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Estimation the Effect of Copper Nanoparticles on the Levels of Some Antioxidants Enzymes to Eliminate Inflammatory Damages

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

((وَعَلَّمَ آدَمَ الْأَسْمَاءَ كُلَّهَا ثُمَّ عَرَضَهُمْ عَلَى الْمَلَائِكَةِ فَقَالَ أَنْبِئُونِي بِأَسْمَاءِ هَؤُلَاءِ إِنْ
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صَدَقَ اللهُ العليُّ العظيمُ

(سورة البقرة/آية ٣١-٣٢)

CERTIFICATION

We certify that this thesis entitled **(Estimation the Effect of Copper Nanoparticles on the Levels of Some Antioxidants Enzymes to Eliminate Inflammatory Damages)** was prepared under my supervision at the Department of chemistry/ College of Science / University of Babylon , in partial requirements for the Degree of Master of Science in Biochemistry and this work has never been published anywhere .

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DEDICATIONS

To my dear mother's soul....may God have mercy on her.
To my esteemed father..... my God prolong his life, and grant him health and wellness.
I dedicate a special appreciation to my dear wife,
For girls and boys.
And to all the souls of the martyrs who sacrificed for the sake of Iraq.

Mahmood ali

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"In The Name of God Most Gracious Most Merciful"

Thanks and praise be to Allah prayers and peace be upon His Prophet Muhammad and his pure good family to complete this stage.

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Summary

Nanotechnologies are critical components in healthcare. Copper has been examined for its anti-inflammatory properties in its (copper ion) form. In comparison to typical treatments, nano - copper may have a better biological effect and has a variety of uses in healthcare.

In the present study, synthesis and characterization of copper Nano- particles and their application as anti- inflammatory agent were investigated. The goal of this study was to produce copper nanoparticles using plant extract and then assess their efficacy in increasing healing ability by influencing the levels of antioxidant enzymes that can reduce inflammatory damage. Purification and characterization of the final product were completed. Copper nano- particles were prepared by green synthesis process from copper sulphate solution through the extract of Artemisia plant.

UV-visible spectroscopy, Fourier Transform Infrared Spectroscopy, X-Ray Diffraction and Scanning Electron Microscopy were all used to characterize the formation of Cu NPs. Average particle size was found to be 54.57 nm. Antibacterial activity of prepared nano-particles was tested against two pathogens (*E- coli, Bacillus subtilis*) , as well as the therapeutic efficacy of the biological material .

In the case of biological substance particularly, fish skin tissue, the activity of some antioxidant enzymes (catalase, superoxide dismutase, and glutathione peroxidase) has been evaluated.

The project's design includes dividing forty fish into two groups for a laboratory research to measure the activity of some antioxidant enzymes. Ten were used as healthy fish (not infected with inflammation), thirty fish on the other hand were infected with dermatitis.

The effects of veterinary and Cu NPs treatment on the levels of each antioxidant enzyme were studied after two weeks treatment. Ten fish were treated with veterinary drug (Fungus Guard), while the other ten were treated with Cu NPs.

The results indicated significant evaluation of the activity of SOD, CAT and GPx in infected fish skin tissue in comparison with those of healthy ones ($p \leq 0.05$).

When copper nanoparticles were utilized for inflammatory treatment in infected fish, the results showed considerable increases in the activity of each antioxidant enzyme and were more efficient than veterinarian treatment ($p\leq0.05$) when compared to healthy fish.

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Abbreviation	Details
Cu NPs	Copper Nanoparticles
cGPX	Cellular Glutathione Peroxidase
Cu Zn-SOD	Copper, zinc superoxide dismutase
DTNB	5,5'- Dithiobis(2-Nitrobenzoicacid)
EC-SOD	Extracellular superoxide dismutase
EDX	Energy Dispersive X-ray
FTIR	Fourier Transform Infrared spectroscopy
FWHM	Full Width at Half maximum intensity
GPx	Glutathione Peroxidase
GSH	Reduced glutathione
GSH-Px	Glutathione peroxidase
GR	Glutathione Reductase
GSSG	Oxidized glutathione
$H_2 O_2$	Hydrogen Peroxide
NADH	Necotine Amid Adenine Dinecleutide
NPs	Nanoparticles

