-3	Synthesis of Nanoparticles (NPs)	9
1-4	Biosynthesis of Nanoparticle	11
1-4-1	Biosynthesis of NPs from plants	12
1-5	Biosynthesis of Copper Nanoparticles	14
1-5-1	Optimized Conditions for Biosynthesis of CuNPs	15
1-6	Biological Applications of Copper Nanoparticles	16
1-6-1	Antioxidant Activity	17
1-6-2	Catalytic Activity	20
1-6-3	Anti-bacterial Activity	22
1-6-4	Anti-inflammatory Activity	23
1-6-5	Hemolysis	24
1-7	The aims of study	26

	Chapter Two: Materials and Methods	
2-1	Materials	27
2-1-1	Apparatuses	27
2-1-2	Chemicals	28
2-2	Methods	30
2-2-1	Summary of Experimental part	30
2-2-2	Collection of plant	31
2-2-3	Qualitative phytochemical analysis	31
2-2-4	GC-mass spectroscopy	33
2-2-5	Preparation of *Myrtus communis* Leaves Extract	34
2-2-6	Synthesis of Copper Nanoparticles (CuNPs)	34
2-2-7	Effect of Heating Time of Extract	34
2-2-8	The Influence of the Extract Volume	34
2-2-9	Effect of pH of Extract	35
2-2-10	Effect of $CuSO_4 \cdot 5H_2O$ Concentration on Synthesis of CuNPs	35
2-2-11	Characterization of Synthesized Copper Nanoparticles	35
2-2-11-1	UV-Vis Spectroscopy	35
2-2-11-2	Fourier Transform-Infrared (FT-IR) Spectroscopy	35
2-2-11-3	X-ray Diffraction Analysis	36
2-2-11-4	Atomic Force Microscopy (AFM)	37
2-2-11-5	Scanning Electron Microscopy (SEM)	37
2-2-11-6	Transmission Electron Microscopy (TEM)	37
2-2-12	Biochemical Applications	38
2-2-12-1	Antioxidant Assay	38
2-2-12-1-1	1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Assay	38

2-2-12-1-2	Reducing Power	39
2-2-12-1-3	Determination of Total antioxidant Activity	41
2-2-12-2	Catalytic Activity	42
2-2-12-3	Anti-bacterial Assay	43
2-2-12-4	In vitro Anti-inflammatory Activity	44
2-2-12-4-1	Inhibition of Albumin Denaturation	44
2-2-12-4-2	Protein inhibitory action	45
2-2-12-4-3	Membrane Stabilization Test	46
2-2-12-5	Hemolytic Assay	47
	Chapter Three: Results and Discussion	
3	Results	49
3-1	Qualitative Phytochemical analysis of *Myrtus communis* Leaves Extract	49
3-2	GC-Mass Spectroscopy	51
3-3	Synthesis of Copper Nanoparticles	54
3-4	Characterization of the Synthesized Copper Nanoparticles	56
3-4-1	UV-Visible Spectroscopy	56
3-4-2	Fourier Transform Infrared Spectroscopy (FT-IR)	61
3-4-3	X-Ray Diffraction (XRD)	66
3-4-4	Atomic Force Microscopy (AFM)	67
3-4-5	Scanning Electron Microscopy (SEM)	68
3-4-6	Transmission Electron Microscopy (TEM)	69
3-5	Biological Activities	71
3-5-1	Antioxidant Activity	71
3-5-2	Catalytic Activity	74
3-5-3	Anti-bacterial Assay	78
3-5-4	Anti-Inflammatory Activity	82

3-5-5	Hemolytic Activity	85
	Conclusions	88
	Future studies	89
	References	
	References	90

List of Tables

Table No.	Subject	Page No.
2-1	The apparatuses and equipment used in this study	27
2-2	The chemicals and kits used in this study	28
3-1	Qualitative phytochemical analysis of *M. communis* leaves extract.	49
3-2	GC-mass analysis of *M. communis* leaves extract	54
3-3	The result of the XRD for synthesized and standard CuNPs.	67
3-4	Anti-bacterial activity of CuNPs and *M. comminus* extract against some pathogenic bacteria.	80

List of Figures

Figure No.	Subject	Page No.
1-1	*Myrtus communis Lin*.	4
1-2	The chemical structure of the essential phytochemicals of *M. communis* leaves extract.	7
1-3	Typical synthetic for nanoparticles for top-down and bottom-up approach.	11
1-4	Factors affecting to synthesis CuNPs.	15
1-5	Different applications of Copper nanoparticles in biomedical fields.	17
1-6	The main enzymes responsible for oxidative stress and scavenging free radicals.	20
1-7	Mechanism of Reduction of 4-nitrophenol.	21
2-1	The general Scheme of synthesizing CuNPs and their applications.	30
3-1	GC-MS of *M. communis* leaves extract	53
3-2	The gradual change in the color of the synthesized CuNPs with time.	55
3-3	UV-visible spectra of CuNPs at a different time of incubation.	56
3-4	UV-Visible spectra of CuNPs peak at 481 nm	58
3-5	UV-Visible spectra for CuNPs at different boiling times of extract.	59
3-6	UV-Visible spectra for CuNPs at different volumes of extract.	59
3-7	UV-Visible spectra for CuNPs at different $CuSO_4 \cdot 5H_2O$ concentrations.	60

3-8	UV-Visible spectra for CuNPs at different pH .	60
3-9	FT-IR spectrum of *M. communis* leaves extract.	64
3-10	FT-IR spectrum of synthesized CuNPs.	65
3-11	X-Ray diffraction (XRD) of synthesized CuNPs from *M. communis* leaves extract.	67
3-12	AFM assay of CuNPs synthesized by 10 mL of *M. communis* aqueous leaves extract for boiling 10 minutes. (a) Two-dimensional image (b) Three-dimensional image.	68
3-13	SEM image of biosynthesized copper nanoparticles (a) The particle size at different average diameters D1, D2, and D3. (b) SEM shows the spherical shape of particles .	69
3-14	TEM images of biosynthesized CuNPs. TEM image shows CuNPs with spherical and semispherical forms.	70
3-15	Distribution of flow diameter of the copper nanoparticles.	71
3-16	The inhibition percentage for DPPH free radicals scavenging activity of *M. communis* extract (MCE), synthesized copper nanoparticles (CuNPs), and Ascorbic acid (AA) at different concentrations.	72
3-17	Total antioxidant ability of CuNPs, *M. communis* extract (MCE), and Ascorbic acid (AA).	72
3-18	Reducing power of CuNPs, *M. communis* extract (MCE), and Ascorbic Acid (AA).	73
3-19	UV-visible spectrum of the reduction of 4-NP by CuNPs at concentrations (a) 100 µg/mL (b) 50 µg/mL (c) 25 µg/mL (d) 20 µg/mL (e) 10 µg/mL and (f) 5 µg/mL.	76
3-20	UV-visible spectrum of the reduction of 4-NP by *M. communis* at the concentrations (a) 100 µg/mL (b) 50	77

	µg/mL (c) 25 µg/mL (d) 20 µg/mL (e) 10 µg/mL and (f) 5 µg/mL.	
3-21	The antibacterial activity (1) *M. comminus* leaves extract and (2) (CuNPs) (a)*Lactobacillus salivarins* (b) *Staphylococcus aureus* (c) *Klebsiella pneumonia* and (d) *Pseudomonas aeruginosa*.	81
3-22	Effect of *M. communis* leaves extract and CuNPs on albumin denaturation. Aspirin as a reference (positive control) .	84
3-23	Effect of *M. communis* leaves extract and CuNPs on protein inhibitory activity. Aspirin as a reference (positive control).	84
3-24	Effect of *M. communis* leaves extract and CuNPs on membrane stabilization. Aspirin as a reference (positive control) .	85
3-25	The percentage of hemolysis induced by CuNPs. Triton X-100 was used as a positive control and normal saline as a negative control	86
3-26	The percentage of hemolysis induced by *M. communis* extract. Triton X-100 was used as a positive control and normal saline as a negative control.	86

List of Abbreviations

AFM	Atomic Force Microscopy
AP	Aminophenol
CAT	Catalase
$CuNP_S$	Copper nanoparticles
CVD	Chemical Vapor Deposition
DPPH	1,1-diphenyl-2-picrylhydrazyl
FT-IR	Fourier- Transform Infrared
GC-mass	Gas Chromatography and Mass spectroscopy
GP_X	Glutathione Peroxidase
GR	Glutathione Reductase
nm	Nanometer
NP	Nitrophenols
NPs	Nanoparticles
NSAIDs	Nonsteroidal Anti-inflammatory Drugs
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SEM	Scanning electron microscopy
SOD	Superoxide Dismutase
SPR	Surface plasmon resonance
TEM	Transmission Electron Microscopy
XRD	X-ray diffraction

Chapter One

Introduction

and

Literature Review

1-1 Introduction

Nanotechnology is a vital area of modern research that deals with the design, synthesis, and manipulation of particle structures ranging in size from 1 to 100 nm (1).

Nanoparticles (NPs) have numerous applications in fields such as health care, cosmetics, food and feed, environmental health, mechanics, optics, biomedical sciences, chemical industries and electronics, space industries, drug-gene delivery, energy science, optoelectronics, catalysis, single-electron transistors, light emitters, nonlinear optical devices, and photoelectrochemical applications (2).

The prefix nano is derived from the Greek word Nanos, which means "dwarf," and refers to one billionth (10^{-9}m) in size (3).

Nanoparticles have been synthesized from a variety of animal, plant, and microorganism sources. Copper has been widely used among metallic nanoparticles due to its stable and catalytic properties. Copper nanoparticles have sparked a lot of interest due to their excellent physical and chemical properties, as well as their low preparation cost (4).

Top-down and bottom-up approaches are commonly used to synthesize nanoparticles. In the top-down approach, bulk materials are gradually broken down into nanosized materials. Atoms or molecules are assembled into nanometer-scale molecular structures using the bottom-up approach. For nanoparticle chemical and biological synthesis, a bottom-up approach is commonly used (5).

Copper nanoparticles can be synthesized using plant extract and have been shown to have a wide range of applications including ,antimicrobial activity (6).

Novel secondary metabolites found in plant crude extract include phenolic acid, flavonoids, alkaloids, and terpenoids. These compounds are primarily responsible for the reduction of ionic nanoparticles into bulk metallic nanoparticles (7).

1-2 Literature Review

1-2-1 *Myrtus communis Linn.*

Myrtle (*Myrtus communis Linn*.) is an evergreen shrub with white fragrant flowers that is native to the Mediterranean littoral and is found primarily in France, Tunisia, Morocco, Lebanon, and the former Yugoslavia (8). Myrtus is a flowering plant genus with about 16 species that grow in temperate, tropical, and subtropical climates. This evergreen shrub grows to a height of about (1–5 m) and blooms in the wild at altitudes ranging from 0–600 m. The ovate-lanceolate leaves are (2–5 cm) long, coriaceous, glabrous, punctate-glandular, and entire. These leaves a delicate aromatic odor when crushed. Five petals, five sepals, and a mass of tufted stamens characterize the white star-like flowers. The berries are pea-sized, orbicular, or ovoid-ellipsoid in shape, blue-black or white, and have hard kidney-shaped seeds. They vary in size (0.7-1.2 cm) and shape. The glabrous berry is rounded (vase-like) in shape, with a swollen central part and a persistent 4-5 partite calyx at the outer part, and it ranges in color from dark red to violet (9). Insects pollinate the flowers, and birds, mammals, and ants use a specialized seed dispersal strategy. Seeds do not appear to be dormant. As a result, germination can occur soon after dispersal, and their rapid establishment strategy may allow seedlings to benefit from autumn and winter rains during the critical first stages of growth. These characteristics can be beneficial for maximizing reproductive success, seedling establishment, and survival in Mediterranean environments (10).

Because of the high essential oil content in its leaves and flower, *Myrtus communis* is an important medicinal and aromatic plant. The essential oil found in the leaves and berries of certain plants provides astringent, antiseptic, anti-hypertensive, anti-inflammatory, and insecticide

activity. The essential oil found in the leaves and berries of certain plants also has nematicidal activity (11). Furthermore, myrtle berries and leaves are used primarily in the industrial formulation of sweet liquors with digestive properties. *M. communis* has been used for medicinal, food, and spice purposes since antiquity. *M. communis* has been used as an infusion in Iranian folk medicine for a variety of purposes including skin discords, antiseptic (smoking), women's diseases, wound (antimicrobial), digestive discords, astringent, for good hair, a bronchodilator, and many other activities(12).

Figure (1-1): Picture of *Myrtus communis Lin.*

1-2-2 The Constituents of Plants

Phytochemistry is a branch of chemistry concerned with the chemical nature of plants or plant products (chemistry of natural products). Plants with a high concentration of chemical constituents may be therapeutically active or inactive. Active components can be found in all parts of the plant,

including the bark, leaves, flowers, roots, fruits, and seeds (13). Phytochemicals are divided into two categories: primary and secondary metabolites. Sugars, amino acids, proteins, and chlorophyll are examples of primary metabolites, whereas secondary metabolites are substances produced by plants to help them compete in their environment (14). Secondary metabolites such as alkaloids, flavonoids, saponins, tannins, and others (Figure 1-2) have been shown to have anti-diarrheal, antiulcer, antidiabetic, antihypertensive, antioxidant, antimicrobial, and antimutagenic properties. These small molecules have a variety of effects on the plant and other living organisms. In the plant kingdom, over 50,000 secondary metabolites have been discovered. Secondary plant metabolites are responsible for the actions of medicinal herbs and many modern medicines (15). The study of active compounds which were found in plants has resulted in the discovery of new medicinal drugs with effective protection and treatment roles against a variety of diseases, including cancer and Alzheimer's disease (16). Other research has found these secondary metabolites in the *M. communis* plant (17).

Phenols are the most common type of secondary metabolite. They range from simple compounds with a single aromatic ring to complex polymers such as tannins and lignin. These compounds have biological properties such as anti-apoptosis, anti-carcinogenesis, anti-inflammatory activity, anti-atherosclerosis, cardiovascular protection, endothelial function improvement, and inhibition of angiogenesis and cell proliferation (18).

Flavonoids are a type of polyphenolic secondary metabolite that occurs naturally in plants. That have numerous biological activities, including anti-carcinogenic, anti-inflammatory, anti-radical, and antioxidant properties. In addition , Flavonoids may have anti-oxidative properties as

free radical scavengers, hydrogen-donating compounds, singlet oxygen quenchers, and metal ion chelators, in particular. The phenolic hydroxyl groups attached to the ring structures are responsible for these properties (19).

The plant extracts were also found to contain saponins, which are known to have an anti-inflammatory effect. Saponins are known for their ability to precipitate and coagulate red blood cells. Saponins' properties include the formation of foams in aqueous solutions (20).

Terpenes are necessary plant metabolites. Floral fragrances, which serve as insect attractants, pine oil, growth inhibitors, the two plant hormones, gibberelic acid, abscisic acid, and some insecticidal substances are among them. Tri-terpene has anti-tussive, expectorant, analgesic, anti-inflammatory, and cytotoxic properties (21).

Alkaloids are plant-derived compounds that contain one or more nitrogen atoms, usually in a heterocyclic ring (an amine functional group), and have a significant effect on animals, including humans. Their pharmacological activity, which includes anti-arrhythmic, anti-cancer, and anti-malarial effects, distinguishes them (22).

Tannins are a type of plant secondary metabolite. Tannins and alkaloids in plants are known to play anti-herbivore defense roles. As a result, the presence of tannins and alkaloids in medicinal plants may deter grazers. Tannin-containing herbs are widely used as diuretics, anti-diarrhea, and hemostatic agents for a variety of conditions, including inflammation, liver damage, kidney problems, arteriosclerosis, hypertension, stomach problems, and suppression of adequate oxygen (23).

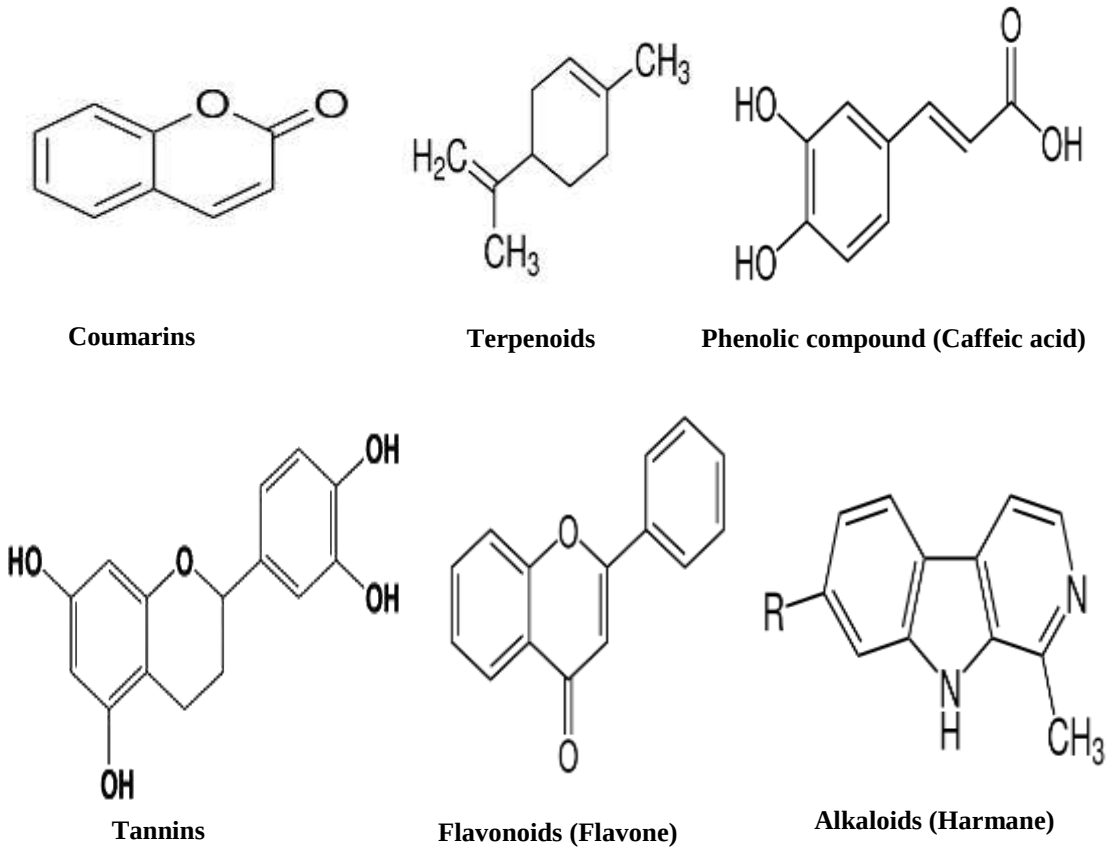

Figure (1-2): The chemical structure of the essential phytochemicals of *M. communis* leaves extract (24).

1-2-3 Biological Activity of *Myrtus communis*

Plants have long been used in folk medicine as natural healing remedies with therapeutic effects such as preventing cardiovascular disease, inflammation disorders, and cancer risk. Furthermore, the pharmacological industry uses medicinal plants as agents for drug synthesis due to the presence of active chemical substances. They are also useful as additives in the food and cosmetic industries due to their preservative properties and the presence of antioxidants and antimicrobial constituents (25).

Myrtle has been used as a spice and medicinal agent, as well as for food preparation, since ancient times. Despite its pleasant odor, myrtle limited

widely used as a spice due to its bitterness. Because the flavor is intense, unpleasant, and bitter, its culinary application is limited to the regions of origin, such as Italy (26). In Italy, particularly in Sardinia, the berries and leaves of this plant are used to make two well-known liquors (Mirto Rosso and Mirto Bianco, respectively); in Italian folk medicine, the fruit of this plant is used to treat many types of infectious disease, including diarrhea and dysentery; and the leaves are used as an antiseptic and anti-inflammatory agent, as well as a mouthwash, for the treatment of candidiasis (27). Foods flavored with myrtle smoke are popular in rural areas of Italy. Some of the plant's parts are used in the food industry to flavor meat and sauces (26), and its berries and leaves are primarily used in the industrial formulation of sweet liquors with advertised digestive properties. The fragrant leaves have been widely used in the perfume and cosmetic industries, particularly in Portugal and Turkey (28). It's been used for centuries as an antiseptic, disinfectant, and hypoglycemic agent. In villages throughout Turkey, myrtle leaves and fruits have been used as an antiseptic medicine (27). The essential oil extracted from myrtle leaves has been used to treat lung conditions. Myrtle is commonly used as an infusion and decoction (10). Infusions of the leaves and young branches are used as stimulants, antiseptics, astringents, and hypoglycemic. They are used to treat conditions such as asthma, eczema, psoriasis, diarrhea, gastrointestinal disorders, and urinary infections. The leaf decoction is used for vaginal washing, enemas, and to treat respiratory diseases; the fruit decoction is used as an antidiarrheal and anti-hemorrhoidal agent, as well as to treat mouth and eye diseases (29).

Myrtus communis has previously been used to treat a variety of ailments, according to previous research. It has long been used to treat diarrhea, hemorrhoids, bleeding, headache, leucorrhoea, excessive

perspiration, pulmonary and skin diseases, as well as for hemostatic, stimulant, and the treatment of constipation and respiratory diseases (30).

1-3 Synthesis of Nanoparticles (NPs)

Two basic approaches, which include various preparation methods and have been used since ancient times, are used in the synthesis of nanoparticles, which can be of natural or synthetic origin and exhibit unique properties at the nanoscale. The first method is the "top-down" method, which uses external force to break down solid materials into small pieces. Many physical, chemical, and thermal techniques are used in this approach to provide the necessary energy for nanoparticle formation. These include photolithography, e-beam lithography, dip-pen lithography, molecular beam epitaxy, and others. The second approach, is known as "bottom-up," implies that nanostructures are created using atoms as building blocks. This approach includes chemical solution-phase synthesis, vapor deposition, and plasma/flame spraying synthesis. Bottom-up approaches have gotten a lot of attention because they are generally less expensive, can be controlled by manipulating many experimental parameters, and have the potential to reach an industrial scale Figures (1-3) (31).

Due to cavities and roughness in nanoparticles, it is impossible to obtain perfect surfaces and edges in the top-down approach, which is more expensive to implement, whereas excellent nanoparticle synthesis results can be obtained in the bottom-up approach. Furthermore, the bottom-up approach produces no waste materials that must be removed, and nanoparticles with smaller sizes can be obtained (32).

Biogenic Reduction, like chemical Reduction, is a "Bottom-Up" approach in which a reducing agent is replaced by an extract of natural

products with inherent stabilizing, growth terminating, and capping properties. Researchers are focusing on green NP synthesis, in which plant extract is primarily used to synthesize NPs. Green synthesis of NPs from metal ions is more environmentally friendly, free of chemical contamination, less expensive, and safe for biological applications (33), (34).

There are numerous physical, chemical, biological, and hybrid methods for the synthesis of various types of nanoparticles. To synthesize NPs, the most common chemical approaches, such as chemical reduction using a variety of organic and inorganic reducing agents, electrochemical techniques, physicochemical reduction, and radiolysis, are widely used (35).

The distinct properties of biologically synthesized NPs are preferred over physical-chemically produced nanomaterials. Physical-chemical procedures can be used to create nanoparticles. However, these methods are capital intensive and have numerous issues, such as the use of toxic solvents, the generation of hazardous by-products, and surface structure imperfections. Chemical methods are typically made up of multiple chemical species or molecules, which can increase particle reactivity and toxicity while also harming human health and the environment due to composition ambiguity and lack of predictability (33).

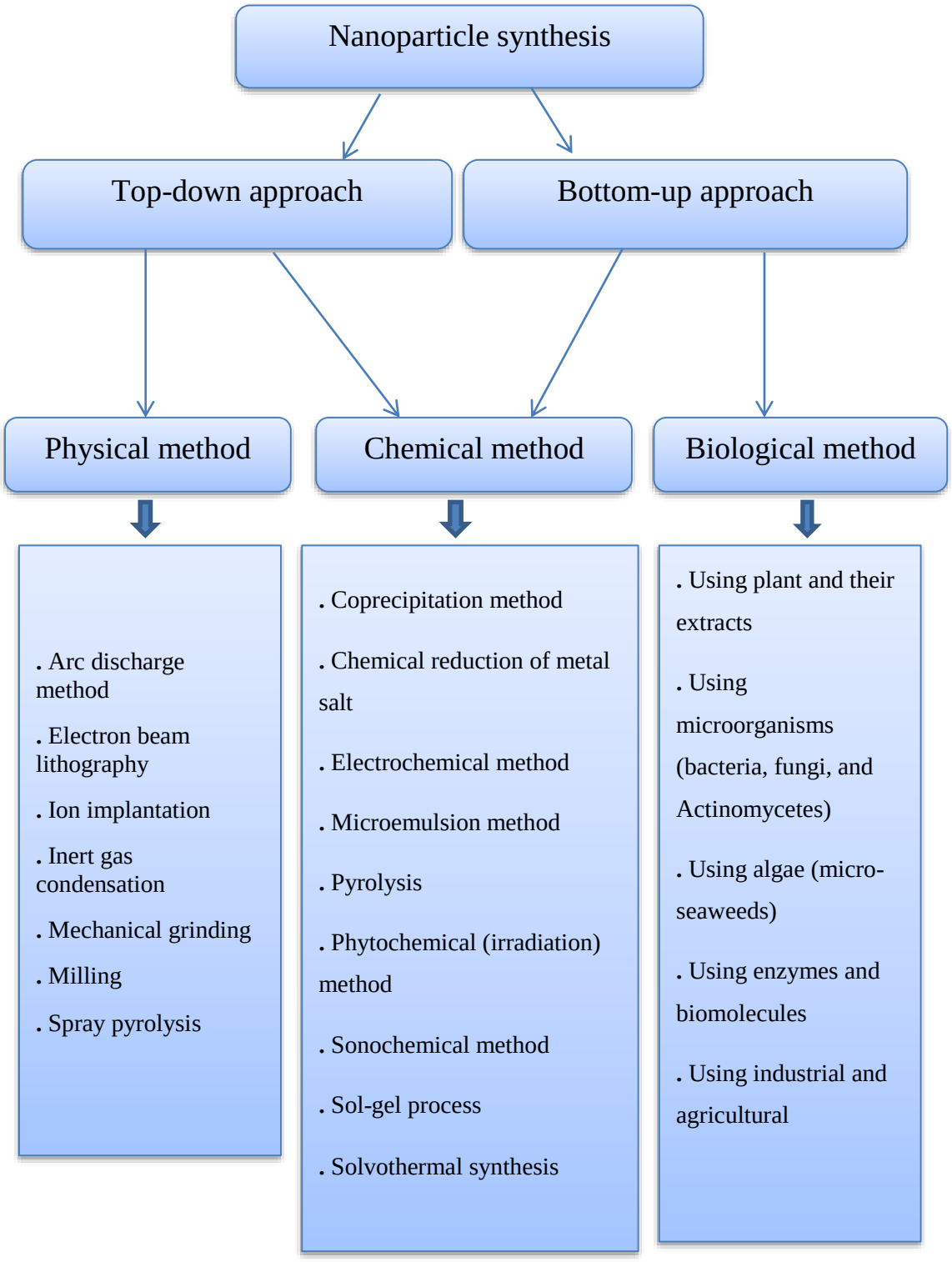

Figure (1-3): Typical synthetic for nanoparticles for top-down and bottom-up approach (36).

1-4 Biosynthesis of Nanoparticle

Many studies in the literature indicate that chemical approaches to nanoparticle synthesis are both environmentally unfriendly and costly. As a

result, there is an increasing need to develop environmentally and economically friendly processes that do not rely on toxic chemicals in the synthesis protocols. The use of plant parts and microorganisms in the biosynthesis of nanoparticles has been proposed as a cost-effective and environmentally friendly alternative to physical and chemical methods (37), (1).

Reductase enzymes, microorganisms can accumulate and detoxify heavy metals by reducing metal salts to metal nanoparticles with narrow size distribution and, thus, less polydispersity. Microorganisms, such as bacteria (such as Actinomycetes), fungi, and yeasts, have been studied extra- and intracellularly to synthesize metal nanoparticles in recent years (38). In another study, *Bacillus, Pseudomonas, Klebsiella, Escherichia, Enterobacter, Aeromonas, Corynebacterium, Lactobacillus*, and other microorganisms were used to synthesize metal nanoparticles (35).

Plants and plant extracts appear to be the most promising alternative to chemical synthesis and the more complex culturing and isolation techniques required for many microorganisms. Furthermore, plant extract-derived molecule combinations act as both reducing and stabilizing (capping) agents during nanoparticle synthesis (39). These biological molecules are chemically complex, but they are environmentally friendly. Nanoparticles have been created using *Azadirachta indica* (40), *Enicostemma axillare (Lam.)* (41), *Cissus arnotiana* (42), and other plant extracts (43).