NADPH	Reduced Nicotinamide Adnine Dinucleotide phosphate
Ni-SOD	Nickel superoxide dismutase
NMs	Nanomaterials
NO•	Nitric Oxide
nm	Nanometer
$O_2^{\cdot -}$	Superoxide radical
$OH^{\cdot}$	Hydroxyl radical
$ONOO^-$	Peroxynitrite
PHGPX	Phospholipid Hydroperoxide Glutathione Peroxidase
PUFA	Polyunsaturated fatty acids
RNS	Reactive Nitrogen Species
ROH	Ethanol alcohol
ROOH	Carboxylic acid
ROS	Reactive Oxygen Species
SEM	Scanning Electron Microscope
SOD	Super Oxide dismutase
UV.Vis	Ultra violet-Visible
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic Acid
Tris	Tris- (hydroxymethyl)amino methane
XRD	X-Ray Diffraction
α-TQ	α-Tochopherol

CHAPTER ONE
INTRODUCTION

1. Introduction

1.1 Nanoparticles

Nanoparticles (NPs) are classified as particles with dimensions varying from 1 to 100 nm and having unique characteristics including a large surface area due to their small size.[1] It is well known that the atoms and molecules included in the small size particles that behave quite differently from those found in large size or mass materials [2].

NPs are receiving a lot of attention nowadays since they function as a linkage between the atomic or molecular structure and the bulk material, displaying new characteristics depending on specific features including size, shape distribution, capping agent, ionic strength, and morphology [3].

NPs are of a large number of materials, together with particulate substances, that have at least one dimension of less than 100 nm [4]. Due to their small size, high surface area to volume ratio, and high heat transfer, metallic nanoparticles have been employed and applied in "optical, thermal, magnetic, sensoric devices, and catalysis." The production of nanoparticles has gained a lot of interest because of its extraordinary physical and chemical characteristics, such as high conductivity and melting point, minimal electrochemical migration, and cost-effectiveness [5,6].

NPs can be made in a variety of ways, but the most common are physical and chemical techniques. The physical technique has the drawback that the resulting nanoparticles have imperfect surface formation, a poor rate of production, a high manufacturing cost, and a high energy demand [8-10]. Toxic chemicals, intense reducing agents, and high levels of radiation are used in the chemical technique for nanoparticle synthesis [7].

Numerous procedures are available to be used for (NPs) synthesis. These procedures, in general, are classified into mainly two categories**.** The first category is **Bottom-up approach,** while the second one is **Top-down approach** as shown in **Figure 1.1**. These procedures are even further divided into subcategories based on the preparation, reaction conditions, and protocols being used [11,12].