1-4-1 Biosynthesis of NPs from plants

By the wide availability of plant materials, using plants for nanoparticle synthesis can be advantageous over other biological processes. It also eliminates the ease of large scale-up and the process of culture

maintenance, and there is no need to use high pressure, energy, temperature, or toxic chemicals (44). Noble metal nanoparticles are the most promising because they have good anti-bacterial properties due to their large surface area to volume ratio, which is gaining attention among researchers due to the growing microbial resistance to metal ions, antibiotics, and the development of resistant strains. These distinct properties are primarily determined by the size, shape, and surface area of the nanoparticles (45).

The biosynthesis of metal nanoparticles by plants is currently being explored, with (inactivated plant tissue, plant extracts, and living plant) receiving attention as a viable alternative to chemical and physical methods. Metal nanoparticle synthesis using plant extracts is very cost-effective and thus can be used as an economical and valuable alternative for large-scale metal nanoparticle production. Plant extracts have the potential to act as both reducing and capping agents in the synthesis of nanoparticles. Metal nanoparticle reduction via biomolecules found in plant extracts (e.g. enzymes, proteins, amino acids, vitamins, polysaccharides, and organic acids such as citrates) is environmentally friendly but chemically complex (36). Plant NP synthesis can be classified as extracellular, intracellular, or phytochemical. When plant extract is used as a raw material for NP synthesis, extracellular methods are used, whereas intracellular methods use cellular enzymes to synthesize nanoparticles within plant cells. After synthesis, the NPs are recovered by separating the plant cell walls (46).

Nanoparticles are made from plant parts such as roots, stems, leaves, bark, and even seeds. The composition of plant extracts influences NP formation in a salt solution. Aside from the concentration of each extract and salt, pH was also important in the formation of NPs (47).

1-5 Biosynthesis of Copper Nanoparticles

Copper nanoparticles (CuNPs) are highly interested due to their unique properties, which include a high surface-to-volume ratio, high yield strength, ductility, hardness, and flexibility. In a variety of applications, copper nanoparticles exhibit catalytic, antibacterial, antioxidant, and antifungal activities, as well as cytotoxicity and anticancer properties (48).

Copper nanoparticles can be produced using microorganisms (bacteria, fungi, aquatic algae, and yeasts), alcoholic or aqueous plant extracts, and other biosynthesis methods (49). The use of plant extracts to make CuNPs has grown in popularity due to the abundance of biological entities, diversity, and environmentally friendly production methods (50). The synthesis of copper nanoparticles has piqued the interest of researchers because it is less expensive than obtaining silver or gold (51). Copper nanoparticles research has advanced significantly in the last decade as a result of its numerous applications in nanotechnology and nanomedicine, such as catalysis, optics, electrical, and antifungal/antibacterial treatment (52).

Metal ions with monovalent or divalent oxidation states are converted into zero-oxidation copper nuclei during the CuNPs synthesis process, and these nuclei are merged to form various shapes. During nucleation, nuclei aggregate to form various shapes such as wires, spheres, cubes, rods, triangles, pentagons, and hexagons (48). In the literature, copper nanoparticles were synthesized and stabilized using various plants such as *Ocimum sanctum* (53), *Ginkgo biloba L.* (54), *Calotropis Procera L*., and others. However, when compared to other biological methods, the synthesis of CuNPs with plant extracts is advantageous because metal ions are removed much faster, resulting in high particle stability (55).

1-5-1 Optimized Conditions for Biosynthesis of CuNPs

Several controlling factors are involved in the nucleation and subsequent formation of stabilized nanoparticles during the biological synthesis of metallic nanoparticles. Metal salt concentration, reaction time, reaction solution pH, and temperature are among the factors that have a significant impact on the quality, size, and morphology of the synthesized nanoparticles (Figure 1-4).

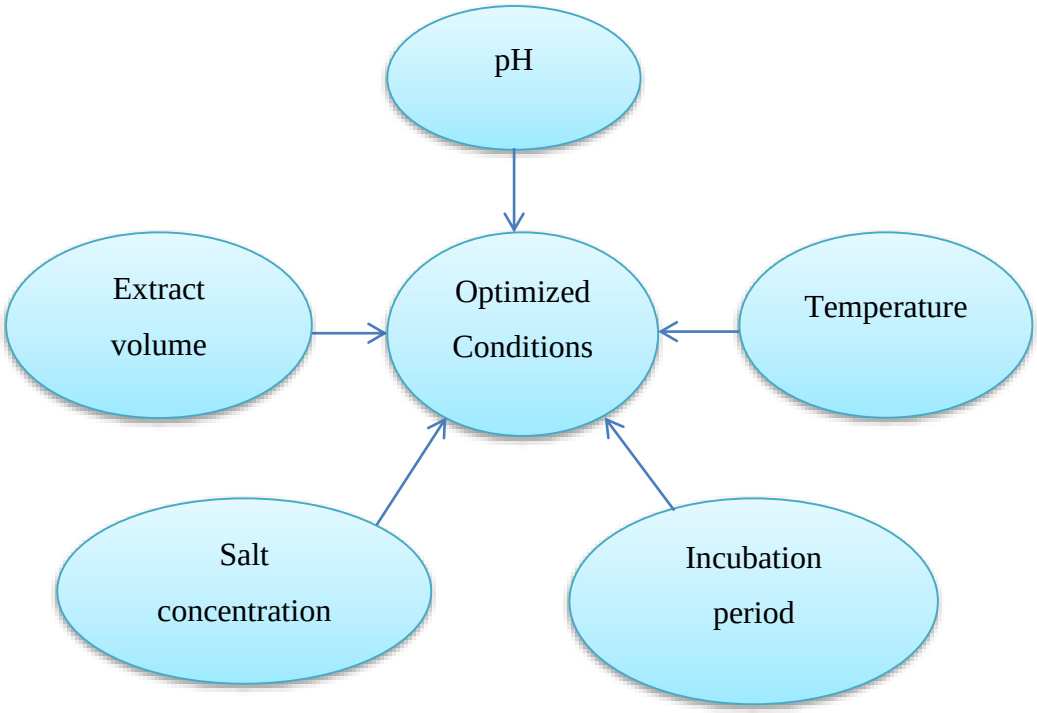

Figure (1-4): Factors affecting to synthesis CuNPs (56).

When plant extract and Cu-salt are combined, the color changes from colorless to dark brown, indicating the first appearance of CuNPs. Copper salt has a significant impact on the size and structure of CuNPs. As the concentration of salt increases, the surface plasmon bands shift to a higher frequency, indicating aggregation. The incubation period of the reaction determines the efficiency of CuNPs (57). The best results for CuNPs synthesis are obtained by varying the pH of the reaction medium within the desired range. The pH of the reaction mixture was changed to control the

size of the nanoparticles. Smaller-sized nanoparticles were obtained at higher pH values compared to those obtained at lower pH values. This difference can be attributed to a difference in the rate at which metal salts are reduced by plant extract. The inverse relationship between pH value and nanoparticle size revealed that increasing the pH value yields small-sized spherical nanoparticles while decreasing the pH value yields large-sized (rod-shaped and triangular) nanoparticles (58). While it is well known that reaction temperature is an important factor in any synthesis, it has recently been discovered that temperature also plays a role in determining the size, shape, and yield of nanoparticles synthesized using plant extracts. Both the reaction and particle formation rates appear to accelerate as the reaction temperature rises; however, the average particle size decreases, and the particle conversion rate steadily rises as the temperature rises (59).

1-6 Biological Applications of Copper Nanoparticles

Copper nanoparticles have piqued the interest of researchers in variety fields (biomedicine, pharmacy, agriculture, and food industry) due to their outstanding chemical and physical properties, constantly renewable surface, low cost, and nontoxic preparation.

In various applications, copper nanoparticles exhibit catalytic activity, antibacterial activity, cytotoxicity or anticancer activity, antioxidant activity, and antifungal activity (Figure 1-5) (52). Copper-NPs are used in catalytic activity for the Huisgen cycloaddition of alkynes and azides in a variety of solvents under ligand-free conditions. Different dyes, toxic organic compounds, and pesticides found in industrial waste are extremely harmful to the environment and living organisms; therefore, CuNPs are used for the degradation and reduction of various dyes such as methylene blue (60), and the reduction of 4-nitrophenol (61). Copper- NPs in high concentrations have been linked to several health problems. When used

properly, these particles are environmentally friendly; however, when used arbitrarily, they become a formidable foe (62).

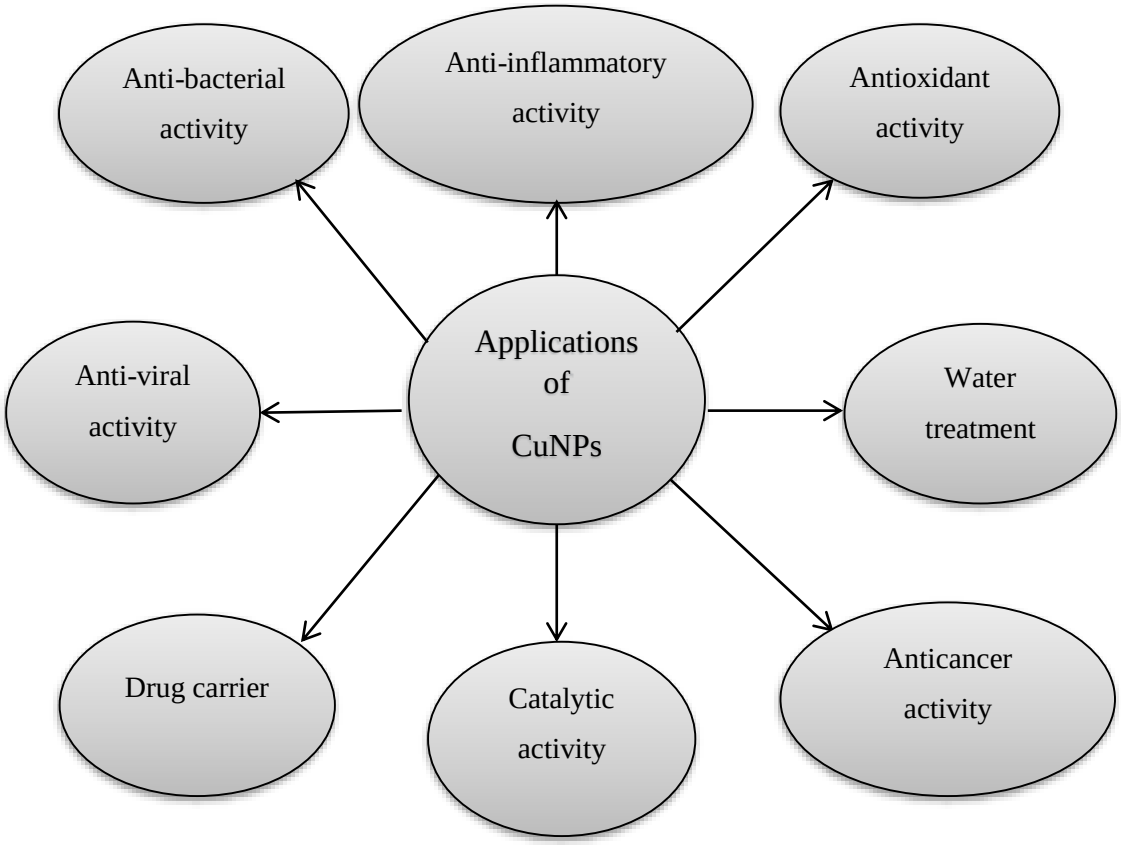

Figure (1-5): Different applications of Copper nanoparticles in biomedical fields (63).

1-6-1 Antioxidant Activity

One of the most important free radical-producing processes in food, chemicals, and even living systems is oxidation. Free radicals play an important role in the degradation of food and chemical materials, as well as in the development of over a hundred human diseases. Antioxidants significantly postpone or prevent the oxidation of easily oxidable substrates (64).

The byproducts of normal cellular metabolism are free radicals. A free radical is an atom or molecule that has one or more unpaired electrons in its valence shell or outer orbit and can exist independently. A free radical's

odd number of electron(s) makes it unstable, short-lived, and highly reactive. They can capture electrons from other compounds to achieve stability due to their high reactivity. As a result, the attacked molecule loses an electron and becomes a free radical, triggering a chain reaction cascade that eventually damages the living cell. Both reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced by both endogenous (mitochondria, peroxisomes, endoplasmic reticulum, phagocytic cells, etc.) and exogenous (pollution, alcohol, tobacco smoke, heavy metals, transition metals, industrial solvents, pesticides, certain drugs such as halothane, paracetamol, and radiation) sources (65). ROS and RNS are both components of free radicals and other non-radical reactive species. ROS/RNS serve a dual purpose in the living system, acting as both beneficial and toxic compounds. ROS/RNS have beneficial effects at moderate or low levels and are involved in a variety of physiological functions such as immune function (i.e. defense against pathogenic microorganisms), some cellular signaling pathways in mitogenic response, and redox regulation. However, at higher concentrations, both ROS and RNS cause oxidative stress and nitrosative stress, respectively, which can damage biomolecules. Oxidative and nitrosative stress occur when there is an excess of ROS/RNS production on one side and a deficiency of enzymatic and non-enzymatic antioxidants on the other (66).

In humans, oxidative processes are responsible for the formation of various forms of activated oxygen. Free radicals, which include hydroxyl ($OH^{\bullet}$), superoxide ($O_2^{\bullet-}$), alkoxyl ($RO^{\bullet}$), and peroxyl ($RO_2^{\bullet}$), are molecules with an unpaired electron in the outer orbit that are generally unstable and highly reactive. Although hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) are not free radicals, they can cause free radical reactions (67). Preventative mechanisms, repair mechanisms, physical defenses, and

antioxidant defenses are all part of the human body's defense against ROS-induced damage. The enzymes catalase and glutathione peroxidase (both of which remove H_2O_2), as well as superoxide dismutase (which catalyzes the dismutation of O_2^- to form H_2O_2), provide an enzymatic antioxidant defense (Figure 1-6). In humans, glutathione peroxidase is thought to be more important than catalase as an H_2O_2-removing system. Furthermore, low-molecular-mass substances like uric acid, ascorbate (vitamin C), glutathione, tocopherols (vitamin E), ubiquinol, ergothioneine, hypotaurine, and lipoic acid may act as antioxidants in the human body (25). "Oxidative stress" is caused by an imbalance between the production of ROS and the activity of antioxidant defenses. Severe oxidative stress can cause cell damage and tissue destruction by causing mutations and mitochondrial and membrane disruption. As a result, when endogenous antioxidant defenses are insufficient to completely scavenge ROS, ongoing oxidative damage to DNA, lipids, proteins, and other molecules can be demonstrated. ROS have been linked to a variety of human diseases, including atherosclerosis, inflammation, cancer, rheumatoid arthritis, and neurodegenerative diseases such as Alzheimer's and multiple sclerosis. However, ROS may contribute to the occurrence of these diseases, but they may not be the primary cause (67).

Plant extracts and products, such as flavonoids and other polyphenolic constituents, are effective radical scavengers and lipid peroxidation inhibitors (68).

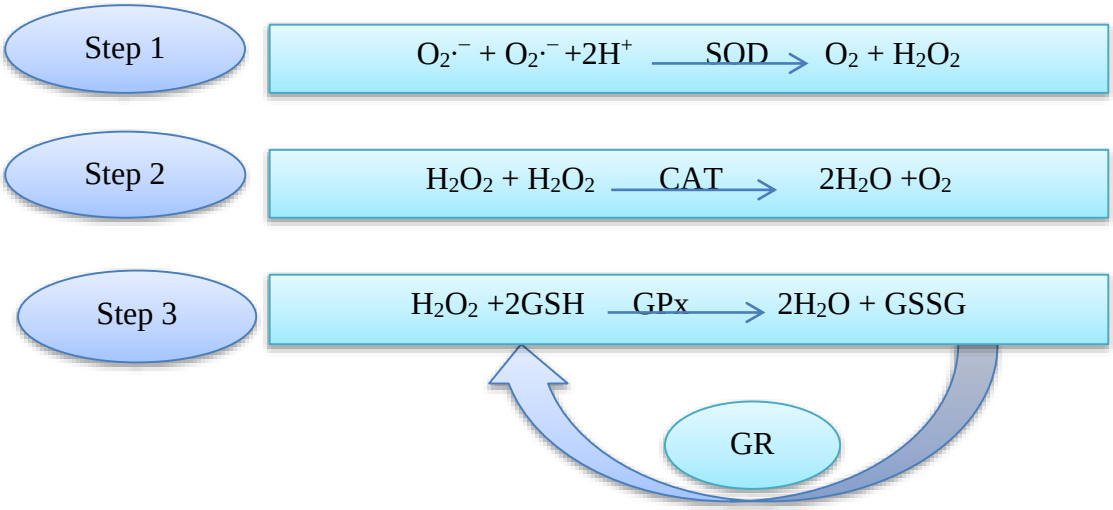

Figure (1-6): The main enzymes responsible for oxidative stress and scavenging free radicals are Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Glutathione Reductase (GR) (69).

1-6-2 Catalytic Activity

The reduction process is a fundamental chemical transformation in organic synthesis and industrial chemistry, with the key step being the transformation of electrons from a donor to an acceptor substance. Nitrophenol (NP) reduction is a common and simple method for producing aminophenol (AP), which are important intermediates in the synthesis of a variety of nitrogen-containing compounds such as agrochemicals, pharmaceuticals, polymers, dyes, pesticides, and cosmetics (70).

Nitrophenols are among the most prevalent organic pollutants in waste-waters generated by agricultural and industrial sources, including companies that manufacture explosives, dyes, and other products. 4-Aminophenol (4-AP) on the other hand, is an important intermediate in the production of antipyretic and analgesic drugs. As a result, various NPs can be reduced to their amino counterparts using catalysts, with the catalysts used playing a significant role (71). Concerning the greener aspect of catalytic processes, it is highly desirable to develop environmentally benign procedures that can be carried out preferably in aqueous media, thus

avoiding the use of volatile organic solvents; sodium borohydride ($NaBH_4$) is one such preferred water-soluble reductant for representative reducting processes (70).

Copper-NPs have been reported to be effective in the reduction of 4-NP. Cu is a low-cost alternative to the more expensive noble metal NPs, with a wide range of potential applications in nanoscience. Because of its reusability and stability, it is regarded as an effective catalyst, particularly with the assistance of various supports. Furthermore, for 4-NP Reduction, CuNPs exhibit higher catalytic activity than other metal NPs such as Ag, Au, Pt, and Pd (72), (73). A hypothetical mechanism (Figure 1-7) depicts the reduction of 4-NP; in the mechanism, 4-NP and sodium borohydride are present in the solution as ions. The protons of the borohydride ion adsorb on the surface of the copper nanoparticles, producing BO_2. 4-Nitrophenolate ions adsorb on the surface of CuNPs as well. CuNPs overcome the kinetic barrier of reactants due to the adsorption of both protons and 4-nitrophenolate ions, and 4-nitrophenolate ion is converted into 4-aminophenolate ions. After conversion, the 4-aminophenolate ion is desorption and converted into 4-aminophenol(48).

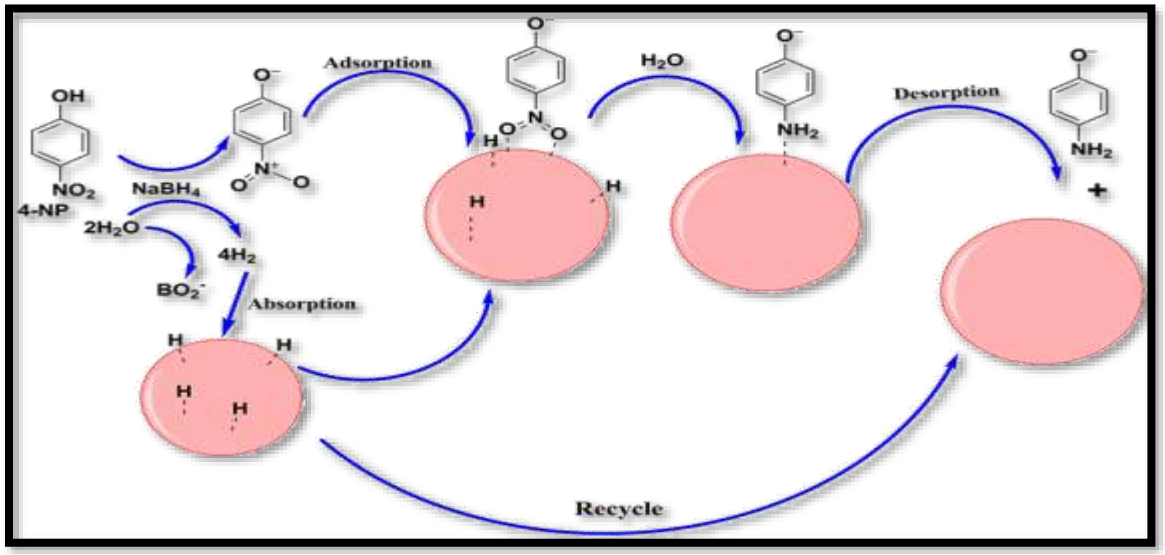

Figure (1-7): Mechanism of Reduction of 4-nitrophenol (48).

1-6-3 Anti-bacterial Activity

Antibiotic resistance in pathogenic bacteria is a challenging global health issue that has prompted the search for new and more effective antibacterial agents. Nanotechnology has been proven to be a powerful weapon in the fight against bacteria. Antibiotics have enabled significant advances in therapeutics, contributing to the rise of modern medicine (74). The antimicrobial activity of nanoparticles (NPs) is known to be proportional to their surface area in contact with microorganisms. As a result, high surface area NPs ensure a diverse range of reactions on the surface of microorganisms, inhibiting normal cell function or causing cell death (75). Copper -NPs have received a lot of attention in recent years due to their low cost, abundance, and exceptional antimicrobial activity (76). In recent years, there has been an increase in the occurrence of multiple resistances in human pathogenic microorganisms, owing largely to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This has compelled scientists to look for new antimicrobial substances in unexpected places, such as medicinal plants. The search for new antibacterial agents should be expanded by screening a wide range of plant families. Antimicrobial activity testing of plant extracts and plant products has revealed that higher plants may be a source of novel antibiotic prototypes (77). Many mechanisms of antibacterial activity have been reported by previous researchers; thus, the action of CuNPs on bacteria has yet to be fully explored. CuNPs are thought to be adsorbed onto bacteria cell walls and interact with electronegative elements within the cell membrane. This results in failed metabolism, which interferes with and disrupts transcription in bacteria, resulting in antibacterial activity by CuNPs. It is also thought that the synergistic effect of CuNPs with extract bioactive compounds would have

had a significant impact on inhibiting the activity of pathogenic bacteria. The helical structure of DNA molecules is thought to have been disrupted by the action of CuNPs. Furthermore, when CuNPs interact with the released Cu metal ion, the electrochemical potential across the cell membrane decreases, compromising membrane integrity (78).

Controlling the growth of bacterial biofilms with nanoparticles (NPs) has gained prominence in the medical field. The use of nanoparticles is a promising therapeutic strategy for combating the emergence of multidrug-resistant bacteria. Although the antimicrobial effects of NPs on various bacterial species have been demonstrated, the bactericidal mechanisms of NPs are still being researched. Several factors, including NP physicochemical properties and bacterial species involved, may play important roles in NP antibacterial activity. Certain bacterial species are more sensitive to specific NPs than others (79).

1-6-4 Anti-inflammatory Activity

Inflammation is a complex reaction to local injury or other trauma; it is distinguished by redness, heat, swelling, and pain. Inflammation is a normal protective response to tissue damage caused by physical trauma, noxious chemicals, and/or microbial agents. It is the body's response to inactivate or destroy organisms, remove irritants, and prepare the environment for tissue repair (80).

Excessive activation of phagocytes, production of $O_2^{\cdot}$, OH, radicals, as well as non-free radical species (H_2O_2), which can harm surrounding tissue either directly or indirectly with hydrogen peroxide (H_2O_2) and OH, radicals formed from $O_2^{\cdot}$, which initiates lipid peroxidation resulting in membrane destruction, is seen in many inflammatory disorders. Tissue damage causes an inflammatory response by releasing mediators and

chemotactic factors. Reactive oxygen species are also known to activate matrix metalloproteinase (e.g., collagenase), resulting in increased tissue destruction, such as collagenase damage seen in various arthritic reactions. As a result, agents that can scavenge reactive oxygen species may be useful in the treatment of inflammatory disorders (81).

There are two types of inflammation: acute and chronic. Acute inflammation is the body's initial response to harmful stimuli, and it is achieved through the progressive movement of plasma and leukocyte-like constituents from the blood into the injured tissues (locations). Chronic inflammation causes a progressive shift in the type of cells present at the site of inflammation and is characterized by the inflammatory process's simultaneous breakdown and healing of the tissue (82).

To reduce inflammation, a variety of nonsteroidal anti-inflammatory drugs (NSAIDs) are used. These anti-inflammatory NSAIDs have several side effects that can be mitigated by the use of medicinal plants instead (83). Traditional medicine has used plant extracts to treat a wide range of disorders, including acute and chronic inflammation. Flavonoids are a family of substances found in these extracts that have many interesting biological properties, including anticancer, antimicrobial, antiviral, anti-inflammatory, immunomodulatory, and antithrombotic properties (84).

1-6-5 Hemolysis

Hemolysis is defined as the breakdown or disruption of the integrity of the red blood cell (RBC) membrane, which results in hemoglobin release. The presence of free hemoglobin in the red cell suspending media, such as plasma or additive solutions, usually indicates hemolysis in blood products. Some diseases, such as hemolytic anemia, or processes, such as blood centrifugation, can cause RBCs to break down prematurely. Despite

significant progress in improving RBC stability during processing, storage, and transfusion, being outside the body increases the risk of hemolysis (85).

The presence of NPs in the environment is significant in terms of their impact on human health; thus, the combination of nanotechnology and toxicology gives rise to the new term 'nanotoxicology.' One of the major contributors to the toxic potential is the release of metal ions from NPs. The dissolution of metal ions from metal NPs, as well as the environment in which the NPs are exposed, play critical roles in inducing toxicity (86).

Copper-NPs can cause toxicity due to their small size and ability to release ions in acidic conditions. In general, the physicochemical properties of any nanoparticle system are critical to comprehending toxicological mechanisms. Particle size, shape, crystallinity, aggregation, and surface coating are examples of these properties (87).

Copper is kept in homeostasis in the human body. If the amount of copper consumed exceeds the range of human tolerance, toxic effects such as hemolysis, jaundice, and even death will occur (88).

The membrane mechanical stability of red blood cells (RBCs) is a good indicator for estimating *in vitro* cytotoxicity as cells with cytotoxic compounds. Lyses can have a variety of health consequences and cause a variety of disorders. Hemolysis has been observed in transferable diseases as a result of microbe action. Phenolic compounds, flavonols, and glycosides have numerous biological activities that may be important in antioxidant and cytotoxic activity (89).

1-7 The Aims of this Study

1. Green synthesis of CuNPs from the environmentally friendly substance (*Myrtus Communis* leaves extract).
2. Optimization of the suitable conditions for the synthesis of the CuNPs
3. Characterization of the synthesized nanoparticles.
4. Applications of synthesized CuNPs.

Chapter Two

Materials

and

Methods

2.Material and Methods

2-1 Materials

2-1-1 Apparatuses

The apparatuses and equipment are summarized in table (2-1)

Table (2-1): The apparatuses and equipment's used in this study

Instruments	**Company**
Atomic force microscopy (AFM)	CSEM
Cooling Centrifuge	D-78532/Germany
Electronic Balance	ABS 220-4/KERN
Fourier transform infrared (FT-IR)	Shimadzu (8400S)/Japan
GC-mass chromatography– mass Spectrophotometer (GC-MS)	Aligant / U.S.A
Gemyy Orbit Shaker	VRN-480/ Germany
Centrifuge	D-78532 Germany
Hot plate with magnetic stirrer	LabTech/Korea
Micro pipette	DragoLAB/China
Oven	D-91126 Schwabach FRG/ Germany
Scanning electron microscopy (SEM)	Tescan Mira3 SEM/French
Transmission electron microscopy (TEM)	(EM10C/Germany)
Water bath	Gallenkamp / England
UV-Vis spectrophotometer	UV-1800/ Kyoto, Japan
Vortex mixer	945307 /THE.U.S.A
X-ray diffraction (XRD)	Philips Xpert /Holland

2-1-2 Chemicals

The chemicals which used in this study are listed in table (2-2)

Table (2-2) Chemicals and kits used in this study

Chemicals	Company
1,1-Diphenyl-2picrylhydrazyl Free Radical	Tokyo Chemical Industry TCI
Ammonium molybdate	BDH Chemical Ltd Pool/England
Anhydrous acetic acid	Chem- Supply
Anhydrous sodium carbonate	Chem- Supply
Basic nitrate bismuth	BDH Chemical Ltd Pool
Calcium chloride	Stago
Chloroform	Gainland Chemical Company
Copper sulphate solution	Analar Trad Mark
Ethanol	Gainland Chemical Company
Iron chloride	BDH Chemical Ltd Pool
L-Ascorbic Acid	Himedia, India
Lead acetate	BDH Chemical Ltd Pool
Mercuric chloride	BDH Chemical Ltd Pool
Potassium citrate	Merck
Potassium dihydrogen phosphate	Merck
Potassium ferricyanide	Analar Trad Mark
Potassium iodide	Analar Trad Mark
Sodium hydroxide	Analar Trad Mark
Sodium Phosphate	BDH Chemical Ltd
Sulfuric Acid	Belgium
Trichloroacetic Acid	Gainland Chemical Company
Bovine serum albumin	Sigma (USA)

Sodium borohydride	Chem- Supply
perchloric acid	Himedia
Trypsin	Sigma (USA)
Triton-X 100	Himedia

2-2 Methods

2-2-1 Summary of Experimental Part

The general steps for synthesis copper nanoparticles and their applications summarized in figure (2-1).