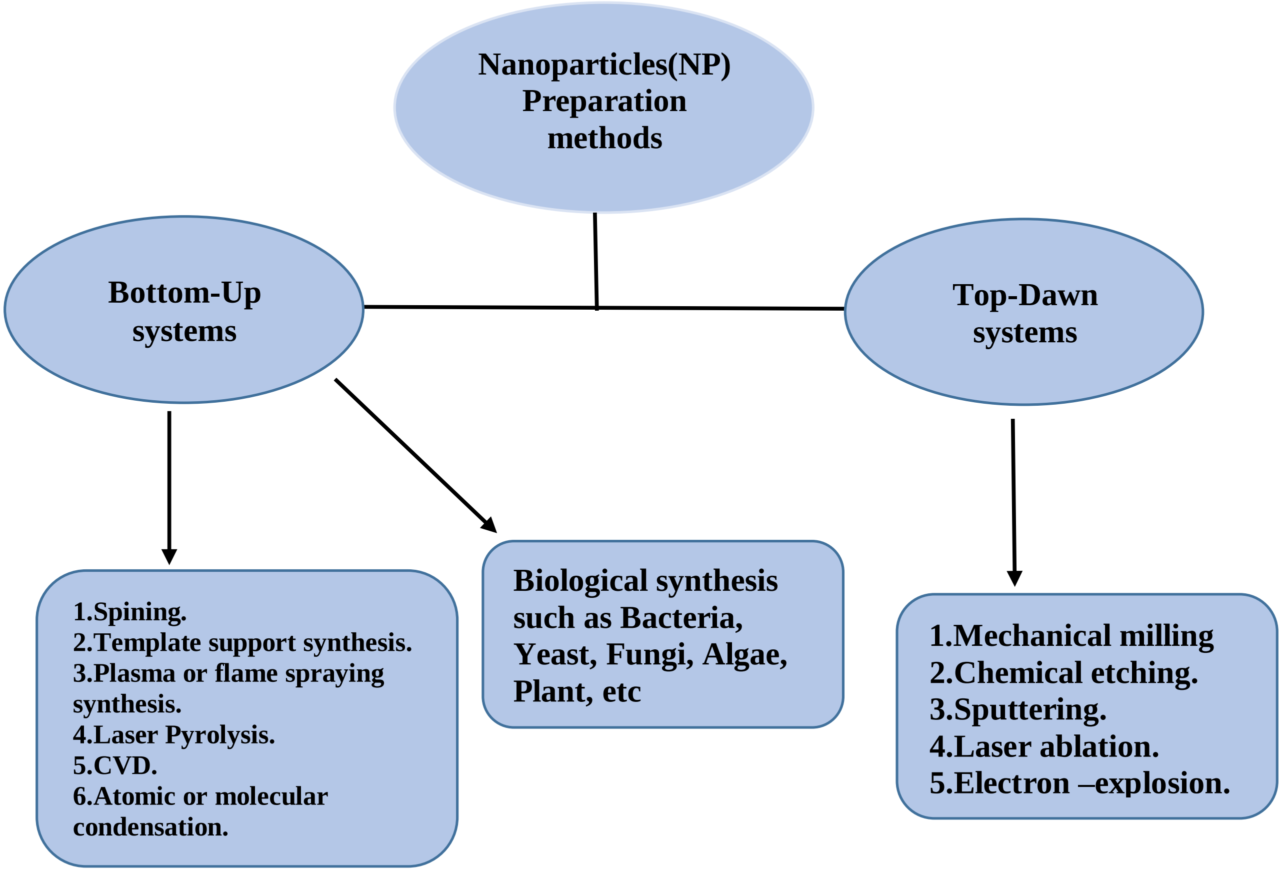


Figure1.1: Typical-synthetic-methods for NPs for top-down and bottom-up approaches [12].

1.2 Literature review

Chemical synthesis of metallic NPs goes back to the 14th and 13th centuries BC, as Egyptians and Mesopotamians began creating glass with metals, marking the commencement of the metallic nanoparticle period [13].

Michael Faraday described the synthesis of a colloidal Au NP solution in 1857, that was the first scientific description of NP production and began the scientific history of Nanomaterials (NMs). He further discovered that the optical properties of Au colloids differ from those of their bulk counterparts [14].

The notion of ‘‘nanoscience’’ has been credited to the Nobel Prize Laureate Richard Feynman who had set an identical visionary claim in 1959. He says that "The principles of physics, as far as I can see, do not speak against the possibility of manoeuvring things atom by atom". He also had stated that we will be allowed, in the future, to be able to "write the entire 24 volumes of the Encyclopedia Britannica on the head of a pin" [15]. Nevertheless, the word ‘‘nanotechnology’’ firstly has been used in 1974 by Norio Taniguchi who was also the one who defined it as the "production technology allowing to reach ultrahigh precision and ultra small sizes" [16].

The word "nano" originates from the Greek "nanos," which means "dwarf". At that point, the improvement of nanotechnologies has been facilitated by the two instruments that have advanced imaging and manipulation of nanoscale objects; the Scanning Tunneling Microscope (STM) was invented in 1981 by Gerd Binnig and Heinrich Rohrer, researchers from (IBM), and the Atomic Force Microscope (AFM) which was invented in 1986 by Gerd Binnig, Calvin Quate and Christoph Gerber [17].