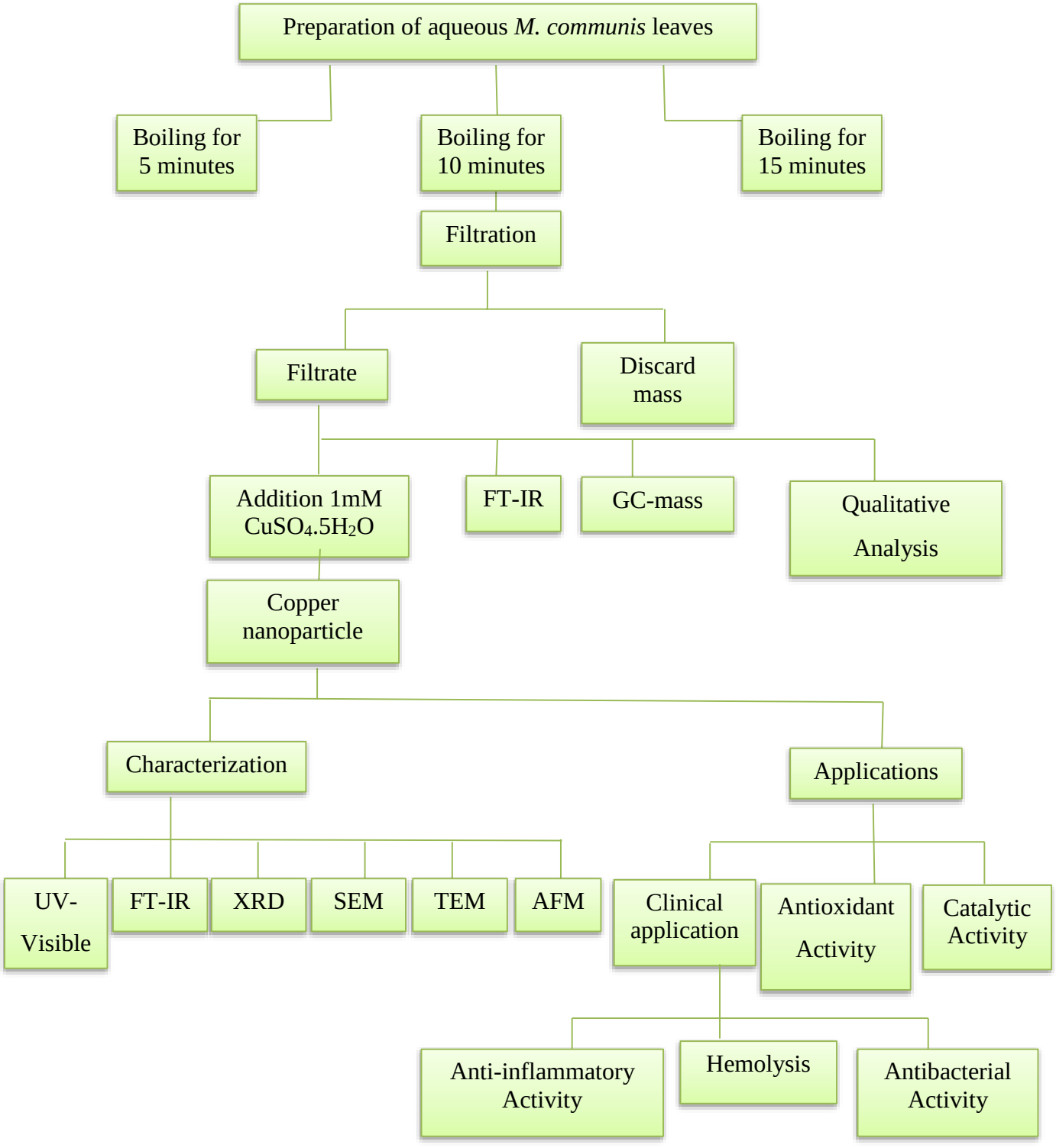

Figure (2-1): Schematic diagram for synthesizing CuNPs and their applications

2-2-2 Collection of Plant

Myrtus communis fresh leaves were obtained from the garden of the University of Kerbala, College of Science. The leaves were cleaned with distilled water and rinsed with deionized water. The clean leaves were dried and crushed into small slices before being stored in a dark and dry place for future use. *Myrtus communis* was identified by the Botanist Dr. Nibal Muteer at the University of Kerbala, College of Education of Pure Science.

2-2-3 Qualitative Phytochemical Analysis.

The crude solution of *M. communis* leaves extract was submitted to phytochemical analysis. These phytochemical included alkaloids, coumarins, flavonoids, resins, saponins, sugars, tannins, phenolic compound, terpenes and steroids

- **Alkaloid test** [90]

Dragendroff's reagent was used for the identification of alkaloids and, prepared as, follows:

Solution A: it was prepared by dissolving 0.85 g of basic nitrate bismuth in a mixture of 10 mL of acetic acid and 40 mL of distilled water.

Solution B: it was prepared by dissolving 8 g of potassium iodide in 20 mL of distilled water.

Stock solution: it was prepared by mixing equal volumes of solution A and B.

Spray reagent: it was prepared by mixing 1 mL of stock solution with 2 mL of acetic acid and 10 mL of distilled water.

Dragendroff's reagent gives orang color when spraying it over a spot of alcoholic extract. That mean presence alkaloid in the plant.

- **Coumarin test** [91]

A 5 mL of alcoholic extract was taken in a test tube and covered using the filter paper and then, spraying it with 5% sodium hydroxide, and boiling in water bath for 5 minutes; intense yellow-green fluorescence develops when dried paper is placed under UV-light for 5-10 minutes.

- **Flavonoid test** [92]

Equal volumes of 50 % ethyl alcohol and 50 % sodium hydroxide were mixed and the alcoholic extract was added within the same volume, the yellow color indicates the presence of flavonoid.

- **Resins test** [93]

A 25 mL of 95 % ethanol was added to 5 g of plant, boiling it for 2 minutes in water bath, the extract was filtered and few drops of 4 % Hcl were added. When strong turbidity appears, indicate a positive test.

- **Saponins test** [20]

A 5 mL of aqueous extract of plant was added in a test tube and was shaken vigorously a thick foaming remained for long time; this is evidence for a positive test.

- **Sugar test** [94]

Benedict reagent was used for testing monosaccharide and prepared by dissolving 173 g of potassium citrate with 100 g of anhydrous sodium carbonate in 800 mL distilled water, then boiling and filtration, the copper sulphate solution 17.3% was added to the filtrate, the volume was completed to 1liter of distilled water.

$$RCHO + 2\ Cu^{2+} + 5\ OH^{-} \rightarrow RCOO^{-} + Cu_2O + 3\ H_2O$$

Plant extract (1 mL) was taken and 2 mL of Benedict reagent was added in a test tube, then boiling in water bath for 5 minutes. A red color

precipitate, indicate the presence of sugar.

- **Phenolic compound test** [94]

A few drops of 1 % $FeCl_3$ were added to 1 mL of alcoholic extract of plant. Green, purple, blue or black color, one of them given in solution contains phenols.

- **Tannins test** [92]

A 1mL of lead acetate was added to 1 mL of alcoholic extract. Appearance of white mucilage precipitate indicates the presence of tannin.

- **Terpene and Steroid test** [94]

A 1 mL of alcoholic extract was mixed with 2 mL of chloroform; one drop of anhydrous acetic acid and one drop of concentrated H_2SO_4 were added. Brown color indicates the presence of terpene while formation blue color after leaving the mixture for a period indicates the presence of steroid.

2-2-4 GC-Mass Spectroscopy

Gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. GC- MS were used to analyze the chemical constituents of *M. communis* leaves extract. This analysis was done at the University of Kashan, Iran. The instrument used electron ionization with two columns, Helium, Nitrogen, and zero air, as a carrier gas. The furnace temperature was 400 ^{0}C; after getting a chromatogram of GC-Mass for organic compound, the constituents were identified by comparing their spectra to those stored on the computer library.

2-2-5 Preparation of *Myrtus communis* Leaves Extract

The crude extract of *M. communis* was made by weighing 10 g of dried leaves and adding them to 100 mL of deionized water, then heating the combination for 10 minutes on a hot plate at 50 °C. The mixture was filtered using Whatman No. 1 filter paper. The filtrate was used for synthesis CuNPs (95).

2-2-6 Synthesis of Copper Nanoparticles (CuNPs)

For the synthesis of CuNPs, aqueous solution of (1mM) of copper sulfate ($CuSO_4.5H_2O$) was prepared, then various volumes of aqueous leaves extract of *M. communis* 10% (3, 5, and 10 mL) (the pH of the extract was adjusted to pH 10 by the addition of 0.1 mM NaOH) was mixed with 10 mL aqueous solution of (1mM) $CuSO_4 \cdot 5H_2O$ and stirred continuously for 30 minutes at room temperature. The reduction took place rapidly, which is indicated by the change in color of the solution. The mixture was incubated at room temperature overnight. The mixture was centrifuged at 8000 xg for 15minuets to get copper nanoparticles. The nanoparticles were purified and washed three times by deionized water with repeated centrifugation; finally, the pellets of CuNPs were dried using an air oven and collected for further use.

2-2-7 Effect of Heating Time of Extract

To measure the influence of heating time to prepare the aqueous leaves to extract *M. communis*. A 10 g of cut leaves were boiled with 100 mL of deionized water for (5, 10, and 15 min) respectively. The color change in the solution from yellow to brown was followed using UV-visible spectroscopy at different time periods.

2-2-8 The Influence of the Extract Volume

To measure the effect of extract volume, various volumes of aqueous

extract (3, 5, and 10 mL) respectively was added into 10 mL of (1 mM) $CuSO_4 \cdot 5H_2O$; the synthesized CuNPs were observed as a function of time of reaction using UV-visible spectroscopy at periods and follow the exchange of color with the time.

2-2-9 Effect of pH of Extract

pH is an essential factor for any synthesis reaction, especially for biochemical reactions. The development of pH values of leaves extract on the synthesis of CuNPs was studied using various pH (5, 6, 7, 8,9 and 10) with the constant volume and concentration of $CuSO_4 \cdot 5H_2O$ (10 mL, 1mM) and temperature (50˚C). The effect of pH values was analyzed using UV–visible spectroscopy.

2-2-10 Effect of $CuSO_4 \cdot 5H_2O$ Concentration on Synthesis of CuNPs

The concentration of precursor is also a significant parameter for nanoparticles synthesis. The concentration of $CuSO_4 \cdot 5H_2O$ was optimized by varying the concentration of $CuSO_4 \cdot 5H_2O$ (1mM, 2mM, and 3mM) with the constant other factors, the volume of extract (10 mL), pH of the medium (10), and temperature (50˚C). The effect of $CuSO_4 \cdot 5H_2O$ concentration was evaluated using UV–visible spectroscopy.

2-2-11 Characterization of Synthesized Copper Nanoparticles

2-2-11-1 UV-Vis Spectroscopy

The synthesis of CuNPs was confirmed by a color change and UV-visible absorption spectra. The absorbance of the solution mixture was measured at different periods, and the maximum absorption was determined with the scanning at the range from 300 to 700 nm.

2-2-11-2 Fourier Transform-Infrared (FT-IR) Spectroscopy

FT-IR analysis was done for both extract and synthesized CuNPs. FT-

IR spectroscopy is commonly used to examine interactions between NPs and capping agents. Using this method, the interactions of the functional group and the metal NPs can be checked when two or more functional groups are present in the capping agent.

- **Sample Preparation for FT-IR Spectroscopy**

Copper nanoparticles were purified then filtrate and pellet washed in deionized water. Process was repeated three times, removes the filtrate and dried pellet. After drying pellet powder well, the pellet was measured with the KBr disk in the wavelength ranging from 400 to 4000 cm^{-1}.

2-2-11-3 X-ray Diffraction Analysis

X-ray diffraction (XRD) is an effective non-destructive method for the characterization of crystalline materials. This provides information on structures, phases, desired crystal orientation (texture), and other structural parameters, such as average crystal size, crystallinity, strain, and crystal defects.

The synthesized CuNPs were characterized using (XRD) (Philips Xpert /Holland XRD) with Cu-Kα radiation (λ =1.50456) in the range of (10^0 to 80^0). Different phases present in the synthesized samples were determined by X′ pert high score software with search and match facility. The crystal size of the prepared samples was calculated using the Scherer equation as follows:

$$D = 0.9\,\lambda / \beta \cos\theta \text{ ------} \qquad (2\text{-}1)$$

Where D is the average of crystal size, λ is the wavelength of X-ray, β is the half maximum of the full width, and θ is Bragg's angle in radians. Cu-kα1 radiation (λ =1.50456) was used in the range of 10^0 to 80^0. This analysis was done in Iran.

2-2-11-4 Atomic Force Microscopy (AFM).

The Atomic Force Microscope (AFM) is commonly used for the topological investigation of surfaces of biomaterials also in the presence of adhered cells with atomic resolution. In addition to topographic measurements, AFM is also capable of quantifying surface roughness of samples down to the nano-scale.

The size and the surface properties of biosynthesized nanoparticles were visualized by an atomic force microscopy (CSPM Scanning Probe Microscope). Highest measurement was obtained using AFM image analysis software.

- **Sample preparation for AFM**

The powder of copper nanoparticles mixed with ethanol, dropped on glasses slide, and then left on air to dry at room temperature after that the sample was examined.

2-2-11-5 Scanning Electron Microscopy (SEM)

A scanning electron microscope (SEM) scans a focused electron beam over a surface to create an image. The electrons in the beam interact with the sample, producing various signals that can be used to obtain information about the surface topography and composition. This analysis was done in Iran.

- **Sample Preparation for SEM**

The powder of CuNPs was mixed with ethanol and dropped on glasses slide, and then left on air to dry at room temperature.

2-2-11-6 Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) is a microscopy technique whereby a beam of electrons is transmitted through an ultra-thin specimen,

interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen. It provides information about the structure, crystallization, morphology and stress of a substance.

- **Sample Preparation for TEM**

The powder of CuNPs was mixed with ethanol then dropped on glasses slide, and then left on air to dry at room temperature after that the sample was examined.

2-2-12 Biochemical Applications

2-2-12-1 Antioxidant Assay

The synthesized CuNPs from *M. communis* leaves extract was evaluated for their antioxidant activity using three methods. Ascorbic acid was used as a reference.

2-2-12-1-1 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Assay

- **Principle**

At room temperature, the DPPH reagent (1,1-diphenyl-2-picrylhydrazyl) is a stable radical with a strong absorption band centered at about 517 nm. It has a deep violet color in solution and becomes colorless or pale yellow when neutralized by radical scavengers. It thus offers a quick and easy method to measure the radical scavenging activity (90).

$DPPH^{\bullet}$ is characterized as a stable free radical due to the delocalization of the spare electron over the molecule. Thus, the molecule cannot dimerize, as would happen with other free radicals (63). When $DPPH^{\bullet}$ reacts with a hydrogen donor, the reduced (molecular) form (DPPH) is generated, accompanied by the disappearance of the violet colour. Therefore, the absorbance diminution depends linearly on the antioxidant

concentration (96).

- **Solutions:**

DPPH (0.135 mM) was prepared freshly by dissolving 0.0053g in 100 mL ethanol.

- **Procedure:**

The free radical scavenging capacity of the extracts and CuNPs were determined using DPPH (97).

1. A serial of dilutions of each CuNPs , *M.communis* extract ,and ascorbic acid were prepared (5, 10, 20, 25, 50,and 100) µg/mL respectively.
2. An equal volume 1 mL of DPPH (0.135 mM in ethanol) and CuNPs or *M.communis* extract was mixed.
3. The mixture was vortexed vigorously and it was left in the dark at room temperature for 30 minutes.
4. The control was prepared as above without addition extract or CuNPs.
5. The absorbance of the samples and control were measured at 517 nm. Ascorbic acid was used as a positive control.

- **Calculations**

The ability of *M. communis* extract and CuNPs to scavenge the DPPH radical was calculated as a percentage of inhibition.

$$\% \text{ Inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100 \text{ ------ (2-2)}$$

where A is the Absorbance.

2-2-12-1-2 Reducing Power.

- **Principle**

The reducing power was determined according to the method described

by (98). In this process, an absorbance increase can be correlated to the reducing ability of antioxidants. The compounds with antioxidant capacity react with potassium ferricyanide, to form potassium ferrocyanide. The latter reacts with ferric trichloride, yielding ferric ferrocyanide, a blue coloured complex, with a maximum absorbance at 700 nm.

• Solutions:

- Phosphate buffer (pH 6.6) was prepared by dissolving 34.83 g of K_2HPO_4 and 2.7 g of KH_2PO_4 in 1L of the deionize water.
- K_3 $Fe(CN)_6$(1%w/v) was prepared by dissolving 1 g of it in 100 mL deionize water.
- Trichloroacetic acid (TCA) solution (10%): was prepared by dissolving 10 g in100 mL deionize water.
- Ferric chloride ($FeCl_3$) (0.1%) was prepared by dissolving 0.1 g in 100 mL deionize water.

• Procedure [(99)]:

1. A 1 mL of each CuNPs and *M.communis* extract at various concentration (100, 50, 25, 20, 10 , and 5) µg/mL respectively, was mixed with equal volume of phosphate buffer (0.2 M ,pH 6.6) and K_3 $Fe(CN)_6$ (1% w/v).
2. The mixture was incubated at 50 ^{o}C for 20 minute and the 1 mL of trichloroacetic acid (TCA) solution (10%) was added to stop reaction.
3. The mixture was centrifuged at 6000 xg for 15 minutes.
4. The supernatant solution (1.5 mL) was mixed with distilled water (1.5 mL) and 0.1 mL of ferric chloride (0.1%) for 10 minutes.
5. The absorbance was measured at 700 nm. As the absorbance of the reaction mixture increased, reducing power of the CuNPs increased.

2-2-12-1-3 Determination of Total antioxidant Activity

- **Principle**

The antioxidant capacity of CuNPs and *M.communis* extract was measured spectrophotometrically through phosphomolybdenum method described by (98), which was based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 695 nm.

- **Solutions**

- **Reagent Solutions**

The reagent solution (4 mM ammonium molybdate, 28 mM sodium phosphate and 0.6 M sulfuric acid) was prepared by mixing 3.2 mL of concentrated sulfuric acid (18 M), 0.0397 g of anhydrous sodium phosphate, and 0.494 g of ammonium heptamolybdate, all these materials were dissolved in 100 mL of deionized water.

- **Procedure [100]:**

1. An aliquot of 0.3 mL of each diluted extract and synthesized CuNPs at concentration (5, 10, 20, 25, 50 and 100 µg/mL) respectively was mixed with 3 mL for reagent solution.
2. The tubes were incubated at 95 ^{0}C for 90 minutes.
3. The mixture was cooled.
4. The absorbance of the solution was measured at 695 nm.
5. Control (Ascorbic acid) was prepared as above accepting CuNPs or extract.

- **Calculations**

The antioxidant activity was calculated as a percentage (%) as

following:

$$\% \text{ Total antioxidant} = [(A_{Control} - A_{Sample}) / A_{Control}] \times 100 \text{ ----- (2-3)}$$

where A is the Absorbance.

2-2-12-2 Catalytic Activity

• Principle

The reduction of 4-nitrophenol by an excess of $NaBH_4$ in the presence of metal NPs was often used as a model reaction to evaluate the catalytic activity of different systems. The reduction of 4-nitrophenol to 4-aminophenol is of industrial importance as 4-aminophenol is a commercially important intermediate for the manufacturing of analgesic and antipyretic drugs. The reduction of 4-nitrophenol can be monitored by successive decrease of peak intensity at 400 nm (attributed to the presence of 4-nitrophenate ions) (71).

• Solutions:

- Sodium borohydride ($NaBH_4$) was prepared by dissolving 0.757 gm in 100 mL of the deionize water.
- 4-nitrophenol (4-NP) was prepared by dissolving 0.0028 gm of it in 100 mL deionize water.

• Procedure:

The catalytic activity of CuNPs and *M.communis* extract was determined according to the method described by (101).

1. To a 3 mL cuvette containing freshly prepared sodium borohydride (1mL, 0.2 M) solution, a 4-nitrophenol (1.9 mL, 0.2 mM) solution was added.
2. The cuvette was then placed in a UV-vis spectrophotometer, and the

absorbance against wavelengths was recorded.

3. A 0.1 mL of each CuNPs and *M. communis* extract at various concentrations (5, 10, 20, 25,50, and 100) µg/mL were added to the cuvette and shaken vigorously for mixing.
4. The cuvette was kept in a UV-vis. spectrophotometer and scanned from 200 to 800 nm ranges.

2-2-12-3 Anti-bacterial Assay:

The antibacterial activity of *M. communis* leaves extract and CuNPs was determined using the agar well diffusion method (102). The antibacterial activity was assessed against Gram-negative bacteria (*Klebsiella pneumonia and Pseudomonas aeruginosa*) and Gram-positive bacteria (*Staphylococcus aureus and Lactobacillus salivarins*).

• Procedure:

1. Muller-Hinton agar plates were inoculated with tested bacteria (10^5-10^6 CFU/mL).
2. Agar wells of 10 mm diameter were made using cork borer.
3. A serial of dilutions for both CuNPs and *M. communis* extract were prepared at various concentration (100000,75000, 50000, 25000 µg/mL).
4. All wells were filled with (100 µL) of each test sample (CuNPs and *M. communis* extract)
5. The plates were incubated at 37 °C for 24 hours.
6. The inhibition zones diameter of each sample was measured in millimeters.

2-2-12-4 *In vitro* Anti-inflammatory Activity

The anti-inflammatory activity of plant extracts and synthesized CuNPs was evaluated using three *in vitro*-based assays (protein denaturation, proteinases action, Human Red Blood Cells (HRBC) membrane stabilization) method, Aspirin was used as a reference.

2-2-12-4-1 Inhibition of Albumin Denaturation

Denaturation of proteins is a well-documented cause of inflammation (82). As part of the investigation on the changes in the anti-inflammatory activity, the ability of CuNPs and *M.communis* extract on prevent protein denaturation was studied.

• Solutions:

•Bovine serum albumin (BSA) (1% aqueous solution) was prepared by dissolving 1 gm of it in 100 mL deionize water.

• Procedure:

Method of Govindappa *et al* (103) , was followed.

1. A serial of dilutions of each CuNPs, *M. communis* extract, and aspirin were prepared at various concentration (100, 50, 25, 20, 10, 5) µg/mL.
2. The reaction mixture (0.5 mL) was prepared by adding 0.45 mL bovine serum albumin (1% aqueous solution) into test sample (*M. communis* extract, CuNPs, and aspirin).
3. The pH of reaction mixture was adjusted at 6.3 using a small amount of HCl.
4. Samples were incubated at 37°C for 20 minutes.
5. Samples were heated at 51°C for 20 minutes.
6. After cooling, the turbidity of the samples was measured

spectrophotometrically at 660 nm.

• Calculations

• The Percentage of inhibition of protein denaturation was calculated as follows:

$$\% \text{ inhibition} = [(A_{\text{Control}} - A_{\text{Sample}}) / A_{\text{Control}}] \times 100 \text{ ----- (2-4)}$$

where A control is the absorbance of solution without test sample, A sample is the absorbance of solution with sample extract or CuNPs or standard.

2-2-12-4-2 Protein inhibitory action

Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a rich source of proteinase which carries in their lysosomal granules many serine proteinases. It was reported that leukocyte's proteinase plays an important role in the development of tissue damage during inflammatory reactions and a significant level of protection was provided by proteinase inhibitors (104).

• Solutions:

• Trypsin was prepared by dissolving 0.01 g in 100 mL deionized water

• Tris HCl buffer pH (7.4)

• Casein was prepared by dissolving 0.8 g in 100 mL of sodium hydroxide (NaOH)

• Procedure:

The test was performed according to the method described by Govindappa *et al* (103) .

1. A serial of dilutions of each CuNPs , *M.communis* extract ,and Aspirin were prepared at various concentration (5 , 10, 20, 25, 50, 100) µg/mL.

2. The reaction mixture (2 mL) was containing 0.06 mg trypsin, 1 mL (20 Mm) Tris HCl buffer (pH 7.4) and 1 mL test sample (CuNPs , *M.communis* extract , and Aspirin).

3.The mixture was incubated at 37 °C for 5 minutes.

4. Casein 0.8% (w/v) was added to the mixture.

5. The mixture was incubated for an additional 20 minutes.

6. Two mL of 70% perchloric acid was added to terminate the reaction.

7. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank.

• Calculations

The Percentage of inhibition of proteinase inhibitory activity was calculated as follows:

$$\% \text{ inhibition} = [(A_{Control} - A_{Sample}) / A_{Control}] \times 100 \text{ ----- (2-5)}$$

where A is the absorbance

2-2-12-4-3 Membrane Stabilization Test

According to Chippada *et al* (105), lysosomal the stabilization of lysosomal membrane is vital in controlling the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil, such as bactericidal enzymes and proteases, which may lead to further tissue inflammation and damage upon extracellular release or by stabilizing the lysosomal membrane.

• Preparation of Red Blood cells (RBCs) suspension

Fresh whole human blood (10 mL) was collected and transferred to the heparinized tubes. The tubes were centrifuged at 3500 xg for 10 minutes and they were washed three times with an equal volume of normal

saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline (106).

• Procedure:

Heat-induced hemolysis was carried out, as described by Okoli *et al* (107).

1. Serial of dilutions of each CuNPs , *M.communis* extract ,and aspirin were prepared at various concentration (5 , 10, 20, 25, 50, 100) µg/mL.
2. The reaction mixture (2 mL) consisted of 1 mL of test sample (CuNPs, *M. communis* extract ,and aspirin) respectively and 1 mL of 10% RBCs suspension.
3. Saline was added to the control test tube, instead of test sample.
4. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56 °C for 30 minutes.
5. After incubation, the tubes were cooled under running tap water.
6. All tubes containing reaction mixture was centrifuged at 2500 xg for 5 minutes.
7. The absorbance of the supernatants was measured at 560 nm.

• Calculations

The Percentage of Membrane stabilization activity was calculated as follows:

$$\% \text{ inhibition} = [(A_{Control} - A_{Sample}) / A_{Control}] \times 100 \text{ ----- (2-6)}$$

where A is the absorbance

2-2-12-5 Hemolytic Assay

• Principle

Hemolysis in blood products is usually manifested by the presence of free hemoglobin in the red cell suspending media, such as plasma or additive solutions (108).

• Procedure

Ten healthy donors, their ages ranging from 18-40 years old, were subjected to this study. Ethylenediaminetetraacetic acid (EDTA) test tube used to collect blood. This assay was performed as described by the method of Laheeb and Al-saadi (95).

1. A 30 µL of CuNPs or extract solution at various concentrations (5, 10, 20, 25, 50 and 100) µg/mL respectively, was added to 0.2 mL of blood sample and mixed well for 5 seconds.
2. Normal saline (10 mL) was added to prevent excessive hemolysis.
3. The mixture was centrifuged at 3000 xg for 10 minutes.
4. The absorbance of mixture was measured at 540 nm.
5. Complete hemolysis (100%) was achieved by diluting blood with 100 fold of distilled water.
6. Triton X-100 used as positive control.

• Calculations

After measuring the absorbance, the percentage of hemolysis was calculated by following equation:

$$\% \text{ Hemolysis} = [(AT-AS) / (A\ 100\%-AS)] \times 100\ \% \text{ ------- (2-7)}$$

AT: Absorbance of test solution.

AS: Absorbance of normal saline.

A 100%: Absorbance of 100% hemolysis.

Chapter Three

Results

and

Discussion

3.Results

3-1 Qualitative Phytochemical Analysis of *Myrtus communis* Leaves Extract

The chemical constituents of *M. communis* leave extract were investigated. These constituents were flavonoids, resins, saponins, sugar, tannins, phenolic compound, and terpenes (Table 1).

Table (3-1): Qualitative phytochemical analysis of *M. communis* leaves extract

Phytoconstituents	Reagents	Detection indicator	Results
Alkaloids	Dragendroff's reagent	Orange spots	-
Flavonoids	50%ethanol+50% NaOH	Yellow color	+
Resins	95% ethanol+ 4% Hcl	Turbidity	+
Saponins	Shaking the extract vigorously	Foaming remaining for a long time	+
Sugars	Benedict	Red precipitate	+
Tannins	1% lead acetate	White mucilage precipitate	+
Phenolic compound	1% $FeCl_3$	Green color	+
Terpenes	Chloroform + glacial acetic acid	Brown color	+
Steroids		Blue color	-

The plants are considered natural. It has been confirmed through various studies that the reduction of metals into their respective metals nanoparticles has been carried out through plant extracts containing polyphenols, terpenoids, alkaloids, sugars, proteins, and phenolic acids (30).

Qualitative phytochemical analysis was an initial step to investigate the active compound in the extract which assure the presence of phenolic acids, tannins, and flavonoid that have biological properties like antioxidant

activity, antimicrobial effect, anti-inflammatory, anti-diabetic, anti-mutagenic, pro-apoptotic activity in cancer cells, cardiovascular, and anti-atherogenicity effects (21).

Polyphenols are major plant compounds with antioxidant activity, thought to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, squeezing and triplet oxygen, as well as decomposing peroxides (109).

Terpenes are the main group of phytochemicals of the essential oils of various types of medicinal plants which are categorized based on isoprene structure. Various types of these phytochemicals were identified as antiviral, antibacterial, and antifungal agents. It was declared that these chemicals could kill microbes through membrane disruption (110).

The complexity of extracts' chemical composition suggests the involvement of various action mechanisms and consequently, it is difficult to identify just one pathway of molecular action. It is very likely that each of the constituents of the extracts has its mechanism of action or on the other hand, the compounds could act in a synergistic way (29).

Biosynthesis of metal nanoparticles through phytochemicals is usually carried out in an aqueous solution, surface chemistry is a major factor that not only determined the various applications of the nanoparticles but also is very important for the stability of synthesized nanoparticles. Phenolic compound-coated metal nanoparticles represent more stability in comparison with nanoparticles that have been synthesized by other chemical reductants such as citrate or sodium borohydride. In general, reducing agents were adsorbed on the surface of the particles through various mechanisms (111). The assembly pattern on the metal surface depends on various intermolecular interactions between adsorbed molecules and the metal surface. Hydroxyl, carboxylic, or other functional

groups of natural compounds are ideal for adsorption on metal surfaces (112).

Various phytochemicals present in the plant extracts could play a key role in the conversion of the ionic form of copper Cu^{2+} into the metallic nano-form Cu^{0}.

A previous study reported that phenolic compounds, flavonoids, Saponins, tannins, and sugar were found in *M. communis*. These phytochemical compounds and vitamins have significant roles in reductant, capping, and stabilizing the nanoparticles. Probably, the flavonoids present in *M. communis* leaves can perform the reductant of metals salts, and the tannins and saponins may act as the capping agents. As flavonoids have various functional groups, which are capable of reductant metal ions to NPs (113).

3-2 GC-Mass Spectroscopy

In this study, GC-MS was utilized to identify the different biological components of *M. communis* leaves extract. The chromatogram of the study showed eight peaks of most constituents (Figure 1). Most of these included (S)-2-methyl -1-dodecanol, heptadecanoic acid, heptadecyl, thiosulfuric acid, trifluoroacetic acid, n-tridecyl, carbonic acid, decyl dodecyl ester, octadecanoic acid and 9-hexadecenoic acid, methyl ester (Table 2). The heights of different peaks show the relative concentration of the compounds present in the aqueous extract of *M. communis* leaves.

The GC-MS analysis of plant extract results in the identification of several phytochemicals, which have pharmacologically and industrially potential like antioxidants, and several unsaturated fatty acids. The GC-mass analysis displayed the existence of phytochemical components in *M. communis* leaves extract. These compounds have several biochemical and biological activities (114).

Gas chromatography and mass-analysis of aqueous leaves extract of *M. communis* detected the chemical compounds as a percentage of the area which represents the major compounds. Palmitic acid or n-hexadecanoic acid, ethyl ester is recommended to be a saturated fatty acid and it might act as an antioxidant, anti-androgenic, hypocholesterolemic, hemolytic, and alpha-reductase inhibitor. Hexadecanoic acid has earlier been reported as a component in the alcohol extract of the leaves of *Melissa officinalis* (115).

A 9-octadecanoic acid is the main fatty acid in the extract. This fatty acid is characterized for several applications in the biomedical field (anti-arthritic, anti-inflammatory, anti-fungal, anti-tumor, and anti-bacterial). These molecules indicate the medicinal roles of *M. communis* (116). A previous study showed that Octadecanoic acid is present in most plants such as *Aesculus hippocastanum, Cassia Obtusifolia, Jasminum Sambac Linn, Cenchrus setigerus*, etc. Parasuraman *et al*. (2009) identified 17 compounds with n- Octadecanoic acid and hexadecanoic acid as the major compounds in the leaves of *Cleistanthus collinus* (117).

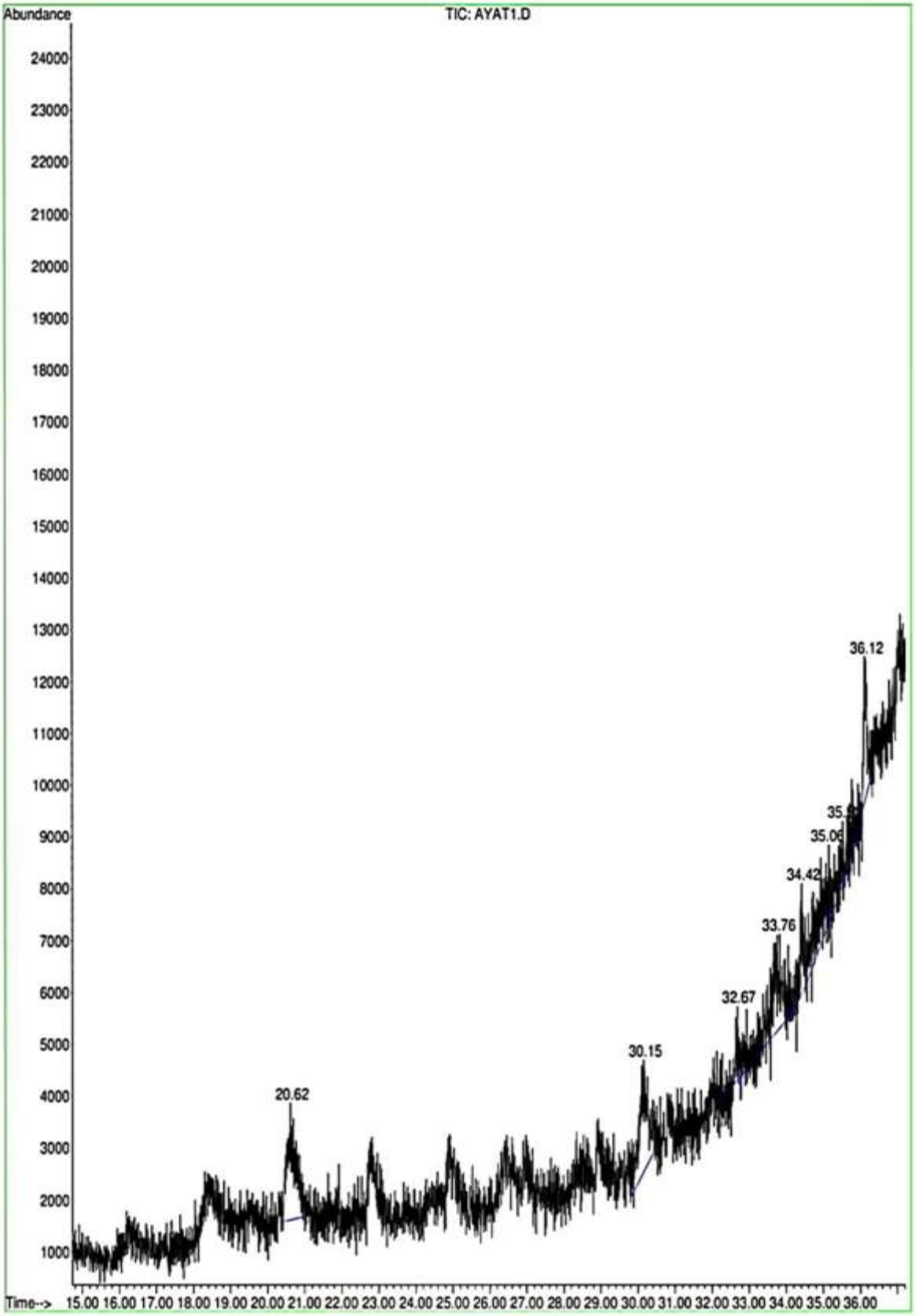

Figure (3-1): GC-MS of *M. communis* leaves extract

Table (3-2): GC-mass analysis of *M. communis* leaves extract

Peak	Retention Time	Area %	Name
1	20.62	18.38	(S)-2-methyl -1-dodecanol Hexyl octyl ether
2	30.15	20.36	9-Hexadecenoic acid, methyl ester, (Z)- 1-Hexacosanol
3	32.67	2.73	Pentadecafluorooctanoic acid, octadecyl ester Hexatriacontyl pentafluoropropionate
4	33.76	20.51	N-(2-Phenylethyl) undeca-(2Z,4E) Heptadecanoic acid, heptadecyl
5	34.42	7.36	9-Octadecenoic acid (Z) Heptadecanoic acid, heptadecyl
6	35.06	12.99	Thiosulfuric acid ($H_2S_2O_3$) cis-Vaccenic acid 9-Octadecenoic acid
7	35.51	4.37	Phenol, 4-bromo-2-(1,2-dimethyl Trifluoroacetic acid, n-tridecyl
8	36.12	13.30	Heptadecanoic acid, heptadecyl Carbonic acid, decyl dodecyl ester

3-3 Synthesis of Copper Nanoparticles

The addition of copper sulfate solution to the aqueous extract of *M. communis* resulted in a color change from yellow to brown due to the synthesis of CuNPs and the color intensity increased with time (Figure 3-2). Copper ions of $CuSO_4.5H_2O$ were reduced to CuNPs. The crude extract of *M. communis* is considered a reducing agent for Cu^{2+} and stabilizer for CuNPs. The first indication for CuNPs construction was the color change within 24 hours, indicating the excitation of surface plasmon resonance of CuNPs. It is the oscillatory movement of electrons existent in the conduction band that causes the surface plasmon resonance (SPR) phenomenon. The color change gradually was followed via measuring UV-visible absorption with time.

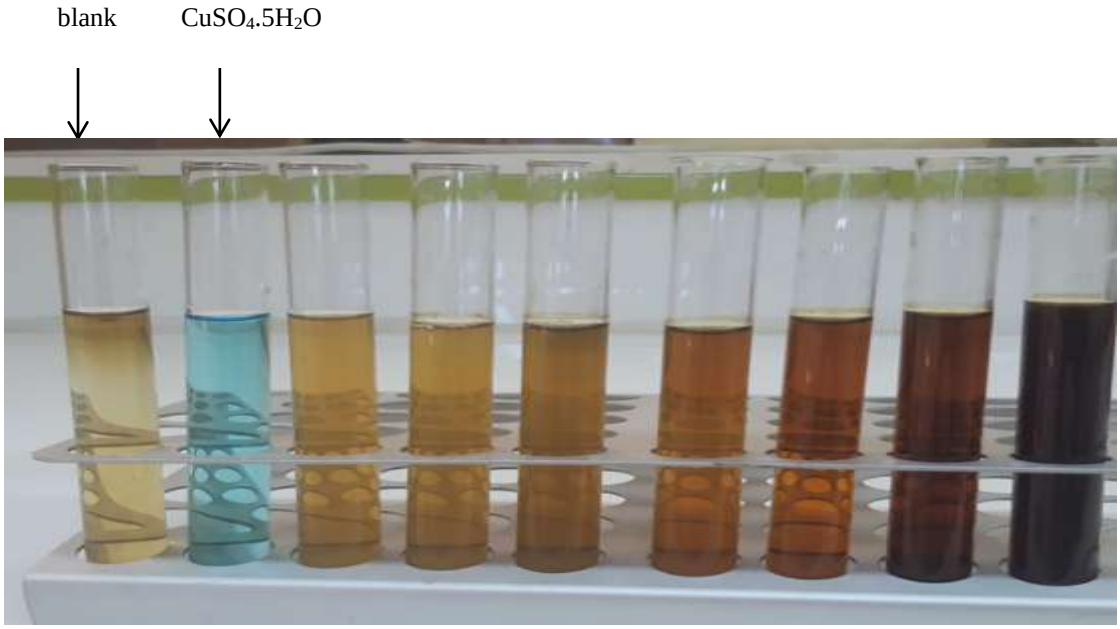

Figure (3-2): The gradual change in the color of the synthesized CuNPs with time.

The exposure time of reducing agents to metal ions is responsible for the synthesis of nanoparticles so, the synthesis is increased as the reaction incubation time of copper with the reductant increases (118).

The color change in the aqueous extract with copper sulfate solution may be due to the presence of bioactive compounds in the aqueous extract. Various phytochemicals are responsible for the reduction of copper ions Probably, the flavonoids or phenolic compounds in *M. communis* leaves act as the reductions of metal ions, whereas saponins and tannins may act as the capping agents. Moreover, polyhydroxy groups may be responsible for the reduction of Cu^{2+} ions into metal NPs (60).

A previous study reported that the color changes in the reaction mixture strongly indicate the reduction of Cu^{2+} ions to Cu^{0}, it was reported that *Magnolia Kobus* leaves extract reduced Cu^{2+} ion into CuNPs was followed by color changes in the reaction mixture from yellow to dark brown due to the excitation of surface plasmon resonance (119).

The intensity of absorption peak increased with increasing period of

mixing plant extract with an aqueous solution of $CuSO_4.5H_2O$ (Figure 3-3), the absorbance was constant even after 24 hours, indicating the completion of the reaction that is meaning that CuNPs prepared are not aggregate. This increase in the absorbance intensity was due to the growth of copper nanoparticles (54).

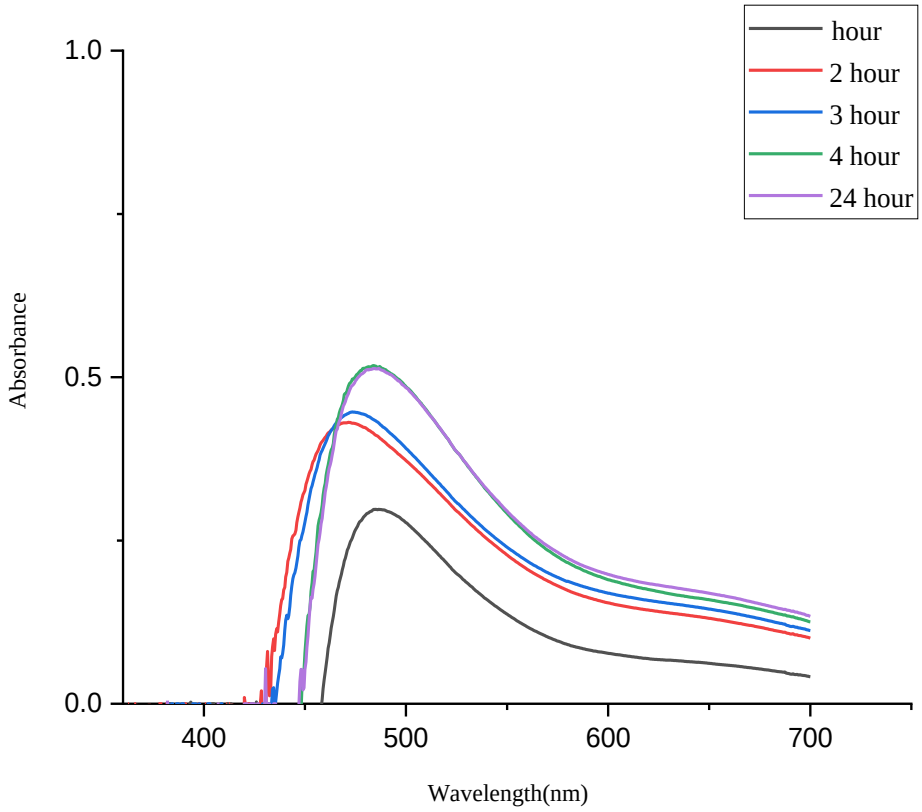

Figure (3-3): UV-visible spectra of CuNPs at a different time of incubation

3-4 Characterization of the Synthesized Copper Nanoparticles

3-4-1 UV-Visible Spectroscopy

The synthesis of CuNPs from *M. communis* leaves extract was firstly characterized with their absorbance spectra using UV-Vis spectroscopy, where Surface Plasmon Resonance (SPR) absorption band at 481 nm after 24 hours (Figure 3-4). The broad, single, and strong absorption peaks showed the formation CuNPs in the sample. To estimate the effect of the boiling time of the leaves extract of *M. communis*, the dry leaves were

boiled for 5, 10, and 15 minutes respectively. The UV-Vis spectrum of synthesized CuNPs was measured at different times. The results revealed that there was very little difference in the formation of CuNPs in 5 and 15 minutes boiling time whereas 10 min boiling time increased the formation of CuNPs (Figure 3-5). The effect of the amount of plant extract of *M. communis* to be added to an aqueous solution of copper sulfate for the formation of CuNPs, different volumes of 10 minutes boiled leaves extract (3, 5 and, 10 mL) respectively were added to 10 mL of $CuSO_4.5H_2O$ (1mM) solution. The UV-vis spectra showed an increase in the intensity of surface plasmon absorbance when increasing filtrate volume (Figure 3-6). In 10 mL of filtrate, the maximum absorbance indicates the complete reducting of copper ions and rapid synthesis of CuNPs. At the wavelength 481 nm, the volume 10 mL of *M. communis* was the best than 3 and 5 mL.

The result of the effect substrate concentration on the synthesis CuNPs showed that a concentration of (1mM) copper sulfate yields the best results, whereas the concentrations of 2mM and 3mM showed the lowest intensity of reaction (Figure 3-7).

The addition of leave extracts to $CuSO_4.5H_2O$ had no effect on the formation of nanoparticles when pH was considered. Copper-NPs were obtained after the pH of the extract medium was changed to basic by adding 0.1 mM NaOH (Figure 3-8). The solution turned light brown at pH 5, 6, 7, and 8 showed the peaks at the range of wavelengths (378-412 nm)respectively, which refers to form Cu_2o (120). It was found that the formation of CuNPs began at pH 10 and absorption peaked at 481 nm, revealing the formation of CuNPs. It was observed that with an increase in pH, the absorption peak shifted towards a higher wavelength, indicating an increase in the size of synthesized CuNPs. As the diameter of the particle increases, the energy required to excite the surface plasmon electrons decreases as a result, the absorption maximum shifted towards a longer

wavelength. In addition to the spectral shift, there was an increase in the absorption intensity with an increase in pH . Moreover, it was observed that higher pH increases the rate of reduction as the color of the solution turned brown more instantly as compared to a solution of lower pH hence, alkaline pH is fantastic for the synthesis of CuNPs (121).

Hence, the optimal formation of CuNPs was obtained using 10 minutes boiling time of the leaves extract, 10 mL volume of *M. communis* extract, the concentration of copper sulfate solution (1mM), pH 10 of leaves extract, and incubation at 50^{o}C, all these conditions were the best and selected for synthesizing CuNPs.

These conditions were chosen due to the rapid change color of the reaction mixture and increased absorbance which indicated increases in the synthesized CuNPs.

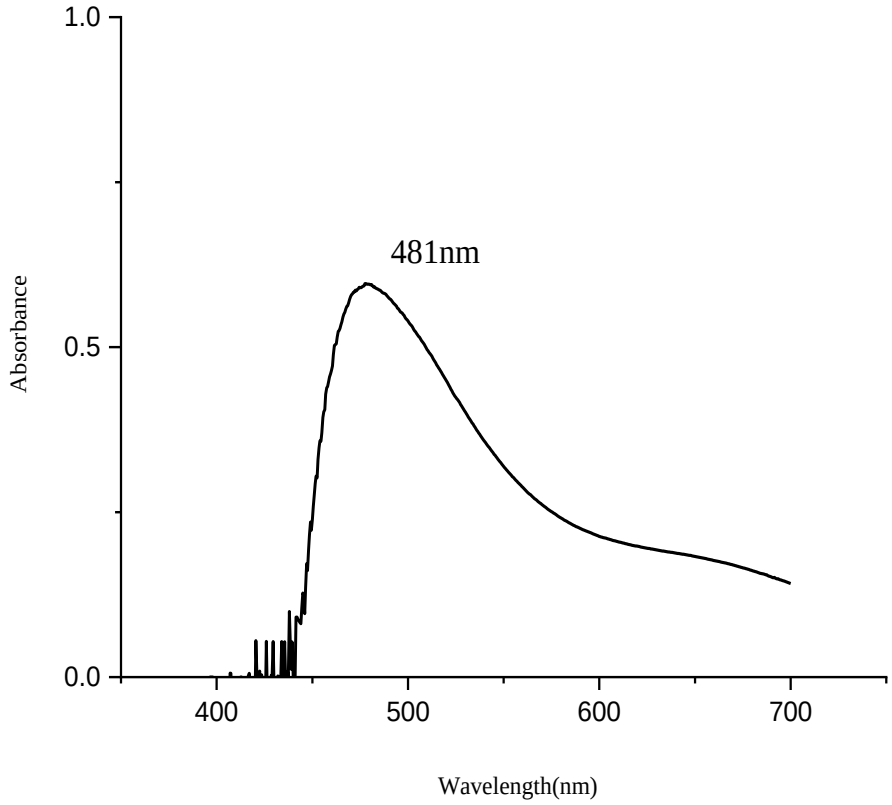

Figure (3-4): UV-Visible spectra of CuNPs at 481 nm.

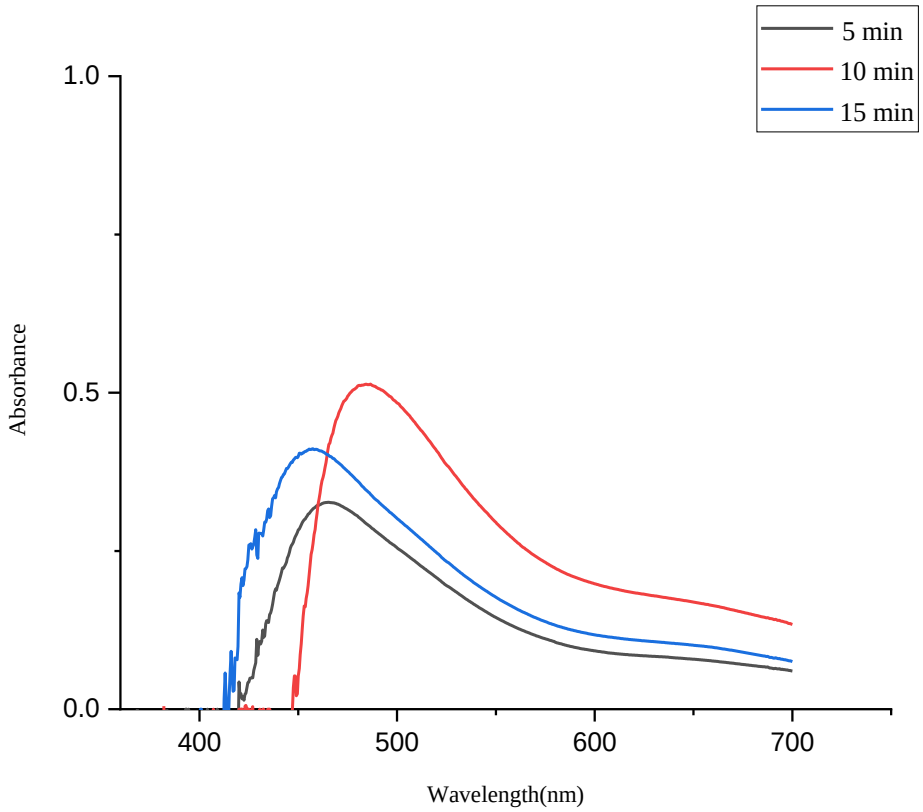

Figure (3-5): UV-Visible spectra for CuNPs at different boiling times of extract.

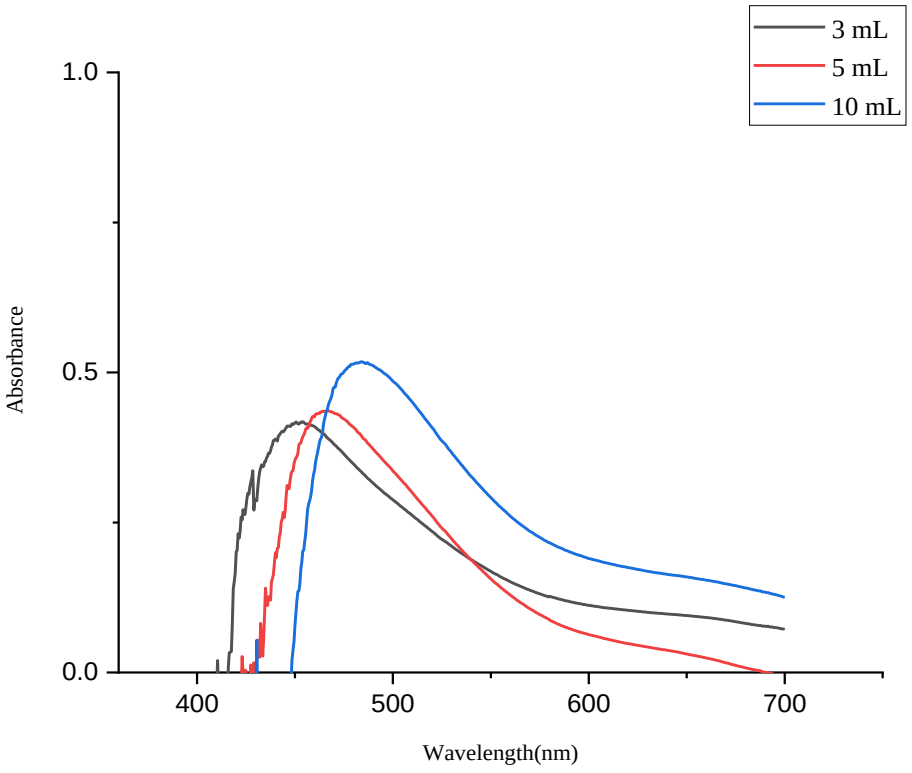

Figure (3-6): UV-Visible spectra for CuNPs at different volumes of extract

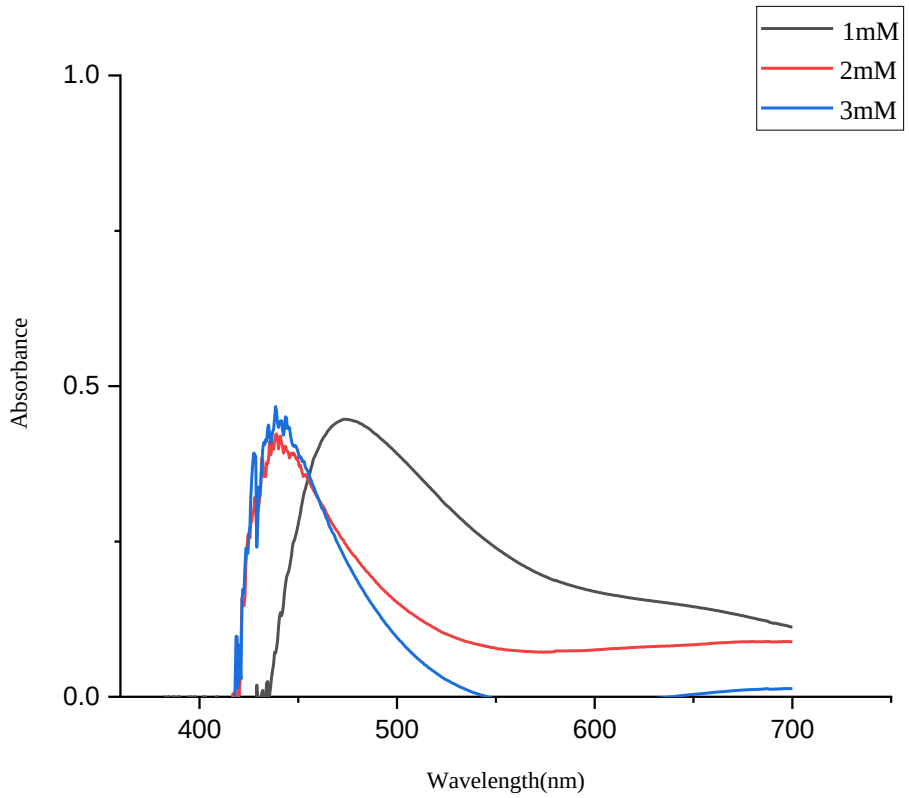

Figure (3-7): UV-Visible spectra for CuNPs at different $CuSO_4 \cdot 5H_2O$ concentrations.