Despite the fact that nanoscience’s and nanotechnologies seem to be novel concepts developed at the end of the 20th century, it is shown that metallic nanoparticles have been employed in glass and ceramic areas from ancient times, particularly as a colorant. Furthermore, the mineral contains a large number of natural (NMs) [18].

NPs, remarkably, have a long history. They are not just the outcome of contemporary research, nor are they limited to man-made materials. Weathering, volcano eruptions, wildfires, and microbial activities all create naturally occurring nanoparticles, which comprise organic (proteins, polysaccharides, viruses, and so on) along with inorganic compounds (iron oxyhydroxides, aluminosilicates, and metals, among others) [19,20]. Nanoparticles are not always manufactured by contemporary synthesis laboratories, but they have clearly been in nature for a long period of time, and hence their usage dates back to ancient times [21]. Though the use of clay minerals as natural nanomaterials appears to be simple, the controlled reinforcing of a ceramic matrix with natural asbestos nanofibers over 4500 years ago is more fascinating [22]. Metal nanoparticles, however, as color pigments in luster and glass technologies, on the other hand, produced the most spectacular effects [23]. Glazed ceramics with metallic luster decorations first emerged in Mesopotamia in the 9th century [24]. The existence of separate silver and copper nanoparticles distributed inside the glaze's outermost layers gave these decorations remarkable optical characteristics [25,26].

Metal nanoparticles that show iridescent bright green and blue colours under specific reflection circumstances are an example these decorations [27].

1.3 Copper Nanoparticles and its Importance's

Among different metal particles such as gold, silver, iron, zinc, palladium and quantum dots, copper is the most commonly utilized material around the world attributed to its electrical, optical, biomedical, catalytic, and antifungal/antibacterial applications [28]. When compared to other conventional catalysts, it can provide higher yields and reaction rates under mild reaction conditions [29]. Cu NPs are used as an antibacterial agent in a variety of applications. Copper is extremely toxic to microorganism like bacteria (*E. coli*, *Staphylococcus Aureus, Pseudomonas aeruginosa*) yet non-toxic to animal cells; as a result of these properties, it is regarded an efficient bactericidal metal. It is supposed to be safe for humans in applications such as food packaging and applications of water treatment [30].

Copper is among the most important components in medical science because of its numerous therapeutic effects like anti-inflammatory, anticancer, analgesic, and antibacterial properties [31].

Nevertheless, there have been numerous investigations on the synthesis of Cu NPs by applying different plant extracts [32]. Due to its catalytic, high electrical conductivity, optical, antifungal, and antibacterial characteristics, Cu NPs have received a lot of interest among the NPs [33].

Copper nanoparticles have received much interest by researchers in recent years attributed to their uses in industry and medicine [34]. In studies with Escherichia coli, other nanoparticles, such as platinum, gold, iron oxide, silicon oxides, and nickel, have not demonstrate bactericidal effects [35].

Because plants contain a wide number of bioactive compounds, several plant parts or complete plants have been utilized in the green synthesis of Cu NPs.
Plants extracts have been successfully used for this purpose [34].

The biocidal properties of Cu NPs have been widely used to treat wounds in recent years. They are employed in processed bandages with low preparation costs and remarkable physical and chemical characteristics [36].

In recent times researchers focused their efforts on developing an efficient "green" chemistry method for manufacturing metal (NPs). Green synthesis of Cu and CuO nanoparticles is further beneficial than chemical and physical synthesis because it is a clean, cost-effective, non-toxic, and ecologically friendly method. It does it without the use of harsh, poisonous, and costly chemicals [37].

Copper nanoparticles, particularly, are inexpensive and, in comparison to conventional catalysts, need only mild reaction conditions to create large yields of products in short reaction periods. They can also be recycled [38].

Copper nanoparticles synthesis is a popular issue in academic and, more significantly, applied nanotechnology research. Chemical reduction , electrochemical reduction, chemical vapor deposition, thermal decomposition , and solve thermal reduction are a variety of chemical and physical procedures [39].