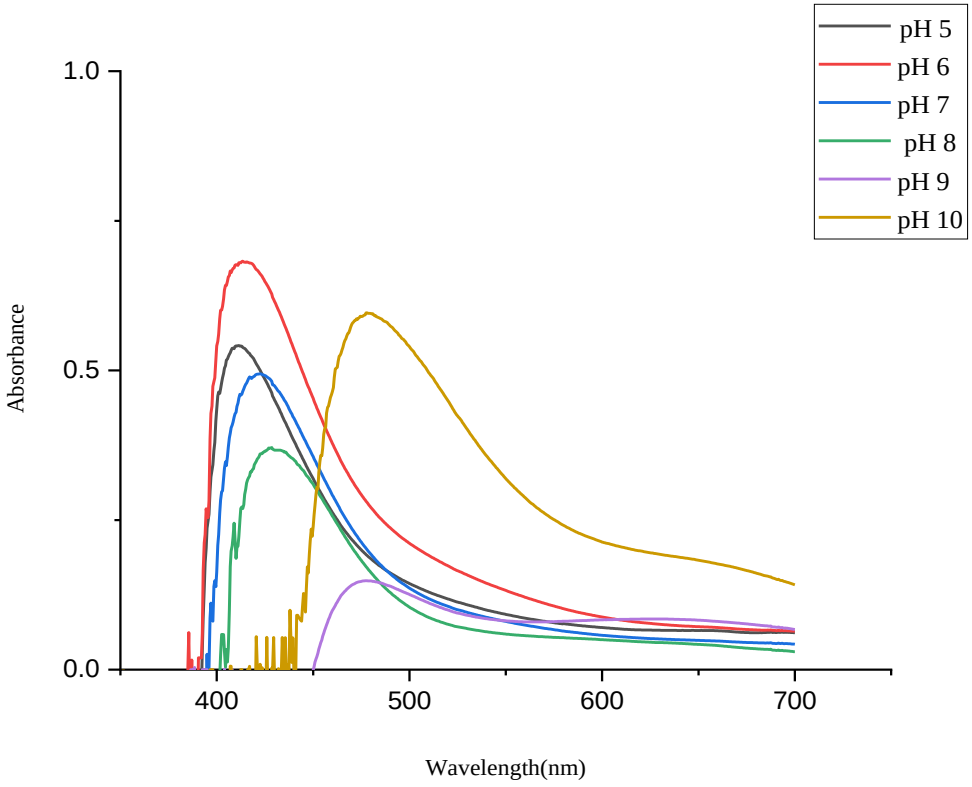

Figure (3-8): UV-Visible spectra for CuNPs at different pH

Characterization of Cu nanoparticles is essential to assure the synthesis of CuNPs and know whether the preparation is suitable for a specific application. Copper-NPs are known to show a maximum UV–visible absorption at the range of 400-600 nm due to surface plasmon resonance. Previous study has shown that the spherical shape of CuNPs participates in the band absorbance at around 450-600 nm in the UV – visible spectra. These bands, absorbance was assumed to match the CuNPs nature with comparatively small size (122).

The exact position of the SPR band may shift depending on the individual particle properties including size, shape, and capping agents (2).

The pH affects the size, shape, and morphology of synthesized CuNPs. The considerable effect of the reaction pH is its potential to alter the electrical charges of the biomolecules present in *M. communis*, which might change their reducing and capping ability and the upcoming growth of nanoparticles (123). A similar result was found in the extract of guava fruit (*psidium guajava L*) and the optimal pH for synthesizing CuNPs was at 10 (124).

3-4-2 Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR characterization is used to identify the functional groups and observe the interaction between biomolecules of *M. communis* extract (Figure 3-9) and CuNPs (Figure 3-10). The FT-IR spectrum of *M. communis* leaves extract showed peaks at 3335.03cm^{-1}(O–H stretching vibrations), 2937.68 cm^{-1} (C–H) and CH_2 vibration of aliphatic hydrocarbons, 1726.35 cm^{-1} (C=O stretching vibration), 1616.40 cm^{-1} (C=C stretching vibrations), 1452.45 cm^{-1} (O–H bending vibrations), 1367.58 cm^{-1} (C–O stretching of the ester group), 1240.27 cm^{-1} (C–O asymmetric stretching in cyclic polyphenolic compounds) and 1039.67 cm^{-1} (O–H deformation), whereas synthesized CuNPs had spectrum peaks at 3429.55

cm^{-1} due to formation aromatic Cu-NPs pheromone(O-H groups), also some signals emerged in 2922.25, 1649.19, 1554.68, and 1066.67 cm^{-1} are related to C-H asymmetric, stretching C=O of aromatic rings, C=C stretching and C-OH bending, respectively. The *M. communis* extract peak locations and absorption intensities were compared to those produced from CuNPs, and results showed that some band positions and absorption intensities from the plant extract peak were replicated in the CuNPs FT-IR spectrum with a slight shift in peak position. Moreover, peaks at 3335.03 cm^{-1} and 3140.22 cm^{-1} in the FT-IR spectrum of the leaves extract are shifted to 3429.55 cm^{-1} in the FT-IR spectrum of CuNPs. Moreover, a band at 1726.35 cm^{-1} is present in the plant extract which has disappeared in the FT-IR spectrum of CuNPs.

Another band at 713.69 cm^{-1} was observed in the FT-IR spectrum of CuNPs due to the presence of CuNPs, as it is not present in the FT-IR spectrum of leaves extract. Therefore, the absence of the carbonyl band of leave extract and appearance of a new peak at 713.69 the cm^{-1} in the FT-IR spectrum of CuNPs indicated that interaction of biomolecules of leaves extracts occurred the through carbonyl band with CuNPs.

The FT-IR spectrum of CuNPs was characteristic of proteins suggesting their role in the stabilization of the nanoparticles (125). The analysis of FT-IR studies confirmed that the carbonyl groups from the amino acid residues have a stronger ability to bind metal that is capping of copper nanoparticles to prevent agglomeration and thereby stabilize the nanoparticles (126). This suggests that the biological molecules perform dual functions of formation and stabilization of CuNPs in the aqueous medium. Other study have proven that proteins can bind with minerals and metallic nanoparticles through free amine groups or reduce cysteine in the proteins (127). Many other researchers found similar FT-IR spectra of CuNPs (128). Similarly, the same phytochemical compounds (flavonoids, phenolic, triterpenoids,

proteins or organic acid, and polysaccharides) and the active groups (O-H, C-H, C=O, and C=C) that are present in plant extract of *Ocimum Sanctum* have been reported to be capable of acting as the important roles of reducing and capping agents in the synthesis of CuNPs (129).

As a result, it appears more likely that many functional groups, such as alcohols, ketones, aldehydes, alkenes, and carboxylic acids, that are present as plant metabolites and reducing sugars of *M. communis* plants are responsible for the reduction of copper ions and stabilization of synthesized (CuNPs).

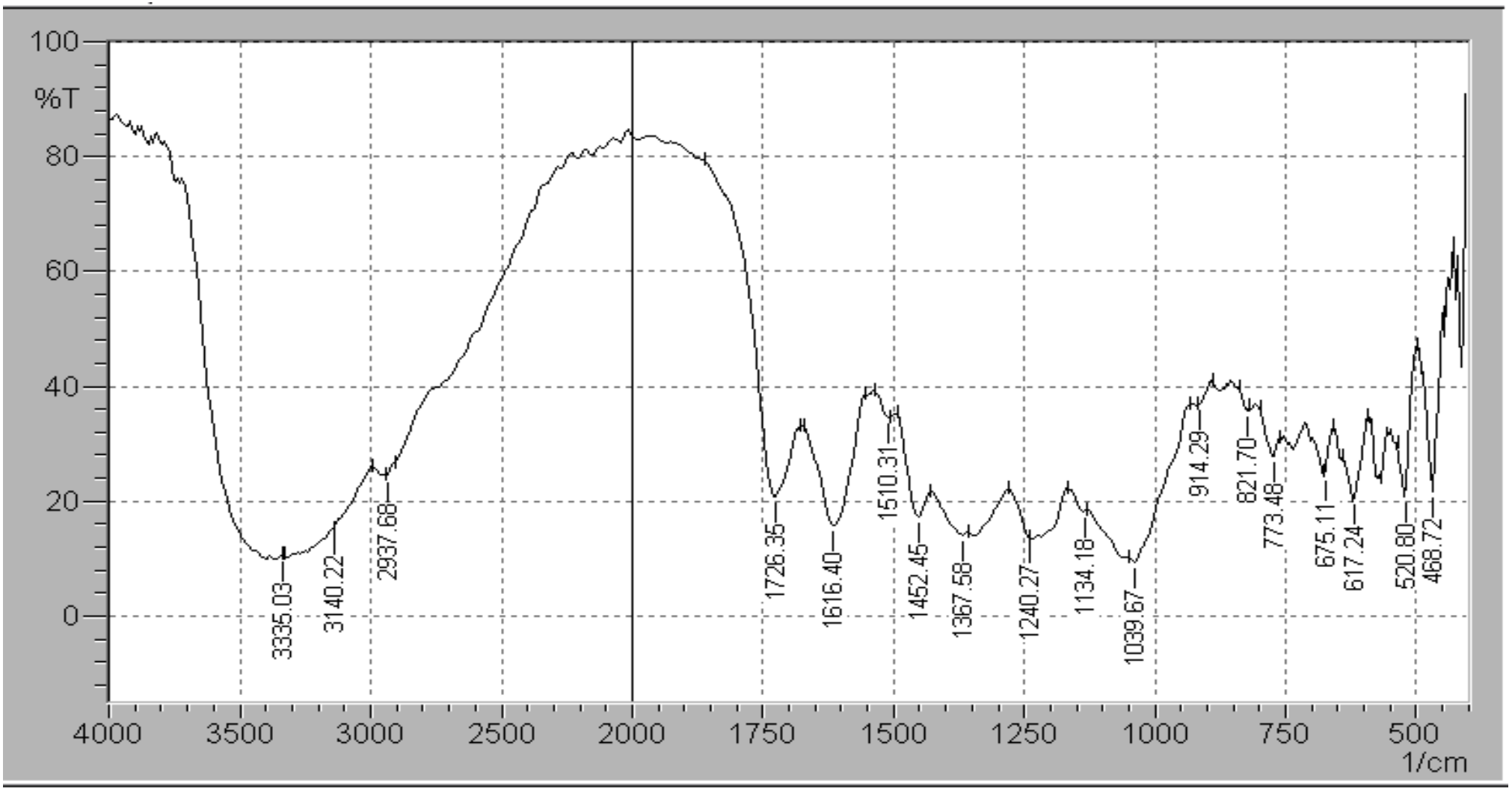

Figure (3-9): FT-IR spectrum of *M. communis* leaves extract

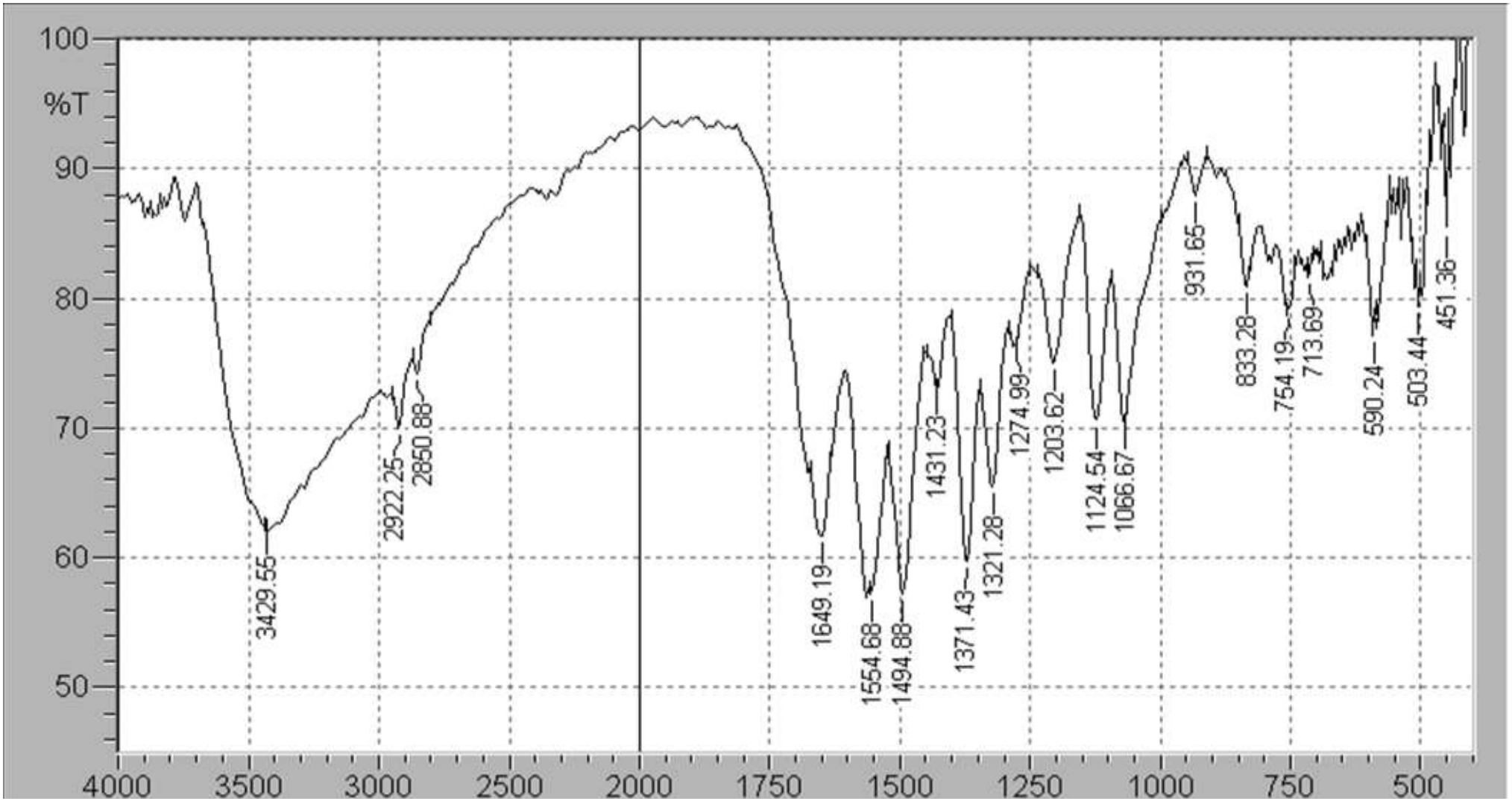

Figure (3-10): FT-IR spectrum of synthesized CuNPs

3-4-3 X-ray Diffraction (XRD)

XRD is an easy technique to determine the size and the shape of the unit cell for any compound. Diffraction pattern gives information on translational symmetry - size and shape of the unit cell from peak positions and information on electron density inside the unit cell, namely where the atoms are located from peak intensities (130).

The crystalline nature of the CuNPs was verified using X-ray diffraction analysis. The X-ray diffraction analysis revealed three featured peaks at 2θ values of 43.43, 50.58, and 74.23°, corresponding to (111), (200), and (220) planes of copper (Figure 3-11). The diffraction peaks and Miller indices (hkl) to each peak are assigned, values of these angles were compared with those 2θ of CuNPs standard sample (JCPDS PDF card 04–0836), which confirmed the Face Centered Cubic (FCC) structure of the formed CuNPs using aqueous extract of *M. communis* and indicated that the particles were crystalline. The crystallographic planes of each angle value were also demonstrated and compared with the standard data file of CuNPs. The average crystal size of CuNPs was estimated to be 39.16 nm.

The intensity of the peak reflects the high degree of crystallite of the copper nanoparticles. The diffraction peak was broad signalize that the crystallite size is very small (131). X-ray diffraction results show that the synthesized copper nanoparticles are crystalline. The Brag reflection at 43.43, 50.58, and 74.23°, at 2θ values, confirmed the crystalized structure of copper nanoparticles.

Table (3-3) shows a comparison of our XRD spectrum with a standard value of the theoretical values of standard X-ray diffraction powder patterns.

Previous study showed the crystalline structure using XRD analysis of biosynthesized CuNPs. Kalpana *et al*, (2016) confirmed the crystalline

nature of *Tridax procumbens* leaves extract (132).

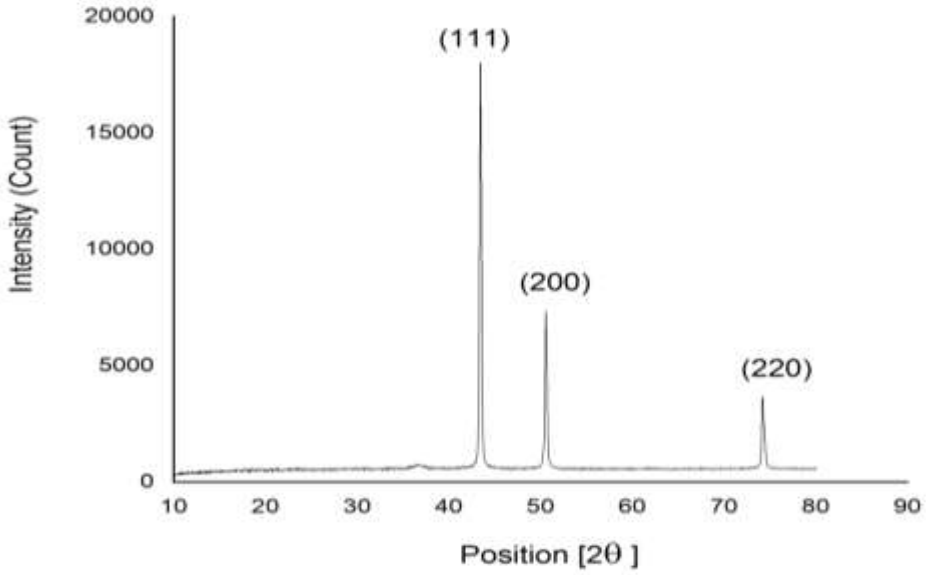

Figure (3-11): X-Ray diffraction (XRD) of synthesized CuNPs from *M. communis* leaves extract.

Table (3-3): The result of the XRD for synthesized and standard CuNPs.

2θ (Deg.)	**FWHM (Deg.)**	**d_{hkl} Exp (Å)**	**d_{hkl} Std. (Å)**	hkl
43.4300	0.2331	2.0819	2.0860	(111)
50.5800	0.2661	1.8031	1.8065	(200)
74.2300	0.3458	1.2766	1.2774	(220)

3-4-4 Atomic Force Microscopy (AFM)

The topography of the surface and the size of particles were determined by atomic force microscopy. This technique holds many advantages in measuring dispersion and particles, as this test would not be affected by the surface oxidation and conductivity (133). The measured CuNPs in this study had an average size of 55 nm. The two-dimensional and three-dimensional images of the particles, revealed homogenous

uniform size and shape of synthesized CuNPs by 10 mL of *M. communis* aqueous leave extract for boiling 10 minutes (Figure 3-12). In another study, the average size of CuNPs synthesized from the *Ziziphus mauritiana L.* plant extract was the same average size as the particles in the current study (134).

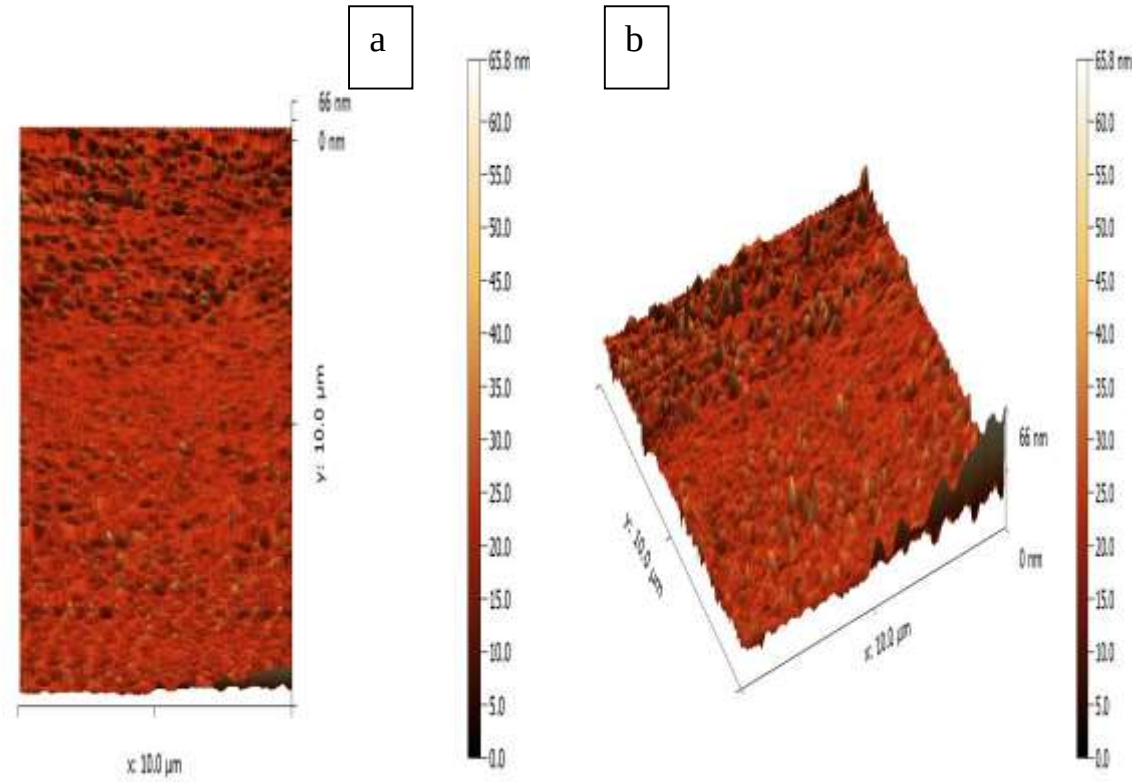

Figure (3-12): AFM assay of CuNPs synthesized by 10 mL of *M. communis* aqueous leaves extract for boiling 10 minutes. (a) Two-dimensional image (b) Three-dimensional image.

3-4-5 Scanning Electron Microscopy SEM

The morphological characteristics (number, dimension, and shape) of the biosynthesized CuNPs were investigated by Scanning Electron Microscopy (SEM) analysis. SEM images showed that the CuNPs are predominately spherical have a smooth surface and well dispersed with a close compact arrangement. The average particle diameter was found to be 34.87, 40.91, and 55.10 (Fig. 3- 13).

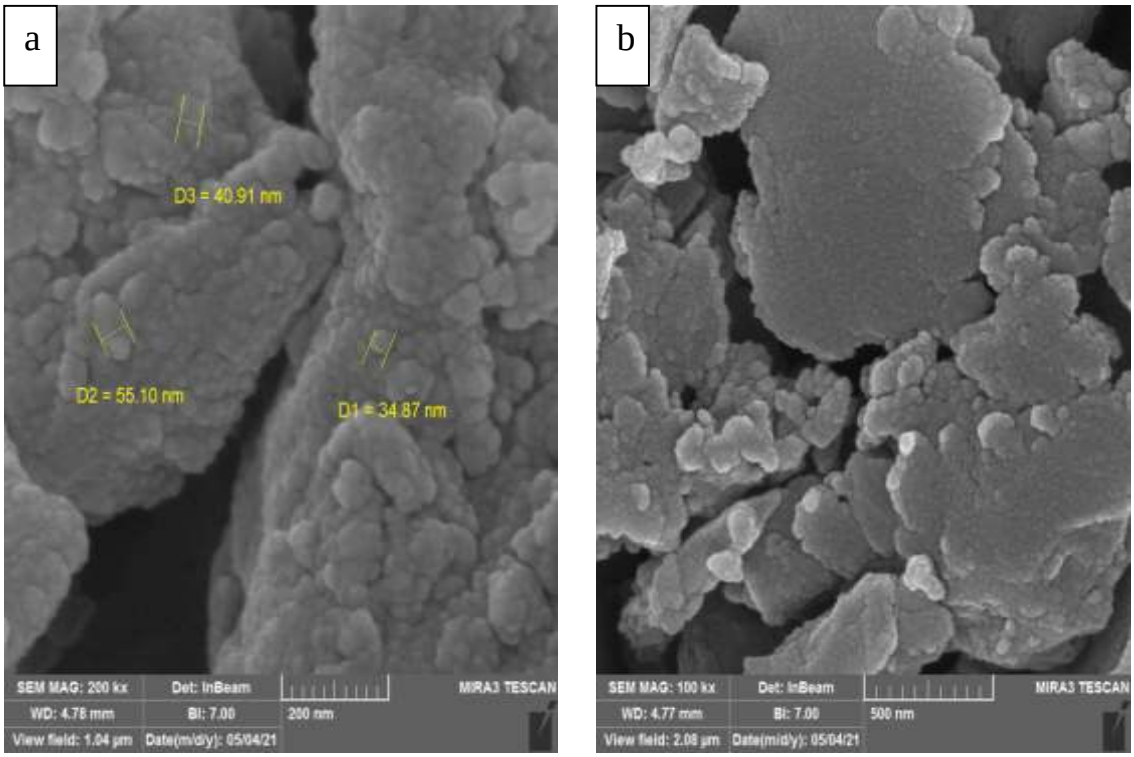

Figure (3-13): SEM image of biosynthesized copper nanoparticles (a) The particle size at different average diameters D1, D2, and D3. (b) SEM shows the spherical shape of particles.

3-4-6 Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) is an important characterization tool for the direct imaging of nanomaterials to obtain quantitative measures of particle size, size distribution, and shape. The particle size was measured using TEM (4).

TEM micrograph of copper nanoparticles is shown in (Figure 3-14) which displays spherical NPs. Figure (3-15) showed the particle size distribution based on TEM data, revealing that most of the distribution falls between 35 and 70 nm in diameter, with an average mean diameter of 55 nm. The presence of an organic compound in plant extract allows particle size to be controlled and at the same time avoids its agglomeration and oxidation. This synthesis method depends on the high reactivity of the copper complex as well as on its concentration and agitation speed during the reaction. Both factors are very specific for controlling the morphology,

particle size, and distribution (75).

These results are consistent with that of FT-IR and XRD studies where the phenolic compounds and flavonoids can facilitate the bioreduction of Cu^{2+} ions to Cu^{0} nanoparticles.

A similar study obtained CuNPs using *Eupatorium glandulosum* extract and displays spherical NPs with an average size of 55 nm (135).

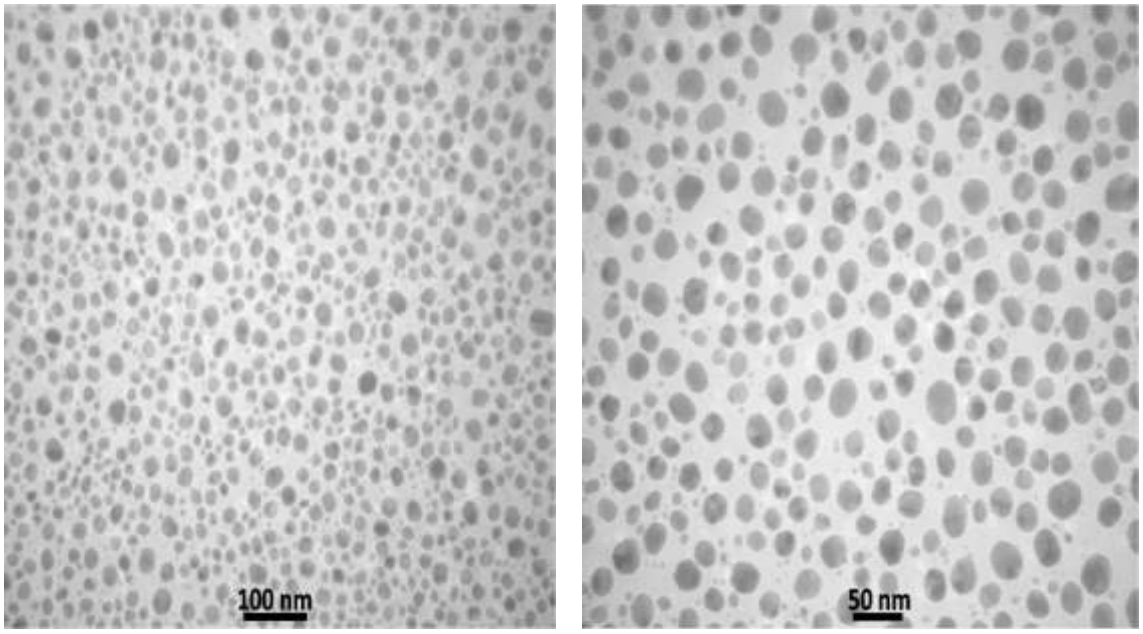

Figure (3-14): TEM images of biosynthesized CuNPs. TEM image shows CuNPs with spherical and semispherical forms.

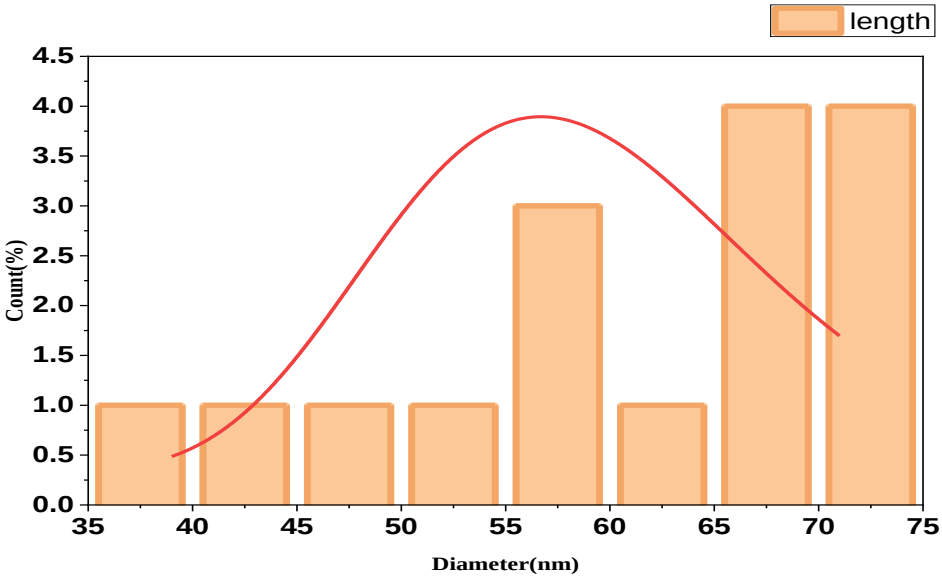

Figure (3-15): Distribution of flow diameter of the copper nanoparticles

3-5 Biochemical Activities

3-5-1 Antioxidant Activity

In this study, three different assays were used to evaluate the antioxidant of the CuNPs because the evaluation of antioxidant activity cannot be carried out accurately by the single universal method, DPPH assay, total antioxidant, and reducing power was used and ascorbic acid was used as a reference. Among the three methods used, DPPH scavenging capacity assay is the best choice because characterized by fast, stability, and simplicity. The results showed that both CuNPs and the aqueous extract of *M. communis* have a high antioxidant activity, which increased gradually with the concentration when using the DPPH method (Figure 3-16). The total antioxidant activity test is based on reducing Mo^{+6} (VI) to Mo^{+5} (V) by the antioxidant sample. The total antioxidant activity of *M. communis* leaves extract was a little more than CuNPs compared with the ascorbic acid (Figure 3-17). Besides, the results revealed that the synthesized CuNPs have a reducing power more than *M. communis* leaves extract (Figure 3-18).

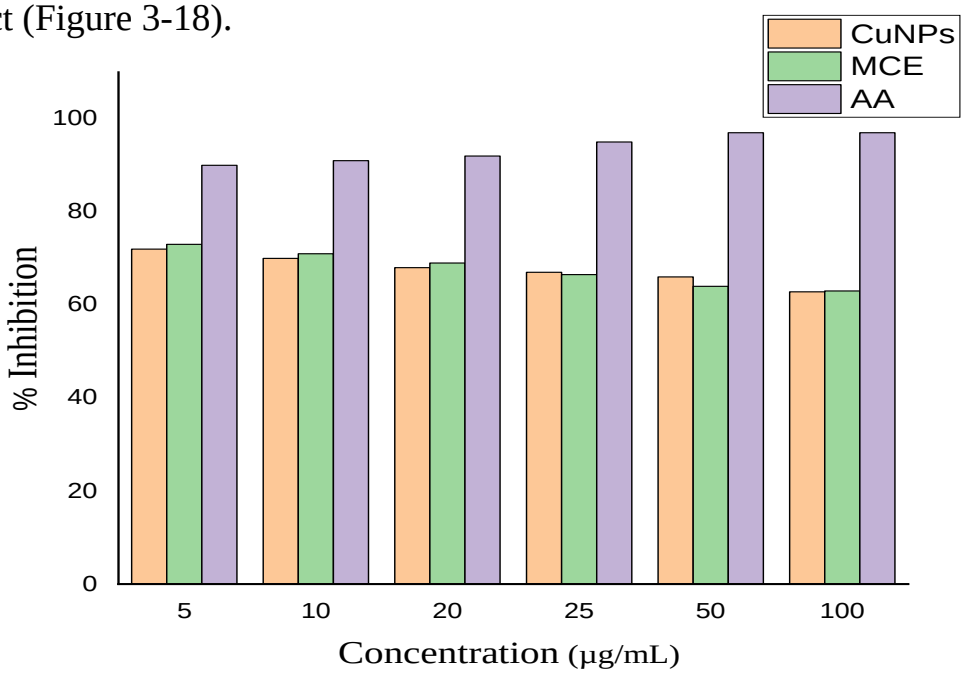

Figure (3-16): The inhibition percentage for DPPH free radicals scavenging activity of *M. communis* extract (MCE), synthesized copper nanoparticles (CuNPs), and Ascorbic acid (AA) at different concentrations.

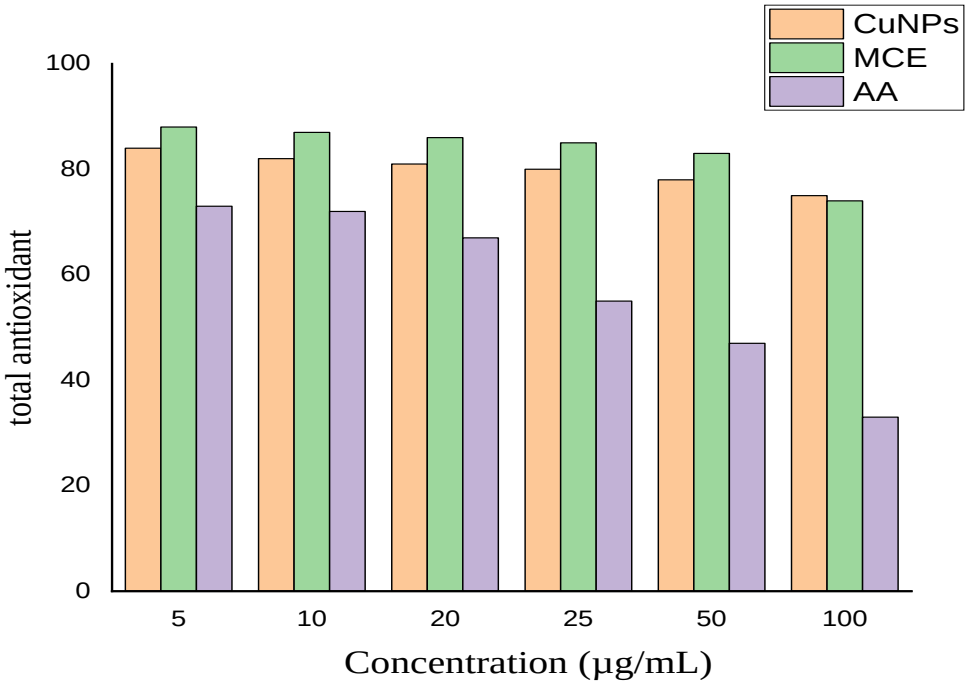

Figure (3-17): Total antioxidant ability of CuNPs, *M. communis* extract (MCE), and Ascorbic acid (AA).

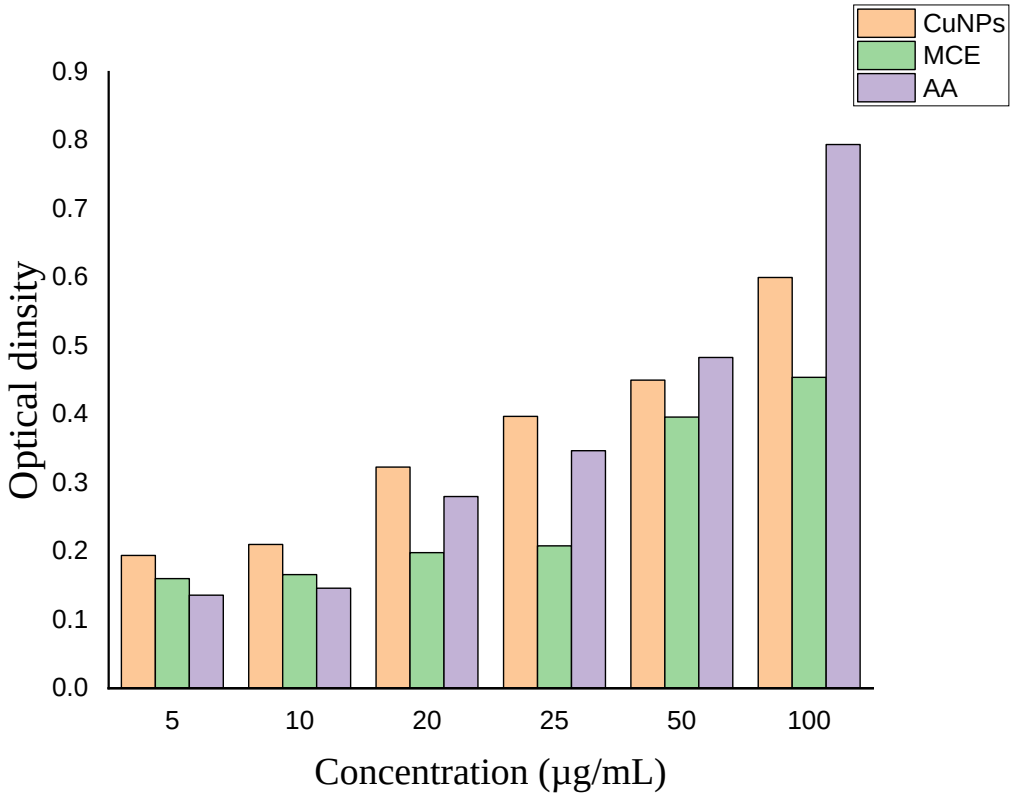

Figure (3-18): Reducing power of CuNPs, *M. communis* extract (MCE), and Ascorbic Acid (AA).

Oxidation is an essential biological process in many living organisms for the production of energy; however, the uncontrolled production of oxygen-derived free radicals. Reactive oxygen species (ROS) caused damage to complex cellular molecules such as carbohydrates, proteins, lipids, and DNA, This led to the appearance of many health problems (65).

Copper-NPs showed a good ability to scavenge free radicals when comparing it with the *M. communis* leaves extract and ascorbic acid, which is used as a well-known standard antioxidant. DPPH is a stable nitrogen-centered free radical widely used to check the radical scavenging ability of a compound or plant extract. When the stable DPPH radical accepts the electron from the antioxidant compound, the purple color of the DPPH radical was reduced to the yellow color, which was measured by a UV-vis spectrophotometer. Substances capable of conducting this reaction can be known as antioxidants and thus radical scavengers. The total antioxidant capacity method determines the ability of a sample to donate electrons and neutralize free radicals (69).

Numerous studies have been carried out on some plants, vegetables, and fruits because they are rich sources of antioxidants, such as vitamin A, vitamin C, vitamin E, carotenoids, polyphenolic compounds, and flavonoids, which prevent free radical damage, reducing the risk of chronic diseases (136).

Polyphenols had great attention is due to their perceptible antioxidant effects and have been associated with health promotion because of their antioxidant properties. They can act as reducing agents, hydrogen-donating antioxidants, singlet oxygen quenchers, and, in some cases, metal chelators. They interfere with the oxidation of lipids and other molecules by rapid donation of a hydrogen atom to radicals (25). Similar reports indicated that the CuNPs have high antioxidant activity against DPPH (137); (42).

3-5-2 Catalytic Activity

In the presence of synthesized CuNPs, 4-nitrophenol (4-NP) reduction was studied using $NaBH_4$. The aqueous solution of 4-NP showed a wavelength absorbance at 317nm, but immediately after the addition of $NaBH_4$ the redshift region at 400 nm is due to the formation of 4-nitrophenolate ion. With the addition of CuNPs, the redshift region at 400 nm dropout and simultaneously appeared the shoulder of the blue-shift wavelength at 298 nm was confirmed the reduction of a nitrophenolate ion to aminophenol as shown in (Figure 3-19), that the reaction terminates within 15 minutes in the presence of CuNPs, consistent with the disappearance of the yellow color (4-NP) at the end of the reaction to form colorless (4-AP) . At high concentrations (100 µg/mL), the UV-visible absorption spectra showed that at 400 nm, the peak completely disappeared after adding CuNPs. Furthermore, a new peak was observed at 298 nm within15 minutes (Figure 3-19a), indicating the formation of 4-aminophenol. At low concentration (50, 25, 20, 10, 5 µg/mL), the peak at 298 nm after 15 minutes not formed. While the addition of extract at all concentrations, the peak at 400 remains, and no other peak formed (Figure 3-20). In the reduction process of 4-NP to 4-AP with aqueous transfers, electrons from surface – hydrogen species transfer to NP when both of the species are absorbed on the surface of the catalyst (138).

The reduction of (NP) in the presence of these composites has been used previously to compare the catalytic activity of different metal nanoparticles immobilized in the same system. Pal *et al* (2002) were the first to identify the reduction of 4-nitrophenol (NP) to 4-aminophenol (AP) by sodium borohydride (BH_4^-) as such a model reaction (139). The finding of this study is in good agreement with previous study where they showed good catalytic activity of produced CuNPs from (*Cassia occidentalis*) leaves extract (105).

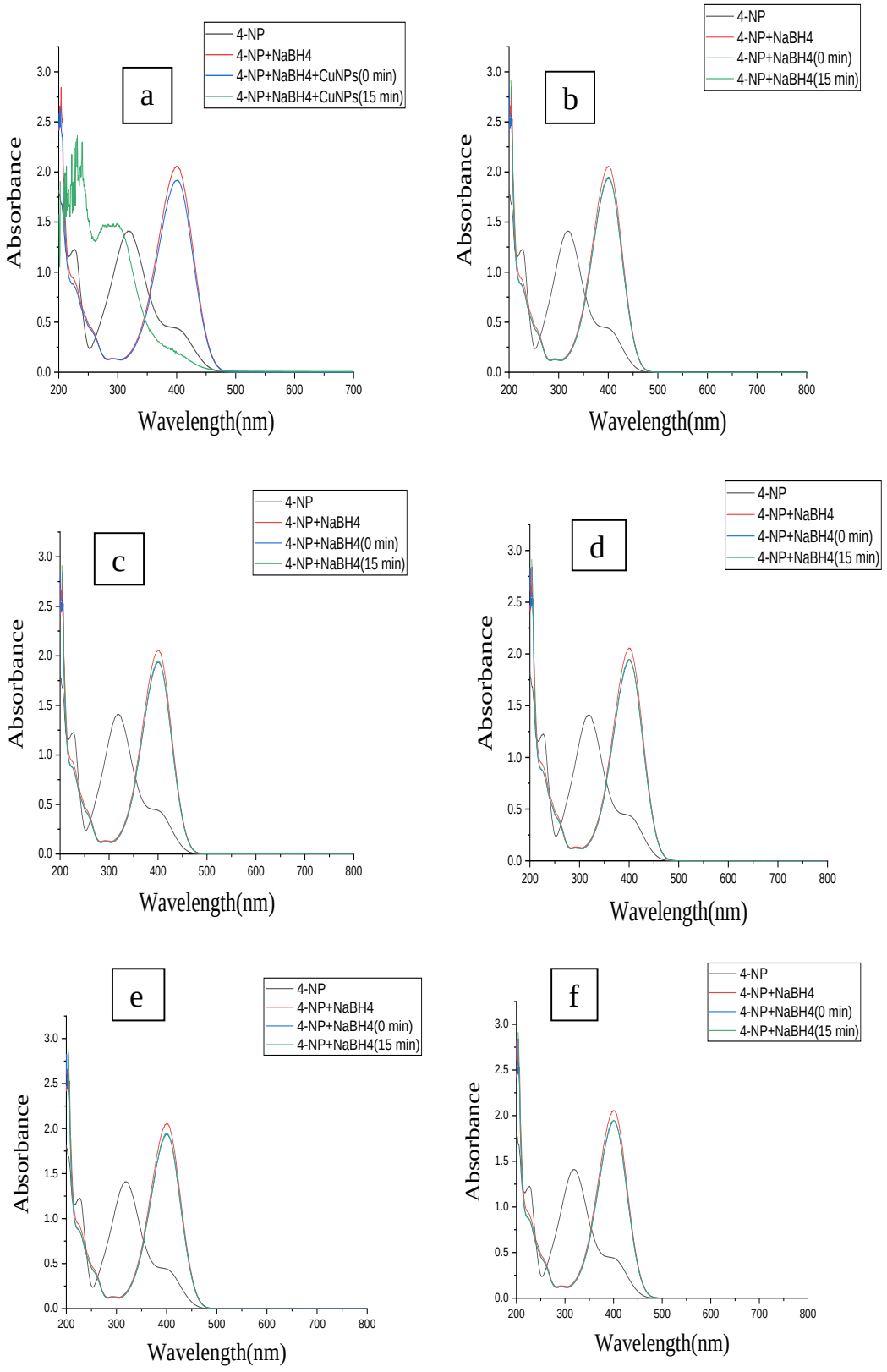

Figure (3-19): UV-visible spectrum of the reduction of 4-NP by CuNPs at concentrations (a) 100 µg/mL (b) 50 µg/mL (c) 25 µg/mL (d) 20 µg/mL (e) 10 µg/mL and (f) 5 µg/mL .

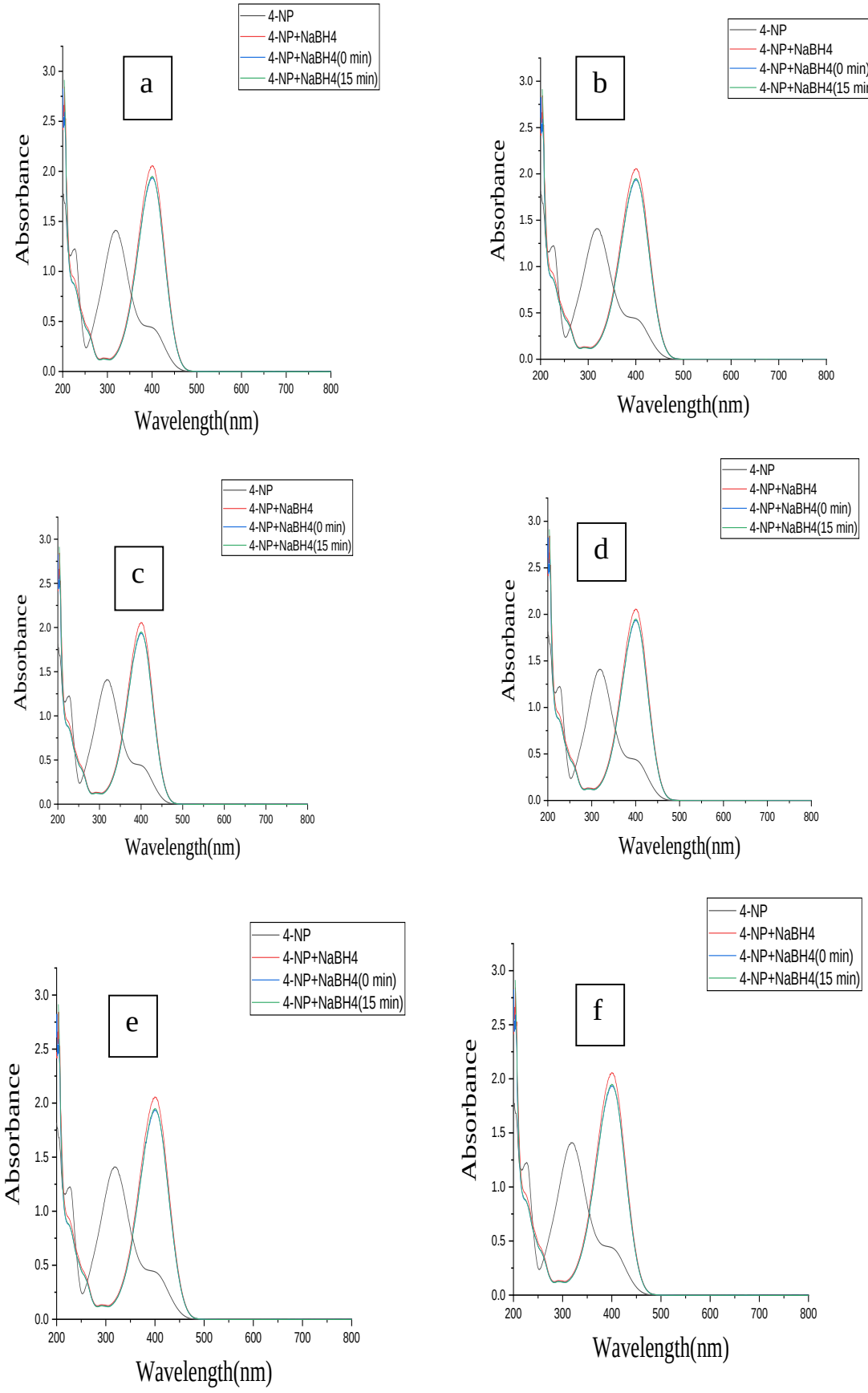

Figure (3-20): UV-visible spectrum of the reduction of 4-NP by *M. communis* at the concentrations (a) 100 µg/mL (b) 50 µg/mL (c) 25 µg/mL (d) 20 µg/mL (e) 10 µg/mL and (f) 5 µg/mL .

3-5-3 Anti-bacterial Assay

The antibacterial activity for each CuNPs and *M. communis* leaves extract were tested against Gram-positive bacteria (*Staphylococcus aureus and Lactobacillus salivarins*) and Gram-negative bacteria (*Klebsiella pneumonia and Pseudomonas aeruginosa*). As shown in (Table 3-4), *M. communis* leaves extract showed a pronounced antimicrobial activity against all the tested bacteria compared to CuNPs. *Staphylococcus aureus* and *Lactobacillus salivarins* were susceptible for *M. communis* at the concentrations (10000,75000, and 50000 µg/ mL) respectively, and they were intermediate at the concentration 25000 µg/ mL compared with antibiotics whereas CuNPs didn't show antimicrobial activity against bacteria under study except for *lactobacillus* which showed intermediate susceptible at the concentrations (10000, and 75000 µg/ mL.

These results of antibacterial activity of *M. comminus* leaves extract were compared with the Antibiotics (Amoxicillin) (Tetracycline), (Trimethoprim), and (Gentamicin), and synthesized CuNPs (Figure 3-21).

The antibacterial activity of the *M. communis* plant was attributed to its unique phytoconstituents, which included tannins, saponins, steroids, cardiac glycosides, and other compounds. It was discovered that the difference in sensitivity between different microorganisms was primarily due to the phytochemical constituents present in the plant (77).

However, it is difficult to compare the data with the literature because several variables influence the results, such as the different chemical composition due to the environmental factors (such as geography, temperature, day length, nutrients, etc) of the plant (140).

And it is not forgotten that probably the synergistic effect between all components of the essential oil of *M. communis* may be attributed to the antimicrobial activity of the essential oil Moreover, it has been reported

that myrtle essential oils of different chemotypes have shown significant inhibitory activity against Gram-positive bacteria more than Gram-negative (74).

The antimicrobial activity of *M. communis* against seven pathogen bacteria was also investigated by Akin *et al*. It was found to have some activity against both Gram-positive and Gram-negative bacteria. The higher efficacy of *M. communis* was confirmed by the agar dilution method (141).

Al-Saimary *et al* used two methods to evaluate the antibacterial activity of various concentrations of aqueous extracts of *M. communis* leave against *Pseudomonas aeruginosa* with comparison to 6 antibiotics; these methods determine the growth inhibition zones and minimal inhibitory concentration. Aqueous leaves extracts gave an excellent effect on bacterial growth and their effects were located within the limits of antibiotic effects (142).

Table (3-4): Anti-bacterial activity of CuNPs and *M. comminus* extract against some pathogenic bacteria.

Test sample	Concentration (µg/mL)	Inhibition zone diameter (mm)			
		Gram-negative		**Gram-positive**	
		Klebsiella pneumonia	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Lactobacillus salivarins*
CuNPs	100000	10	-	-	14
	75000	-	-	-	14
	50000	-	-	-	7
	25000	-	-	-	5
Extract	100000	12	9	25	23
	75000	9	7	20	20
	50000	-	-	20	20
	25000	-	-	19	18
Antibiotic (Amoxicillin)	25	-	-	15	37
(Tetracycline)	30	-	-	-	30
(Trimethoprim)	5	-	-	25	35
(Gentamicin)	10	19	-	7	14
(D.W)		-	-	-	-
(D.W+ ethanol)		-	-	-	-

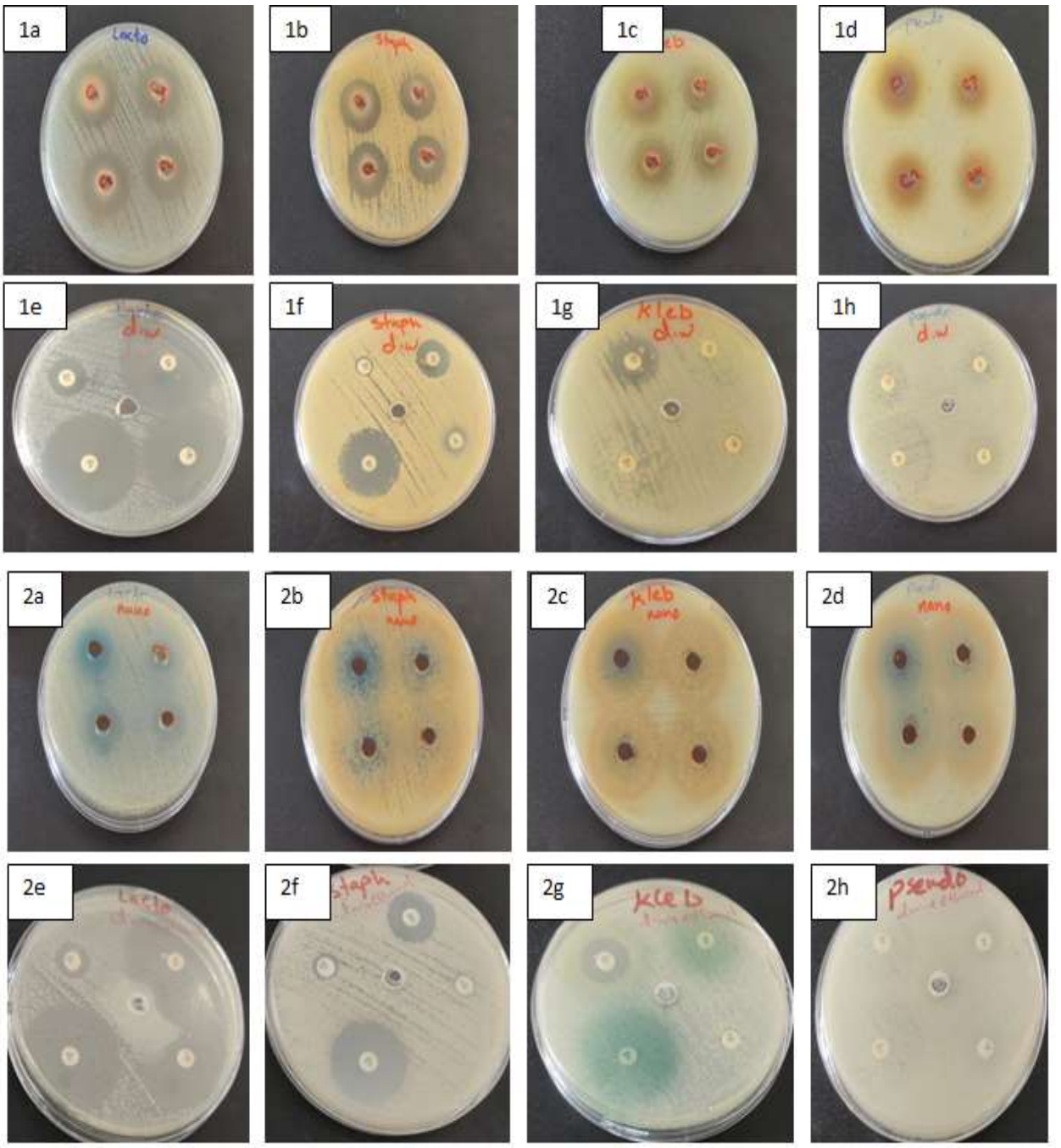

Figure (3-21): The antibacterial activity (1) *M. comminus* leaves extract and (2) (CuNPs) (a)*Lactobacillus salivarins* (b) *Staphylococcus aureus* (c) *Klebsiella pneumonia* and (d) *Pseudomonas aeruginosa*. While, 1(e, f, g, and h)and 2(e, f, g, and h) represent solvent (D.W and D.W+ ethanol) and Antibiotic (Amoxicillin), (Tetracycline), (Trimethoprim), and(Gentamicin) of *Lactobacillus salivarins, Staphylococcus aureus, Klebsiella pneumonia* and, *Pseudomonas aeruginosa* respectively.