These methods, however, have many problems Such as The toxic solvent usage, generation of hazardous byproducts, high consumption of energy and are non-ecofriendly. therefore, developing clean, reliable, biocompatible, cost-effective, a friend of the environment, and sustainable way for nanoparticles production becomes essential [40,41]. Researchers used plant extracts, specific plant parts, or the entire plant for the green synthesis of different nanoparticles, particularly copper nanoparticles, due to the great potentiality of plants as sources for the green synthesis of different nanoparticles, particularly copper nanoparticles [42].

1.3.1 Application of Cu NPs.

Because of their optical, catalytic, mechanical, and electrical properties, Cu NPs have received a lot of attention. They have also been utilized as a gold, silver, and platinum NPs substitution in a variety of applications (for example, microelectronics applications and thermal conducting materials) [43,44]. Cu NPs also have received consideration due to their applications in dressings of wound and biocidal traits, they also used in industrial developments, for instance, gas sensors, catalytic procedure, in high temperature superconductors and solar cells[45]. Cu NPs are employed in antibiotics as for their excellent physical characteristics, they are used as a bactericide agent to cover medical equipment, including heat transfer systems, antimicrobial materials, super strong materials, sensors and catalysts attributed to their disinfecting capabilities and matrix stability [46,47].

Nowadays, NPs have been extensively utilized in cosmetics, textiles, photonics, agriculture, and heavy industries such as catalysts. Moreover, the use of (NPs) in medicine has been expanded. According to the available information that Cu NPs can be utilized as antimicrobial and anti-inflammatory agents for healing wounds [48,49].

1.3.2 Synthesis of copper nanoparticles

Metal and metal oxide NPs development, in recent years, has considerably improved the biomedical sector in terms of bio sensing, imaging, diagnosis, and therapy [50,51]. Metals that are mostly used with their oxides are gold (Au), silver (Ag), and copper (Cu). Among these metals, Cu is a fairly low-cost metal that is further economical than Au and Ag [52]. In addition, Cu NPs are effective catalysts, they have excellent yields and are simple to separate, and they can be reused several times [53].

NPs can be manufactured by using a numeral method together with physical, chemical, biological and hybrid techniques as shown in **Figure 1.2** [54].The biological production of NPs in particular Cu NPs, apply the plants (disabled plant tissue, plant extracts and living plant), enzymes and microorganisms. metal biosynthesis NPs by plants is in use too [55].