3-5-4 Anti-Inflammatory Activity

The *M. communis* plant extracts and synthesized CuNPs were tested to the anti-inflammatory activity using various *in vitro* tests including, protein denaturation, proteinases action, HRBC (Human Red Blood Cells) membrane stabilization method, and aspirin was used as a standard anti-inflammatory drug.

It is well documented that protein denaturation is a contributing factor to inflammation. Aspects of the investigation into the mechanism of anti-inflammatory activity included the ability of the extract and CuNPs to inhibit protein denaturation, which was carried out as part of the investigation. Copper-NPs, *M. communis* leaves extract, and aspirin were all effective in inhibiting heat-induced albumin denaturation at different concentrations, as shown in (Figure 3-22), with the highest levels of inhibition (84.8%,82.4 %, and 88%) achieved at 100 µg/mL for CuNPs, *M. communis* leaves extract, and aspirin as a standard anti-inflammatory drug, respectively.

Protein denaturation is a process in which proteins lose their secondary and tertiary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent, or heat. Most biological proteins lose their biological function when denatured (143).

Copper-NPs and *M. communis* extract demonstrated significant anti-proteinase activity at various concentrations, as demonstrated in (Figure 3-23). They showed the greatest inhibition at 100 µg/mL (88.2 and 83.1 percent, respectively), whereas aspirin showed the greatest inhibition at 100 µg/mL (91.5 percent). Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a rich source of proteinase which carries in their lysosomal granules many serine proteinases. It was

previously reported that leukocyte's proteinase plays an important role in the development of tissue damage during inflammatory reactions and a significant level of protection was provided by proteinase inhibitors (106).

The percentage of inhibition of heat-induced hemolysis of red blood cells at different concentrations of CuNPs and extract is shown in (Figure 3-24). Copper-NPs showed the greatest inhibition (83.5 percent), followed by aqueous extract (76.6 percent), whereas the standard drug, aspirin, showed the highest inhibition of 87.6 percent at 100 µg/mL. RBCs membrane stabilization was studied to know the mechanism of anti-inflammatory action for each nanoparticle and the extract. The effect may prevent neutrophils from releasing their lysosomal content at the site of inflammation (107).

The injury to the RBC membrane will further render the cell more susceptible to secondary damage through free radical-induced lipid peroxidation It is therefore expected that compounds with membrane-stabilizing properties, should offer significant protection of cell membrane against injurious substances (144).

It has been reported that certain saponins and flavonoids exerted a profound stabilizing effect on lysosomal membrane both in vivo and in vitro, while tannins and saponins possess the ability to bind cations, thereby stabilizing erythrocyte membranes and other biological macromolecules (145). Because the extract contains biologically active compounds such as flavonoids and phenolic compounds, the release of neutrophil lysosomal contents at sites of inflammation may be inhibited by these compounds. Some of the components of neutrophil lysosomes are bactericidal enzymes and proteases that, when released into the extracellular environment, cause additional tissue inflammation and damage (83). A similar report indicated that the CuNPs have anti-inflammatory activity using the *Pedalium murex* plant (127).

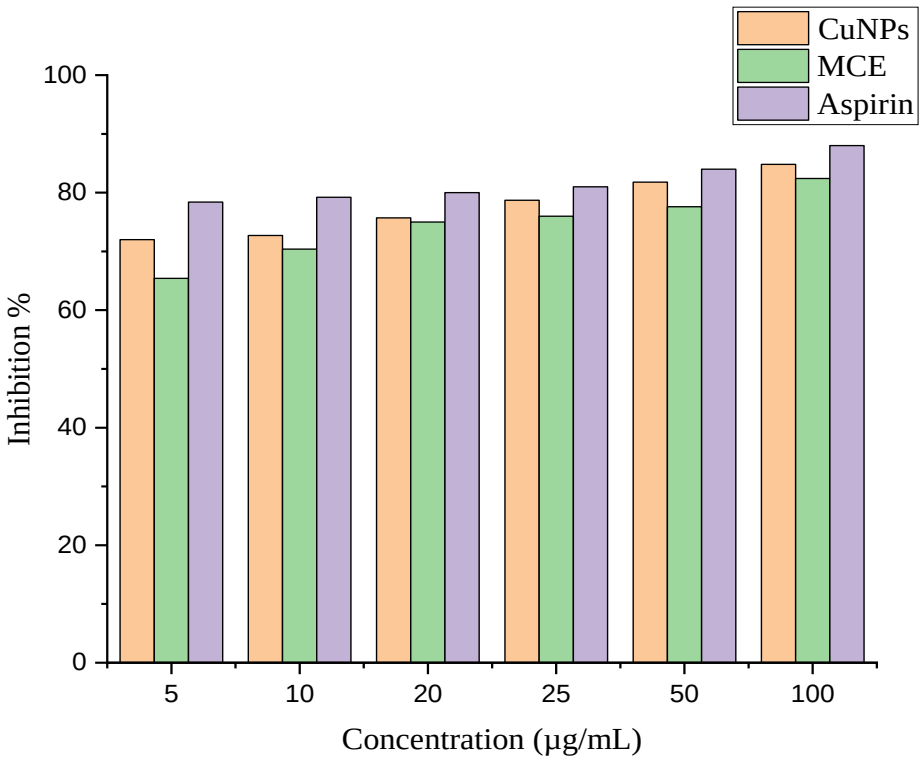

Figure (3-22): Effect of *M. communis* leaves extract and CuNPs on albumin denaturation. Aspirin as a reference (positive control).

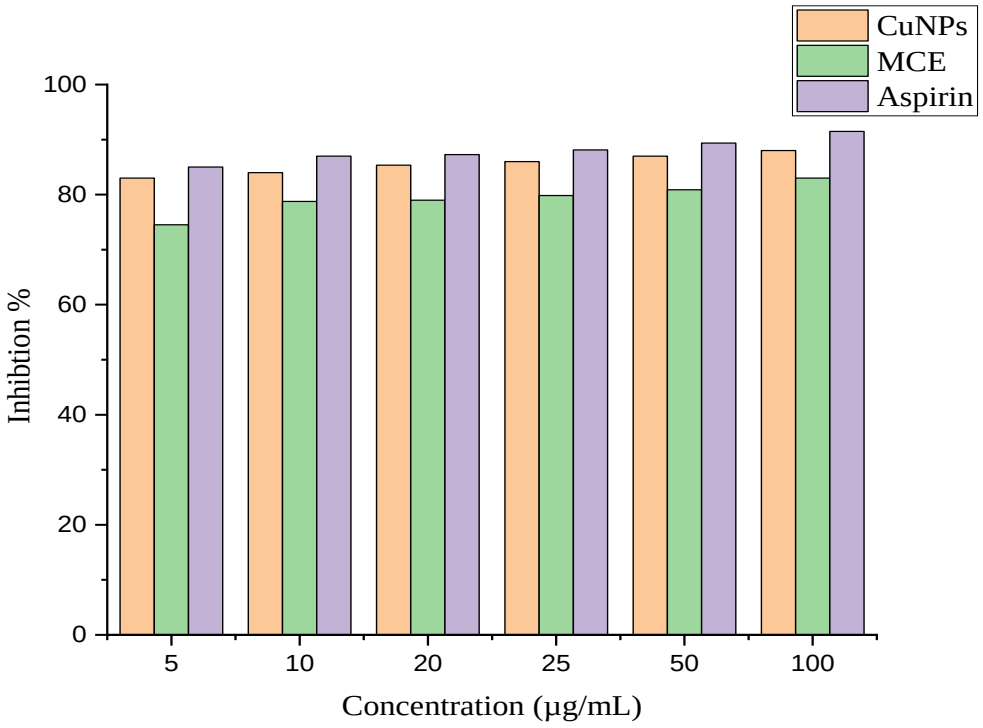

Figure (3-23): Effect of *M. communis* leaves extract and CuNPs on protein inhibitory activity. Aspirin as a reference (positive control).

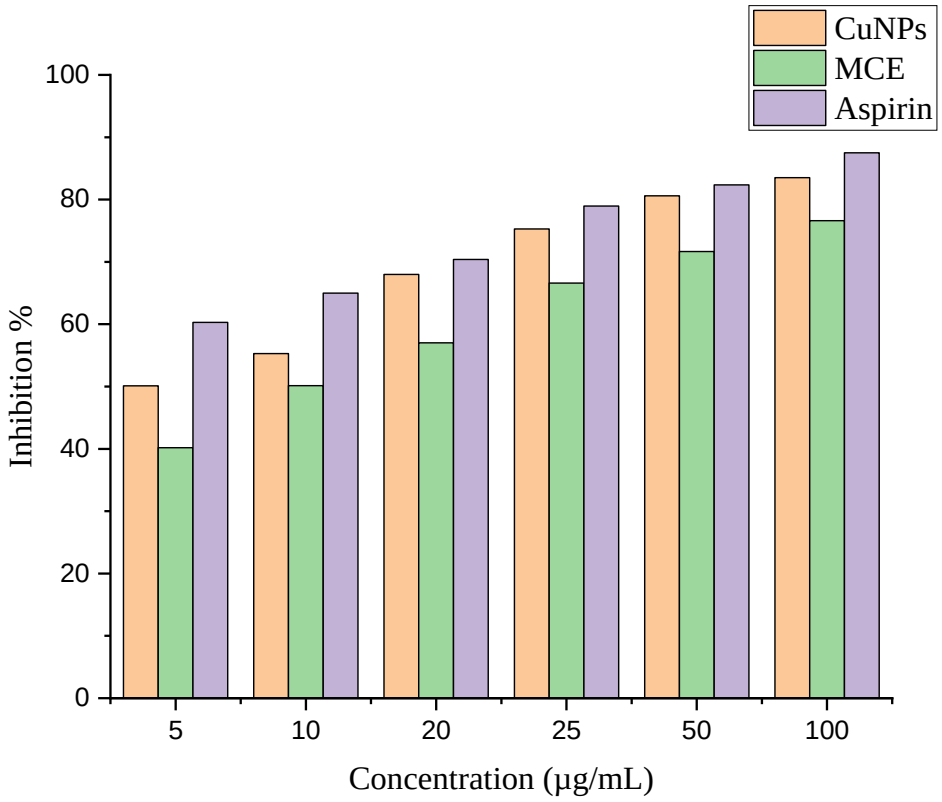

Figure (3-24): Effect of *M. communis* leaves extract and CuNPs on membrane stabilization. Aspirin as a reference (positive control).

3-5-5 Hemolytic Activity

The hemolytic activity of CuNPs from *M. communis* was tested against normal human erythrocytes. Both CuNPs and the extract showed little effect on blood hemolysis compared with negative and positive control. The hemolytic activity of the CuNPs was expressed as percentage hemolysis and reported for 10 healthy nonsmoker donors. The percentages of hemolysis induced by CuNPs were (7.35 %, 6 %, 5 %, 3.40 %, 2.01 %, 1.5 %) respectively (Figure 3-25), whereas for the extract were (7.3, 4.3, 2.67, 1.5, 0.42, 0.34 %) (Figure 3-26), at the concentrations (100, 50, 25, 20, 10, and 5 µg/mL) respectively.

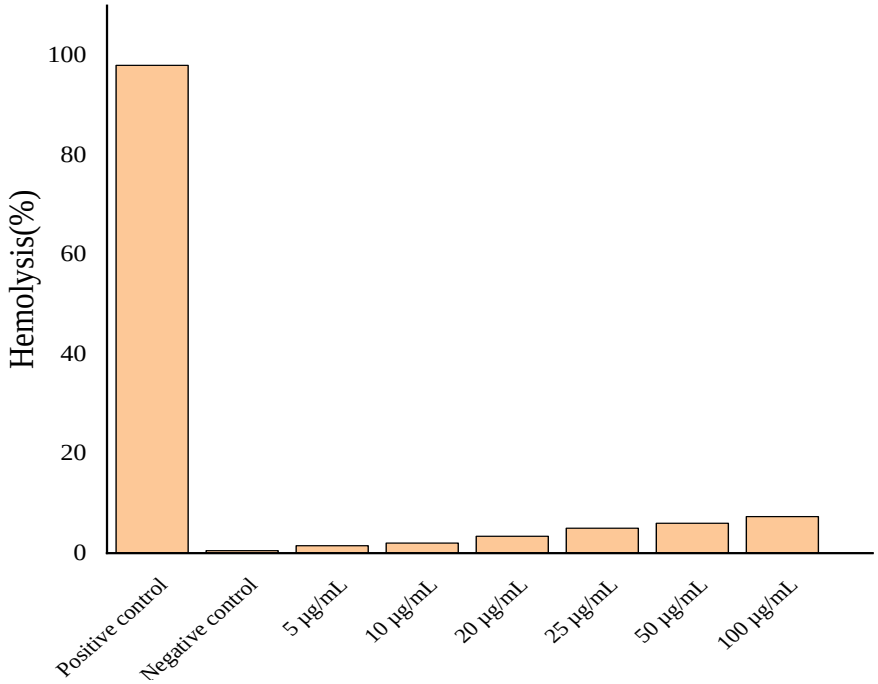

Figure (3-25): The percentage of hemolysis induced by CuNPs. Triton X-100 was used as a positive control and normal saline as a negative control.

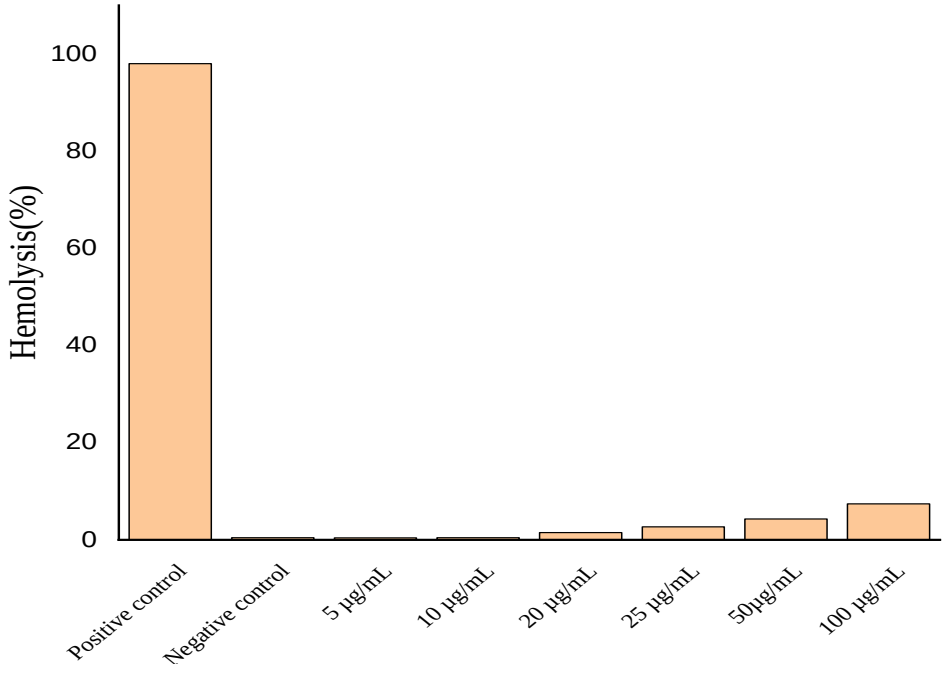

Figure (3-26): The percentage of hemolysis induced by *M. communis* extract. Triton X-100 was used as a positive control and normal saline as a negative control.

The use of bioactive compounds from herbs and medicinal plants can affect the membrane of the erythrocyte. The hemolytic effect has been amplified at increased concentration and is the function of the extract (146).

When red blood cells come into contact with water, hemolysis occurs, and it is critical to thoroughly inspect the implant material before use (101). In previous study on CuNPs has demonstrated that small particles have higher hemolytic activity than large particles, in the contract that in silica nanoparticles increase hemolytic activity increased with large particles (147).

Toxicity of the active molecule is a key factor during drug designing, and hemolytic activity represents a useful starting point in this regard, it provides the primary information on the interaction between molecules and biological entities at the cellular level. Hemolytic activity of any compounds is an indicator of general cytotoxicity towards normal healthy cells. Usually, saponins present in the plants showed hemolytic activity by creating changes in the erythrocyte membrane. In vitro hemolytic assay by spectroscopic method provides an easy and effective method for the quantitative measurement of hemolysis. This method provides the evaluation of the effect of different concentrations of biomolecules on the human erythrocytes (148).

Another study showed the hemolytic activity of *Lantana camera* leave extract increased with the increase in the dose of the extract (149).

Conclusions

1. Copper nanoparticles were successfully synthesized Using aqueous *Myrtus communis* leaves extract.
2. The volume 10 mL of *M. communis* (10%) leaves extract and boiling for 10 minutes are the best conditions for the synthesis of CuNPs.
3. Phytochemicals of plant are responsible for the reducting and stabilizing of nanoparticles.
4. The synthesized CuNPs is considered as a good antioxidant activity.
5. Copper nanoparticles have good catalytic activity.
6. *Myrtus communis* have good antibacterial activity compared with CuNPs.
8. Copper nanoparticles have good anti-inflammatory activity
7. Copper nanoparticles did not cause hemolysis and therefore can be used in pharmaceutical fields.

Future Studies

1. Studying biosynthesis for other metal nanoparticles by different plants, which had high biological activity.
2. Biosynthesis of copper nanoparticles using fungi or bacteria.
3. Studying the clinical applications of the synthesized copper nanoparticles *in vivo*.
4. Studying the anti-diabetic activity of the synthesized copper nanoparticles from *M. communis* leaves extract.
5. Studying the antitumor activity of the synthesized copper nanoparticles from *M. communis* leaves extract.

References

References

1. Singh A, Singh NB, Hussain I, Singh H, Singh SC. Plant-nanoparticle interaction : An approach to improve agricultural practices and plant productivity Plant-nanoparticle interaction : An approach to improve agricultural practices and plant productivity. 2015;7(December):909–17.

2. Joseph AT, Prakash P, Narvi SS. Phytofabrication and Characterization of Copper Nanoparticles Using Allium Sativum and its Antibacterial Activity. Int J Sci Eng Technol. 2016;4(2):463–72.

3. Manivasagan P, Venkatesan J, Sivakumar K, Kim SK. Actinobacteria mediated synthesis of nanoparticles and their biological properties: A review. Crit Rev Microbiol. 2016;42(2):209–21.

4. Ghosh MK, Sahu S, Gupta I, Ghorai TK. Green synthesis of copper nanoparticles from an extract ofJatropha curcasleaves: characterization, optical properties, CT-DNA binding and photocatalytic activity. RSC Adv. 2020;10(37):22027–35.

5. Byrappa K, Ohara S, Adschiri T. Nanoparticles synthesis using supercritical fluid technology - towards biomedical applications. Adv Drug Deliv Rev. 2008;60(3):299–327.

6. Sapna Thakur RR& SS. Study the Antibacterial Activity of Copper Nanoparticles Synthesized Using Herbal Plants Leaf Extracts. Int J Bio-Technology Res. 2014;4(5):21–34.

7. Kuppusamy P, Yusoff MM, Maniam GP, Govindan N. Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications – An updated report. Saudi Pharm J [Internet]. 2016;24(4):473–84. Available from: http://dx.doi.org/10.1016/j.jsps.2014.11.013

8. Chalchat JC, Garry RP, Michet A. Essential oils of myrtle (myrtus communis l.) of the mediterranean littoral. J Essent Oil Res. 1998;10(6):613–7.

9. Sumbul S, Aftab Ahmad M, Asif M, Akhtar M. Myrtus communis Linn. - A review. Indian J Nat Prod Resour. 2011;2(4):395–402.

10. Anwar S, Ahmed N, Awwad N Al, Ansari SY, Wagih ME. Myrtle (Myrtus communis L.) oils [Internet]. Essential Oils in Food Preservation, Flavor and Safety. Elsevier Inc.; 2016. 581–592 p. Available from: http://dx.doi.org/10.1016/B978-0-12-416641-7.00066-3

11. Serce S, Ercisli S, Sengul M, Gunduz K, Orhan E. Antioxidant activities and fatty acid composition of wild grown myrtle (Myrtus communis L.) fruits. Pharmacogn Mag. 2010;6(21):9–12.

12. Pirbalouti AG, Mirbagheri H, Hamedi B, Rahimi E. Antibacterial activity of the essential oils of myrtle leaves against Erysipelothrix rhusiopathiae. Asian Pac J Trop Biomed. 2014;4(Suppl 1):S505–9.

13. Parbuntari H, Prestica Y, Gunawan R, Nurman MN, Adella F. Preliminary Phytochemical Screening (Qualitative Analysis) of Cacao Leaves (Theobroma cacao L.). EKSAKTA Berk Ilm Bid MIPA. 2018;19(2):40–5.

14. Daniel G, Krishnakumari S. Quantitative analysis of primary and secondary metabolites in aqueous hot extract of Eugenia uniflora (L) leaves. Asian J Pharm Clin Res. 2015;8(1):334–8.

15. Teoh ES. Medicinal orchids of Asia. Med Orchid Asia. 2016;1–752.

16. Sofowora A, Ogunbodede E, Onayade A. The role and place of medicinal plants in the strategies for disease prevention. Afr J Tradit Complement Altern Med. 2013;10(5):210–29.

17. Nassar MI, Aboutabl ESA, Ahmed RF, El-Khrisy EDA, Ibrahim KM, Sleem AA. Secondary metabolites and bioactivities of Myrtus communis. Pharmacognosy Res. 2010;2(6):325–9.

18. Gharaati Jahromi S. Extraction Techniques of Phenolic Compounds from Plants. Plant Physiol Asp Phenolic Compd. 2019;

19. Hernández I, Alegre L, Van Breusegem F, Munné-Bosch S. How relevant are flavonoids as antioxidants in plants? Trends Plant Sci. 2009;14(3):125–32.

20. Shi J, Arunasalam K, Yeung D, Kakuda Y, Mittal G, Jiang Y. Saponins from Edible Legumes: Chemistry, Processing, and Health Benefits. J Med Food. 2004;7(1):67–78.

21. Bouaoudia-Madi N, Boulekbache-Makhlouf L, Kadri N, Dahmoune F, Remini H, Dairi S, et al. Phytochemical analysis of Myrtus communis plant: Conventional versus microwave assisted-extraction procedures. J Complement Integr Med. 2017;14(4):2–13.

22. Siddiqui M. Phytochemical Analysis of Some Medicinal Plants. Liaquat Med Res J. 2021;3(8):1–5.

23. Vaghasia Y, Dave R, Chanda S. 567-576.Pdf. Vol. 5, Research Journal of Medicinal Plant. 2011. p. 567–76.

24. Yang L, Wen KS, Ruan X, Zhao YX, Wei F, Wang Q. Response of plant secondary metabolites to environmental factors. Molecules. 2018;23(4):1–26.

25. Škrovánková S, Mišurcová L, Machů L. Antioxidant Activity and Protecting Health Effects of Common Medicinal Plants. Vol. 67, Advances in Food and Nutrition Research. 2012. 75–139 p.

26. Jabri MA, Tounsi H, Rtibi K, Marzouki L, Sakly M, Sebai H. Ameliorative and antioxidant effects of myrtle berry seed (Myrtus

communis) extract during reflux-induced esophagitis in rats. Pharm Biol. 2016;54(9):1575–85.

27. Messaoud C, Laabidi A, Boussaid M. Myrtus communis L. Infusions: The Effect of Infusion Time on Phytochemical Composition, Antioxidant, and Antimicrobial Activities. J Food Sci. 2012;77(9):1–7.

28. Sumbul S, Mohd Aftab Ahmad, Asif M, Saud I, Akhtar M. Evaluation of Myrtus communis Linn. berries (common myrtle) in experimental ulcer models in rats. Hum Exp Toxicol. 2010;29(11):935–44.

29. Aleksic V, Knezevic P. Antimicrobial and antioxidative activity of extracts and essential oils of Myrtus communis L. Microbiol Res [Internet]. 2014;169(4):240–54. Available from: http://dx.doi.org/10.1016/j.micres.2013.10.003

30. Ali Esmail Al-Snafi. Traditional uses of Iraqi medicinal plants (part 2). Int J Biol Pharm Sci Arch. 2021;2(1):022–41.

31. Nadaroğlu H, Alayli Güngör A, İnce S. Synthesis of Nanoparticles by Green Synthesis Method. Int J Innov Res Rev [Internet]. 2017;1(1):6–9. Available from: http://www.injirr.com/article/view/4

32. Makarov V V., Love AJ, Sinitsyna O V., Makarova SS, Yaminsky I V., Taliansky ME, et al. “Green” nanotechnologies: Synthesis of metal nanoparticles using plants. Acta Naturae. 2014;6(20):35–44.

33. Hussain I, Singh NB, Singh A, Singh H, Singh SC. Green synthesis of nanoparticles and its potential application. Biotechnol Lett. 2016;38(4):545–60.

34. Arumugam A, Karthikeyan C, Syedahamed A, Hameed H, Gopinath K, Gowri S, et al. NU Synthesis of cerium oxide nanoparticles using

Gloriosa superba. Mater Sci Eng C [Internet]. 2015; Available from: http://dx.doi.org/10.1016/j.msec.2015.01.042

35. Li X, Xu H, Chen ZS, Chen G. Biosynthesis of nanoparticles by microorganisms and their applications. J Nanomater. 2011;2011.

36. Iravani S. Green synthesis of metal nanoparticles using plants. Green Chem. 2011;13(10):2638–50.

37. Korbekandi H, Iravani S, Abbasi S. Production of nanoparticles using organisms Production of nanoparticles using organisms. Crit Rev Biotechnol. 2009;29(4):279–306.

38. Singh P, Kim YJ, Zhang D, Yang DC. Biological Synthesis of Nanoparticles from Plants and Microorganisms. Trends Biotechnol [Internet]. 2016;34(7):588–99. Available from: http://dx.doi.org/10.1016/j.tibtech.2016.02.006

39. Shah M, Fawcett D, Sharma S, Tripathy SK, Poinern GEJ. Green synthesis of metallic nanoparticles via biological entities. Vol. 8, Materials. 2015. 7278–7308 p.

40. Nagar N, Devra V. Green synthesis and characterization of copper nanoparticles using Azadirachta indica leaves. Mater Chem Phys [Internet]. 2018;213:44–51. Available from: https://doi.org/10.1016/j.matchemphys.2018.04.007

41. Chand Mali S, Raj S, Trivedi R. Biosynthesis of copper oxide nanoparticles using Enicostemma axillare (Lam.) leaf extract. Biochem Biophys Reports [Internet]. 2019;20(November):100699. Available from: https://doi.org/10.1016/j.bbrep.2019.100699

42. Rajeshkumar S, Menon S, Venkat Kumar S, Tambuwala MM, Bakshi HA, Mehta M, et al. Antibacterial and antioxidant potential of biosynthesized copper nanoparticles mediated through Cissus

arnotiana plant extract. J Photochem Photobiol B Biol [Internet]. 2019;197:111531. Available from: https://doi.org/10.1016/j.jphotobiol.2019.111531

43. Długosz O, Chwastowski J, Banach M. Hawthorn berries extract for the green synthesis of copper and silver nanoparticles. Chem Pap [Internet]. 2020;74(1):239–52. Available from: https://doi.org/10.1007/s11696-019-00873-z

44. Singh AK, Talat M, Singh DP, Srivastava ON. Biosynthesis of gold and silver nanoparticles by natural precursor clove and their functionalization with amine group. J Nanoparticle Res. 2010;12(5):1667–75.

45. Geonmonond RS, Da Silva AGM, Camargo PHC. Controlled synthesis of noble metal nanomaterials: Motivation, principles, and opportunities in nanocatalysis. An Acad Bras Cienc. 2018;90(1):719–44.

46. Singh A, Gautam PK, Verma A, Singh V, Shivapriya PM, Shivalkar S, et al. Green synthesis of metallic nanoparticles as effective alternatives to treat antibiotics resistant bacterial infections: A review. Biotechnol Reports [Internet]. 2020;25:e00427. Available from: https://doi.org/10.1016/j.btre.2020.e00427

47. Haverkamp RG, Marshall AT. The mechanism of metal nanoparticle formation in plants: Limits on accumulation. J Nanoparticle Res. 2009;11(6):1453–63.

48. Din MI, Arshad F, Hussain Z, Mukhtar M. Green Adeptness in the Synthesis and Stabilization of Copper Nanoparticles: Catalytic, Antibacterial, Cytotoxicity, and Antioxidant Activities. Nanoscale Res Lett. 2017;12.

49. Theivasanthi T, Alagar M. Studies of Copper Nanoparticles Effects on Micro-organisms. 2011; Available from: http://arxiv.org/abs/1110.1372

50. Mali SC, Dhaka A, Githala CK, Trivedi R. Green synthesis of copper nanoparticles using Celastrus paniculatus Willd. leaf extract and their photocatalytic and antifungal properties. Biotechnol Reports [Internet]. 2020;27:e00518. Available from: https://doi.org/10.1016/j.btre.2020.e00518

51. Lee H, Lee G, Jang NR, Yun JH, Song JY, Kim BS. Biological synthesis of copper nanoparticles using plant extract Characterization of Copper Nanoparticles 2 . 3 Chemical Synthesis of Copper. NSTI-Nanotech. 2011;1(1):371–4.

52. Schröfel A, Kratošová G, Šafařík I, Šafaříková M, Raška I, Shor LM. Applications of biosynthesized metallic nanoparticles - A review. Acta Biomater [Internet]. 2014;10(10):4023–42. Available from: http://dx.doi.org/10.1016/j.actbio.2014.05.022

53. Shende S, Gaikwad N, Bansod S. Synthesis and evaluation of antimicrobial potential of copper nanoparticle against agriculturally important Phytopathogens. Int J Biol Res. 2016;1(4):41–7.

54. Nasrollahzadeh M, Mohammad Sajadi S. Green synthesis of copper nanoparticles using Ginkgo biloba L. leaf extract and their catalytic activity for the Huisgen [3+2] cycloaddition of azides and alkynes at room temperature. J Colloid Interface Sci [Internet]. 2015;457:141–7. Available from: http://dx.doi.org/10.1016/j.jcis.2015.07.004

55. Harne S, Sharma A, Dhaygude M, Joglekar S, Kodam K, Hudlikar M. Novel route for rapid biosynthesis of copper nanoparticles using aqueous extract of Calotropis procera L. latex and their cytotoxicity on tumor cells. Colloids Surfaces B Biointerfaces [Internet].

2012;95:284–8. Available from: http://dx.doi.org/10.1016/j.colsurfb.2012.03.005

56. Kiruba Daniel SCG, Vinothini G, Subramanian N, Nehru K, Sivakumar M. Biosynthesis of Cu, ZVI, and Ag nanoparticles using Dodonaea viscosa extract for antibacterial activity against human pathogens. J Nanoparticle Res. 2013;15(1).

57. Shankar S, Rhim JW. Effect of copper salts and reducing agents on characteristics and antimicrobial activity of copper nanoparticles. Mater Lett [Internet]. 2014;132:307–11. Available from: http://dx.doi.org/10.1016/j.matlet.2014.06.014

58. Din MI, Rehan R. Synthesis, Characterization, and Applications of Copper Nanoparticles. Anal Lett. 2017;50(1):50–62.

59. Song JY, Jang HK, Kim BS. Biological synthesis of gold nanoparticles using Magnolia kobus and Diopyros kaki leaf extracts. Process Biochem. 2009;44(10):1133–8.

60. Plant CL. Synthesis and Characterization of Cu Nanoparticles of Leucas. 2016;3(5):241–2.

61. Karimi J, Mohsenzadeh S. Rapid, green, and eco-friendly biosynthesis of copper nanoparticles using flower extract of Aloe vera. Synth React Inorganic, Met Nano-Metal Chem. 2015;45(6):895–8.

62. Wu SH, Chen DH. Synthesis of high-concentration Cu nanoparticles in aqueous CTAB solutions. J Colloid Interface Sci. 2004;273(1):165–9.

63. Pisoschi AM, Cheregi MC, Danet AF. Total antioxidant capacity of some commercial fruit juices: Electrochemical and spectrophotometrical approaches. Molecules. 2009;14(1):480–93.

64. Phaniendra A, Jestadi DB, Periyasamy L. Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. Indian J Clin Biochem. 2015;30(1):11–26.

65. Gürkan H. The role of free radicals in ethiopathogenesis of diseases. 2008;(January).

66. Shahidi F. 1 - Antioxidants: principles and applications [Internet]. Handbook of Antioxidants for Food Preservation. Elsevier Ltd; 2015. 1–14 p. Available from: http://dx.doi.org/10.1016/B978-1-78242-089-7.00001-4

67. Husain N, Kumar A. Reactive oxygen species and natural antioxidants : A review. Adv Biores. 2012;3(December):164–75.

68. Amir M, Khan A, Mujeeb M, Ahmad MA, Siddiqui NA. Phytochemical screening and in vitro antioxidant activity of jawarish amla- A poly herbal formulation. Pharmacogn J [Internet]. 2011;3(26):54–60. Available from: http://dx.doi.org/10.5530/pj.2011.26.10

69. Peng C, Wang X, Chen J, Jiao R, Wang L, Li YM, et al. Biology of ageing and role of dietary antioxidants. Biomed Res Int. 2014;2014.

70. Zhang K, Suh JM, Choi JW, Jang HW, Shokouhimehr M, Varma RS. Recent Advances in the Nanocatalyst-Assisted NaBH4 Reduction of Nitroaromatics in Water. ACS Omega. 2019;4(1):483–95.

71. Pich A, Richtering W. Polymer Nanogels and Microgels [Internet]. Vol. 6, Polymer Science: A Comprehensive Reference, 10 Volume Set. Elsevier B.V.; 2012. 309–350 p. Available from: http://dx.doi.org/10.1016/B978-0-444-53349-4.00167-9

72. Zhao P, Feng X, Huang D, Yang G, Astruc D. Basic concepts and recent advances in nitrophenol reduction by gold- and other transition

metal nanoparticles. Coord Chem Rev [Internet]. 2015;287:114–36. Available from: http://dx.doi.org/10.1016/j.ccr.2015.01.002

73. Hajfathalian M, Gilroy KD, Yaghoubzade A, Sundar A, Tan T, Hughes RA, et al. Photocatalytic Enhancements to the Reduction of 4-Nitrophenol by Resonantly Excited Triangular Gold-Copper Nanostructures. J Phys Chem C. 2015;119(30):17308–15.

74. El Hartiti H, El Mostaphi A, Barrahi M, Ali A Ben, Chahboun N, Amiyare R, et al. Chemical composition and antibacterial activity of the essential oil of Myrtus communis leaves. Karbala Int J Mod Sci. 2020;6(3):251–8.

75. Betancourt-Galindo R, Reyes-Rodriguez PY, Puente-Urbina BA, Avila-Orta CA, Rodríguez-Fernández OS, Cadenas-Pliego G, et al. Synthesis of copper nanoparticles by thermal decomposition and their antimicrobial properties. J Nanomater. 2014;2014:7–11.

76. Nguyen NY, An BN, Le MV, Hoang HA. Antibacterial activity of copper nanoparticles-chitosan composite against vibrio parahaemolyticus. Biocontrol Sci. 2020;25(3):159–65.

77. Parekh J, Chanda S. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. African J Biomed Res. 2010;10(2):175–81.

78. Murthy HCA, Desalegn T, Kassa M, Abebe B, Assefa T. Synthesis of Green Copper Nanoparticles Using Medicinal Plant Hagenia abyssinica (Brace) JF. Gmel. Leaf Extract: Antimicrobial Properties. J Nanomater. 2020;2020.

79. Alzahrani KE, Aniazy A, Alswieleh AM, Wahab R, El-Toni AM, Alghamdi HS. Antibacterial activity of trimetal (CuZnFe) oxide nanoparticles. Int J Nanomedicine. 2018;13:77–87.

80. Rohini P, Aditya T, Anil M, Arun K. Anti-inflammatory and antioxidant potentiality of Solanum xanthocarpum. African J Biotechnol. 2018;17(37):1188–95.

81. Das B, Choudhury MD, Dey A, Das Talukdar A, Nongalleima KH, Deb L. Antioxidant and anti-inflammatory activity of aqueous and methanolic extracts of rhizome part of drynaria quercifolia (L.) J. Smith. Int J Pharm Pharm Sci. 2014;6(6):43–9.

82. Gunathilake KDPP, Ranaweera KKDS, Rupasinghe HPV. In vitro anti-inflammatory properties of selected green leafy vegetables. Biomedicines. 2018;6(4):1–10.

83. Phukan K, Devi R, Chowdhury D. Green Synthesis of Gold Nano-bioconjugates from Onion Peel Extract and Evaluation of Their Antioxidant, Anti-inflammatory, and Cytotoxic Studies. ACS Omega. 2021;6(28):17811–23.

84. García-Lafuente A, Guillamón E, Villares A, Rostagno MA, Martínez JA. Flavonoids as anti-inflammatory agents: Implications in cancer and cardiovascular disease. Inflamm Res. 2009;58(9):537–52.

85. Sowemimo-Coker SO. Red blood cell hemolysis during blood bank stora. 46 Transfus Med Rev. 2002;16(1):46–60.

86. Naz S, Gul A, Zia M. Toxicity of copper oxide nanoparticles: A review study. IET Nanobiotechnology. 2020;14(1):1–13.

87. Ameh T, Sayes CM. The potential exposure and hazards of copper nanoparticles: A review. Environ Toxicol Pharmacol [Internet]. 2019;71(June):103220. Available from: https://doi.org/10.1016/j.etap.2019.103220

88. Chen Z, Meng H, Xing G, Chen C, Zhao Y, Jia G, et al. Acute toxicological effects of copper nanoparticles in vivo. Toxicol Lett.

2006;163(2):109–20.

89. Motar AA, Hussein RA, Abdulbary M. Study of in vitro and in vivo cytotoxicity effect of some medicinal plants. Kufa J Vet Med Sci. 2016;7(2):211–7.

90. Benoit C, Virginie C, Boris V. The use of NADES to support innovation in the cosmetic industry [Internet]. 1st ed. Vol. 97, Advances in Botanical Research. Elsevier Ltd.; 2021. 309–332 p. Available from: http://dx.doi.org/10.1016/bs.abr.2020.09.009

91. Matos MJ, Santana L, Uriarte E, Abreu OA, Molina E, Yordi EG. Coumarins — An Important Class of Phytochemicals. Phytochem - Isol Characterisation Role Hum Heal. 2015;

92. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African J Biotechnol. 2005;4(7):685–8.

93. Flamini G, Cioni PL, Morelli I, Maccioni S, Baldini R. Phytochemical typologies in some populations of Myrtus communis L. on Caprione Promontory (East Liguria, Italy). Food Chem. 2004;85(4):599–604.

94. Sani I. Preliminary Phytochemical Screening, Antioxidant Potentials and Proximate Composition of Senna occidentalis and Leptadenia pyrotechnica Leaves Extracts. Appl Sci Reports. 2016;14(3).

95. Flaih LS, Al-Saadi NH. Characterization and clinical applications of silver nanoparticles synthesized from cassia obtusifolia leaves extract. Plant Arch. 2020;20:1082–8.

96. Pisoschi AM, Negulescu GP. Methods for Total Antioxidant Activity Determination: A Review. Biochem Anal Biochem. 2012;01(01):1–10.

97. Devi RS, Jeevitha M, Preetha S, Rajeshkumar S. Free Radical Scavenging Activity of Copper Nanoparticles Synthesized from Dried Ginger. J Pharm Res Int. 2020;32(19):1–7.

98. Gomaa EZ. Antimicrobial, antioxidant and antitumor activities of silver nanoparticles synthesized by Allium cepa extract: A green approach. J Genet Eng Biotechnol [Internet]. 2017;15(1):49–57. Available from: http://dx.doi.org/10.1016/j.jgeb.2016.12.002

99. Al-musawi ZF, Al-saadi NH, Ali IM. Antibacterial and Antioxidant Activities of Silver Nanoparticle Synthesized from Dodonaea viscosa L . Extract. 2022;020029(5).

100. Flaih LS, Al-Saadi NH. Green synthesis of silver nanoparticles from Cassia obtusifolia leaves extract: Characterization and antioxidant activity. AIP Conf Proc. 2020;2290(December).

101. Gondwal M, Joshi Nee Pant G. Synthesis and Catalytic and Biological Activities of Silver and Copper Nanoparticles Using Cassia occidentalis. Int J Biomater. 2018;2018.

102. Alavi M, Karimi N. Characterization, antibacterial, total antioxidant, scavenging, reducing power and ion chelating activities of green synthesized silver, copper and titanium dioxide nanoparticles using Artemisia haussknechtii leaf extract. Artif Cells, Nanomedicine Biotechnol [Internet]. 2018;46(8):2066–81. Available from: https://doi.org/10.1080/21691401.2017.1408121

103. Govindappa M, Hemashekhar B, Arthikala MK, Ravishankar Rai V, Ramachandra YL. Characterization, antibacterial, antioxidant, antidiabetic, anti-inflammatory and antityrosinase activity of green synthesized silver nanoparticles using Calophyllum tomentosum leaves extract. Results Phys. 2018;9:400–8.

104. Kulkarni A, Govindappa M, Cp C, Ramachandra YL, Koka PS. Phytochemical analysis of Cassia fistula and its in vitro antimicrobial , antioxidant activities. Adv Med Plant Res. 2015;3(February):8–17.

105. Chippada SC, Vangalapati M. Antioxidant, an anti-inflammatory and anti-arthritic activity of Centella asiatica extracts. J Chem Biol Phys Sci. 2011;1(2):260–9.

106. Sakat SS, Juvekar AR, Gambhire MN. In-vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata linn. Int J Pharm Pharm Sci. 2010;2(1):146–55.

107. Okoli CO, Akah PA. Mechanisms of the anti-inflammatory activity of the leaf extracts of Culcasia scandens P. Beauv (Araceae). Pharmacol Biochem Behav. 2004;79(3):473–81.

108. Kitamura H, Nagano A, Kitano E. Hemolysis of normal human erythrocytes by autologous serum complement. Int Arch Allergy Immunol. 1993;100(3):209–14.

109. Al-Snafi AE. Phenolics and flavonoids contents of medicinal plants, as natural ingredients for many therapeutic purposes-A review. IOSR J Pharm [Internet]. 2020;10(July):42–81. Available from: www.iosrphr.org

110. Zacchino SA, Butassi E, Liberto M Di, Raimondi M, Postigo A, Sortino M. Plant phenolics and terpenoids as adjuvants of antibacterial and antifungal drugs. Phytomedicine [Internet]. 2017;37:27–48. Available from: https://doi.org/10.1016/j.phymed.2017.10.018

111. Amini SM. Preparation of antimicrobial metallic nanoparticles with bioactive compounds. Mater Sci Eng C [Internet]. 2019;103:109809. Available from: https://doi.org/10.1016/j.msec.2019.109809

112. Park JW, Shumaker-Parry JS. Structural study of citrate layers on gold nanoparticles: Role of intermolecular interactions in stabilizing nanoparticles. J Am Chem Soc. 2014;136(5):1907–21.

113. Gortzi O, Lalas S, Chinou I, Tsaknis J. Reevaluation of bioactivity and antioxidant activity of Myrtus communis extract before and after encapsulation in liposomes. Eur Food Res Technol. 2008;226(3):583–90.

114. Pezhmanmehr M, Dastan D, Ebrahimi S, Hadian J. Essential oil constituents of leaves and fruits of Myrtus communis L. from Iran. Planta Med. 2009;75(09).

115. Sharafzadeh S, Khosh-khui M, Javidnia K. O RIGINAL A RTICLE Aroma Profile of Leaf and Stem of Lemon Balm (Melissa Officinalis L .) Grown under. Advances. 2011;5(4):547–50.

116. Mir MA, Bashir N, Alfaify A, Oteef MDY. Gc-ms analysis of myrtus communis extract and its antibacterial activity against gram-positive bacteria. BMC Complement Med Ther. 2020;20(1).

117. Parasuraman S, Raveendran R, Madhavrao C. Gc-Ms Analysis of Leaf Extracts of Cleistanthus Collinus Roxb . (Euphorbiaceae). J Pharmacol Exp Ther. 2009;1(2):284–6.

118. Rocchetti G, Lucini L, Chiodelli G, Giuberti G, Montesano D, Masoero F, et al. Impact of boiling on free and bound phenolic profile and antioxidant activity of commercial gluten-free pasta. Food Res Int [Internet]. 2017;100(May):69–77. Available from: http://dx.doi.org/10.1016/j.foodres.2017.08.031

119. Lee HJ, Song JY, Kim BS. Biological synthesis of copper nanoparticles using Magnolia kobus leaf extract and their antibacterial activity. J Chem Technol Biotechnol.

2013;88(11):1971–7.

120. Amelkovich YA, Nazarenko OB, Sechin AI, Visakh PM. Characterization of copper nanopowders after natural aging. IOP Conf Ser Mater Sci Eng. 2015;81(1).

doi:10.1088/1757-899X/81/1/012072

121. Vaseem M, Lee KM, Kim DY, Hahn YB. Parametric study of cost-effective synthesis of crystalline copper nanoparticles and their crystallographic characterization. Mater Chem Phys [Internet]. 2011;125(3):334–41. Available from: http://dx.doi.org/10.1016/j.matchemphys.2010.11.007

122. Subhankari I, Nayak P. Synthesis of Copper Nanoparticles Using Syzygium aromaticum (Cloves) Aqueous Extract by Using Green Chemistry. World J Nano Sci Technol [Internet]. 2013;2(1):14–7. Available from: http://idosi.org/wjnst/2(1)13/4.pdf

123. Riaz M, Ismail M, Ahmad B, Zahid N, Jabbour G, Khan MS, et al. Characterizations and analysis of the antioxidant, antimicrobial, and dye reduction ability of green synthesized silver nanoparticles. Green Process Synth. 2020;9(1):693–705.

124. Caroling G, Priyadharshini MN, Vinodhini E, Ranjitham AM, Shanthi P. – Characterisation and Study of Antibacterial Effects. 2015;5(2).

125. Kiruba Daniel S, Kumar R, Sathish V, Sivakumar M, Sunitha S, Anitha Sironmani T. Green Synthesis (Ocimum tenuiflorum) of Silver Nanoparticles and Toxicity Studies in Zebra Fish (Danio rerio) Model. Int J Nanosci Nanotechnol [Internet]. 2011;2(2):974–3081. Available from: http://www.irphouse.com

126. Zhou Y, Lin W, Huang J, Wang W, Gao Y, Lin L, et al. Biosynthesis

of gold nanoparticles by foliar broths: Roles of biocompounds and other attributes of the extracts. Nanoscale Res Lett. 2010;5(8):1351–9.

127. Nosiri CI, Arunsi UO, Ngwogu AC, Idume J, Atasie OC. International Journal of Green and Proximate and preleminaryphytochemical analysis of leaves of Cassine Glauca plant. Int J Green Herb Chem. 2019;8(1):30–6.

128. Mittu R. Synthesis, Characterization of Copper Nanoparticles -A Review. Int Adv Res J Sci Eng Technol. 2016;3(5):37–40.

129. Kulkarni VD, Kulkarni PS. International Journal of Chemical Studies Green Synthesis of Copper Nanoparticles Using Ocimum. Int J Chem Stud. 2013;1(3):1–4.

130. Sachin Parmar, Amit Gangwal NSD. *** X-Ray Diffraction Studies of Copper Nanopowder*** Scholars Research Library ***. Sch Res Libr. 2011;2(4):373–83.

131. Elisma N, Labanni A, Emriadi, Rilda Y, Asrofi M, Arief S. Green synthesis of copper nanoparticles using Uncaria gambir roxb. Leaf extract and its characterization. Rasayan J Chem. 2019;12(4):1752–6.

132. Kalpana VN, Chakraborthy P, Palanichamy V, Devi Rajeswari V. Synthesis and characterization of copper nanoparticles using Tridax procumbens and its application in degradation of bismarck brown. Int J ChemTech Res. 2016;9(9):498–507.

133. Chan GH, Zhao J, Hicks EM, Schatz GC, Van Duyne RP. Plasmonic properties of copper nanoparticles fabricated by nanosphere lithography. Nano Lett. 2007;7(7):1947–52.

134. Memon R, Memon AA, Sherazi STH, Sirajuddin, Balouch A, Shah MR, et al. Application of synthesized copper nanoparticles using

aqueous extract of Ziziphus mauritiana L. leaves as a colorimetric sensor for the detection of Ag+. Turkish J Chem. 2020;44(5):1376–85.

135. Subbaiya R, Masilamani Selvam M. Synthesis and characterisation of copper nanoparticles using Eupatorium glandulosum extract and their antimicrobial, antioxidant activities. Res J Pharm Biol Chem Sci. 2015;6(2):1117–27.

136. Miladi S, Mohamed D. In vitro antioxidant activities of Aloe vera leaf skin extracts IN VITRO ANTIOXIDANT ACTIVITIES OF Aloe vera LEAF SKIN EXTRACTS Sonia Miladi and Mohamed Damak. J Société Chim Tunisie. 2008;10(10):101–9.

137. Piyush More SG, Soham Jagtap RN, Chippalkatti R. Antidiabetic and Antioxidant Properties of Copper Nanoparticles Synthesized by Medicinal Plant Dioscorea bulbifera. J Nanomed Nanotechnol. 2015;s6(February 2016).

138. Wunder S, Polzer F, Lu Y, Mei Y, Ballauff M. Kinetic analysis of catalytic reduction of 4-nitrophenol by metallic nanoparticles immobilized in spherical polyelectrolyte brushes. J Phys Chem C. 2010;114(19):8814–20.

139. Pradhan N, Pal A, Pal T. Silver nanoparticle catalyzed reduction of aromatic nitro compounds. Colloids Surfaces A Physicochem Eng Asp. 2002;196(2–3):247–57.

140. Alyousef AA, Arshad M, AlAkeel R, Alqasim A. Biogenic silver nanoparticles by Myrtus communis plant extract: biosynthesis, characterization and antibacterial activity. Biotechnol Biotechnol Equip [Internet]. 2019;33(1):931–6. Available from: https://doi.org/10.1080/13102818.2019.1629840

141. Akin M, Aktumsek A, Nostro A. Antibacterial activity and composition of the essential oils of Eucalyptus camaldulensis Dehn. and Myrtus communis L. growing in Northern Cyprus. African J Biotechnol. 2010;9(4):531–5.

142. Al-Saimary IE, Bakr SS, Jaffar T, Al-Saimary AE, Salim H, Al-Muosawi R. Effects of some plant extracts and antibiotics on Pseudomonas aeruginosa isolated from various burn cases. Saudi Med J. 2002;23(7):802–5.

143. Leelaprakash G, Mohan Dass S. Invitro anti-inflammatory activity of methanol extract of enicostemma axillare. Int J Drug Dev Res. 2011;3(3):189–96.

144. Das B, Choudhury MD, Dey A, Das Talukdar A, Nongalleima KH, Deb L. Antioxidant and anti-inflammatory activity of aqueous and methanolic extracts of rhizome part of drynaria quercifolia (L.) J. Smith. Int J Pharm Pharm Sci. 2014;6(6):43–9.

145. Oyedapo O. Red blood cell membrane stabilizing potentials of extracts of Lantana camara and its fractions. Int J Plant Physiol Biochem [Internet]. 2010;2(October):46–51. Available from: http://www.academicjournals.org/ijppb/PDF/PDF 2010/Oct/Oyedapo et al.pdf

146. Zohra M, Fawzia A. Hemolytic activity of different herbal extracts used in Algeria. Int J Pharma Sci Res. 2014;5(08):495–500.

147. Rothen-Rutishauser BM, Schürch S, Haenni B, Kapp N, Gehr P. Interaction of fine particles and nanoparticles with red blood cells visualized with advanced microscopic techniques. Environ Sci Technol. 2006;40(14):4353–9.

148. Kumar G, Karthik L, Rao KVB. Haemolytic activity of Indian

medicinal plants toward human erythrocytes: an in vitro study. Elixir Appl Bot. 2011;40(June 2014):5534–7.

149. Kalita S, Kumar G, Karthik L, Venkata K, Rao B. A Review on Medicinal Properties of Lantana camara Linn A Review on Medicinal Properties of Lantana camara Linn . 2012;(May 2014).

الخلاصة

ان التخليق البيولوجي للجزيئات النانوية يتم بواسطة كائنات حية مختلفة مثل النباتات والبكتيريا والفطريات والأعشاب البحرية والطحالب الدقيقة. تعد المستخلصات النباتية من أكثر الطرق أمانًا في الكيمياء الخضراء لتصنيع االدقائق النانوية.

صُممت هذه الدراسة لتحضير دقائق النحاس النانوية من مادة آمنة (نباتية) وتقييم نشاطها البيوكيميائي والبيولوجي.

تم استخدام تقنيات مختلفة لتوصف دقائق النحاس النانوية اذ تم استخدام الطرق الطيفية, مثلا استخدام الاشعة فوق البنفسجية-المرئية (UV-Vis) لفحص رنين البلازمون السطحي (SPR) للدقائق النانوية في النطاق من 300 إلى 700 نانومتر وتم تحديد المجموعات الوظيفية الفعالة التي يمكنها اختزال ايونات النحاس Cu^{2+} باستخدام طيف الأشعة تحت الحمراء (FT-IR). تم تحديد التركيب البلوري لدقائق النحاس النانوية باستخدام حيود الأشعة السينية (XRD) ، كما يتضح من القمم عند قيم 2θ البالغة 43.35 و 50.50 و 74.21 ^{0}. اما تقنية المجهر الإلكتروني النافذ (TEM) اظهرت ان الدقائق النانوية تمتلك شكلًا كرويًا اذ بلغ متوسط قطرها 35-75 نانومتر ، بينما كشف فحص المجهري الإلكتروني الماسح (SEM) عن الشكل الشبيه بالكرة لـ دقائق النحاس النانوية. أظهر التحليل المجهري للقوة الذرية (AFM) حجم وخصائص السطح للجسيمات النانوية المركبة حيوياً وكشف عن متوسط حجم 55 نانومتر.

تم اختبار دقائق النحاس النانوية التي تم تحضيرها في هذه الدراسة كمضادات للأكسدة ومحفزات. أظهرت النتائج أن تلك الدقائق كان لها نشاط لكسح الجذورالحرة عند مقارنته بمستخلص أوراق نبات الياس *Myrtus communis*. من الشائع استخدام 4-نيتروفينول (4-NP) كتفاعل نموذجي لتقييم الخواص التحفيزية للمواد النانوية المحضرة ،اظهرت النتائج أن دقائق النحاس النانوية لها نشاط تحفيزي أفضل من المستخلص عند التراكيز العالية .

في هذه الدراسة ، تم اختبار فعالية كل من CuNPs والمستخلص ضد البكتيريا ونشاط هذه الدقائق كمضادات للالتهاب. تم تقييم النشاط المضاد للبكتيريا ل (CuNPs) والمستخلص النباتي ضد البكتيريا سالبة الجرام (*Klebsiella pneumonia* و *Pseudomonas aeruginosa*) والبكتيريا موجبة الجرام (*Staphylococcus aureus* و *Lactobacillus salivarins*) .في الدراسة خارج جسم الكائن الحي ، تم تقييم النشاط المضاد للالتهابات لكل من المستخلص و الدقائق النانوية باستخدام (فحص تمسخ الألبومين ، وفحص تثبيت الغشاء ، وفحص الفعالية المثبطة للبروتينيز) بتراكيز مختلفة. أظهرت نتائج CuNPs نشاطًا قويًا مضادًا للالتهابات مقارنة بالعقار

القياسي (الأسبرين).

في الختام ، يعد نبات الياس *M. communis* مصدرًا جيدًا لأختزال ملح النحاس إلى دقائق النحاس النانوية والأخير له نشاط مضاد للأكسدة ومضاد للالتهابات ، بالإضافة إلى أنه يمكن استخدامه كمحفز.

جامعة كربلاء

كلية العلوم

قسم الكيمياء

التخليق الأخضر لجسيمات النحاس النانوية بأستخدام مستخلص أوراق الياس: التوصيف والتطبيقات

رسالة مقدمة الى

مجلس كلية العلوم – جامعة كربلاء

كجزء من استكمال متطلبات نيل درجة الماجستير في الكيمياء

من قبل

ايات كريم ديلي

بأشراف

أ.د. نرجس هادي السعدي

أ.م. د.محمد عبد الحر كاظم

1443هـ

2022 م