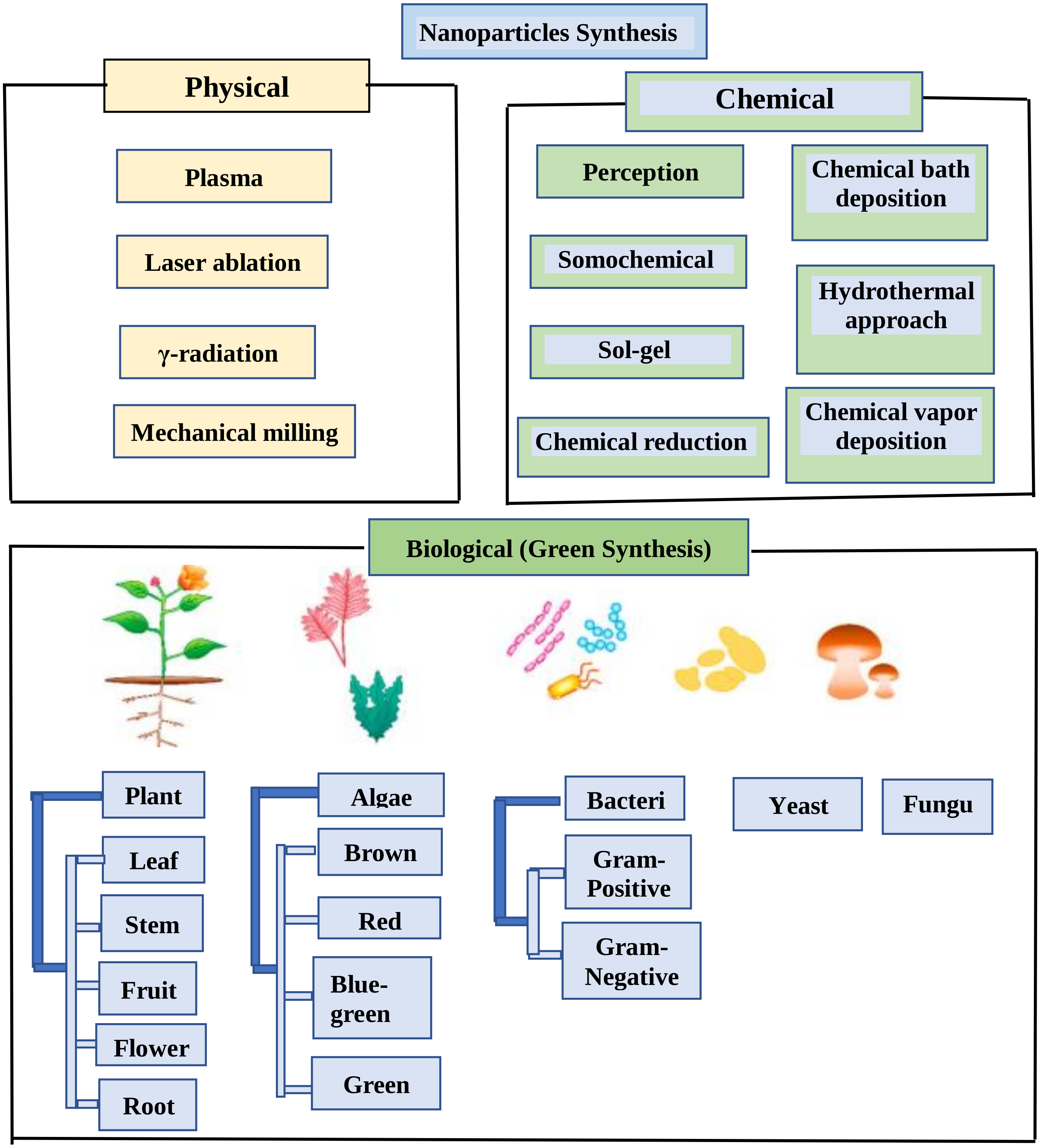


Figure1.2: An outline of several types of synthesis processes used to make a wide range of nanoparticles[49].

For a short period of time, conventional techniques can be utilized to produce NPs in huge quantities with specified sizes and shapes. However, these methods are time-consuming, expensive, and ineffective [56].

Chemical methods for the production of copper nanoparticles have been utilized in the past, but they come with a lot of environmental and health impacts, as well as the use of expensive chemicals. As a result, new and safe techniques for NPs production are needed, such as a biological process using plant extracts such as "Nerium oleander, Punica granatum, Aegle marmelos, and Ocimum sanctum, as well as Zingiber officinale", which enhanced the availability of high yield copper nanoparticles [57].

Green synthesis of environmentally friendly Metal nanoparticles that do not create toxic waste products during the production process has shown a lot of growth in recent years [58-60]. As a replacement for conventional physical and chemical techniques, this can be accomplished by employing biotechnological methods that are considered suitable and environmentally friendly for nanomaterial creation. [61]. As a result, the notion of green technology or green nanobiotechnology has emerged.[48]

Green nanobiotechnology, in general, means producing NPs or nanomaterial by means of biological routes like bacteria, fungi, plants and enzymes or their byproducts, including proteins, with the aid of several biotechnological equipment **Figure 1.2**.The twelve criteria of green science now have turned out into a referential guidance for experts, researchers, chemical technologists and scientific experts all over the world for developing a smaller amount dangerous and further stable compounds [62,63]. In such manner, green nanobiotechnology is a likely backup for the creation of biocompatible stable NPs [64].

Biological based production of Metal nanoparticles employs a bottom-up methodology in which NPs are manufactured by using Reducing and stabilizing agents [65]. Three important steps that are followed for the production of NPs. first, selection of solvent medium. Second, selection of an environmentally friendly reducing agent. Third, selection of a non-toxic substance as a capping agent to settle down the created NPs [55,66]. Nanotechnology offers an advantage over other traditional approaches since it allows for the creation of additional components by the biological system for the development of NPs. The high biodiversity has been studied for the production of bionanomaterials that are environmentally friendly and could be used in a number of therapeutic applications [48].

1.3.3 Green synthesis of Cu NPs.

Many researchers have attempted to investigate green techniques for the production of metal NPs in earlier decades, with a focus on approaches involving microorganisms and organisms instead of plants and plant extracts [67]. The approach of using plants and plant extracts is underutilized but has potential outcomes. Plants for the synthesis of metallic NPs could offer more benefits than some other biological approaches since they do not require the time consuming process of maintaining cell cultures [68].Similarly, the absence of pathogenicity is an important aspect of using plants. This study discusses plant and plant extract techniques in order to determine the best method for production of Cu NPs. The basic synthesis method is displayed in **Figure 1.3**. Depending on the synthesis circumstances, different scientists applied various plant extracts to form Cu NPs of various sizes and forms. Cu NPs are perceived to be extremely oxidant, and several difficulties with stability, oxidation resistance, and aggregation have been identified [48].

As a result, the production of Cu NPs has not received as much attention than that of other metals. At normal temperature, Cu NPs are simply oxidized off the surface, according to later studies [69]. Gold and silver NPs, on the other hand, are more resistant to oxidation than Cu, although Cu remains an attractive for future conducting materials due to its availability and low-cost. The use of various capping agents, such as polymers and natural ligands, can complicate the problems of aggregation and oxidation [48,70].

To take use of the benefits of this approach, the focus has shifted to synthesis of Cu NPs by utilizing plants and plant extracts. For instance, in this research, Cu NPs have been produced in $CuSO_4$ aqueous solution employing worm-wood extract as a reducing agent. (NPs) with a cubical form and a size of 57 nm were produced for twelve hours. Both gram positive and gram negative pathogens were used to test the antibacterial properties of the Cu NPs (as *Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa*) [69,71].

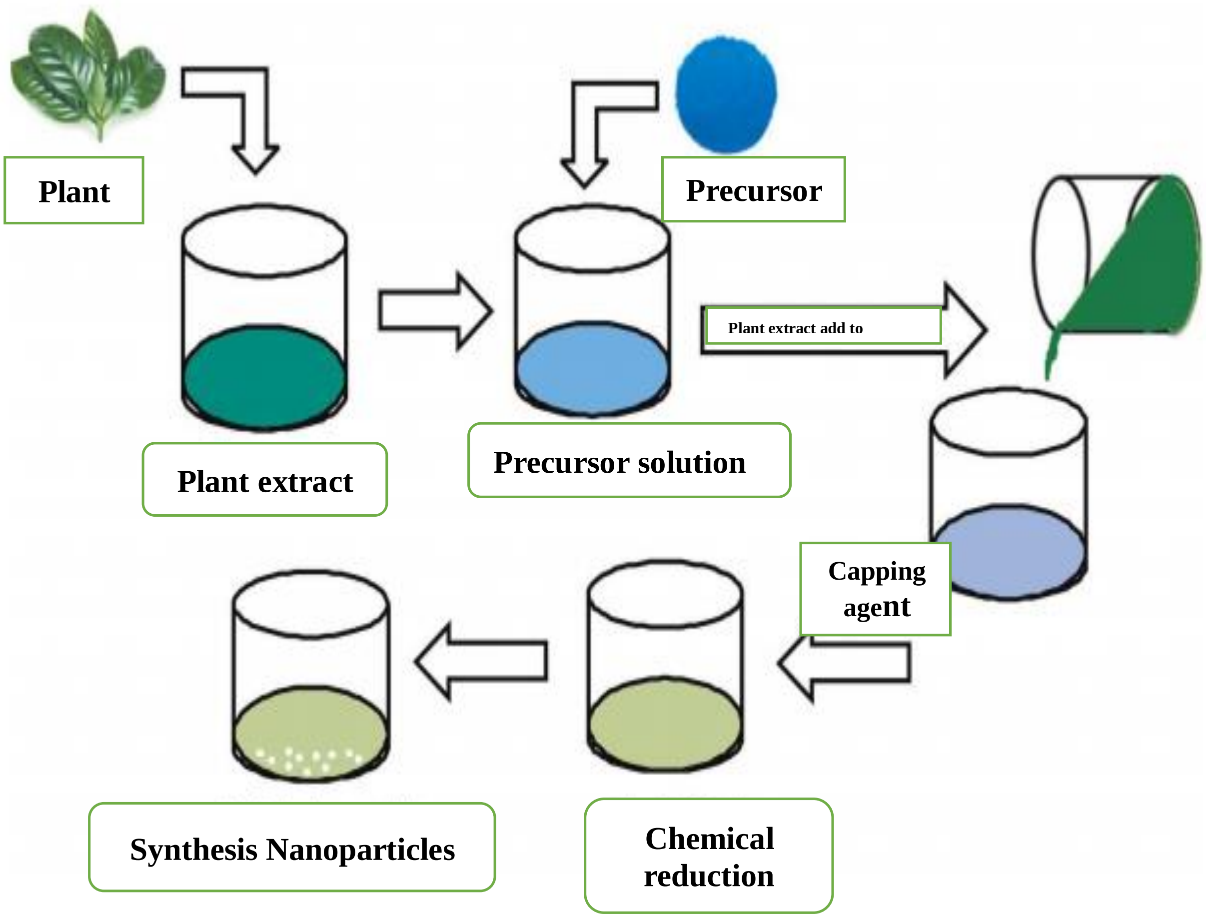


Figure 1.3: Biological synthesis of NPs [48].

Due to several benefits over traditional physical procedures and chemical synthesis methods, green synthesis is becoming a popular approach for producing nanoparticles. The most important advantages are Aqueous room temperature, cost-effective, stable for a long period, and reproducible. Additionally, simple ethanoic wash can remove the capped polyphenols from the particle surface [72].

Cu is a necessary component of all living organisms and is involved in a variety of physiological processes such as protein metabolism, antioxidant activity, cell wall metabolism, hormone sensing...etc. Cu in excess can create Reactive oxygen species (ROS) and free radical. Copper is a structural catalytic component of numerous proteins and enzymes engaged in a number of metabolic processes at the cellular level [73].

The medical characteristics of plant extract are found to secure the NPs Production, which is being used in medicine, drug targeting, and cosmetic activities [74].

Researchers are interested in plant-based synthesis because of various active compounds in the plant can be presented, the method is relatively fast and safe, and it can work at room temperature without requiring a lot of physical effort [75].differnt substances have been used to synthesis Cu NPs as shown in **Table 1.1**

Table 1.1: Certain green substrates for the production of Cu NPs.

Green substrate	Substrate scientific name	Part of substrate	Size range	morphology	References
plant	Cissus arnotiana	Leaves	60–90 nm	spherical shape	[76]
Plants	Tinospora Cardifolia	Leaves	63.3 ± 0.64 nm	spherical shape	[78]
Plants	Quisqualis indica	Floral	39.3 ± 5.45 nm	spherical shape	[79]
Plants	Zingiber officinale and Curcuma longa	Fruits	20-100 nm	spherical shape	[80]
Plants	Solanum Lycopersicum	Fruits	40-70 nm	spherical shape	[81]
Microorganism	Botryococcus braunii	Algae	10-70 nm	irregular shape	[82]
Microorganism	Escherichia sp. SINT7	Bacteria	22.33-39 nm	spherical shape	[83]
Microorganism	Aspergillus niger strain STA9	Fungal	5-100 nm	spherical shape	[84]
Biopolymer	Starch	Polysaccharide	50-70 nm	irregular shape	[85]
Carbohydrate	Glucose	Monosaccharide	200 ± 50 nm	spherical shape	[86]

1.3.4 Factors affecting the green synthesis of copper nanoparticles

The reaction temperature, pH, timeframe, amount of plant extracts, precursor utilized, and mixing velocity are all important factors in influencing the properties of the Cu and CuO NPs being produced. Multiple factors, in fact, influence the synthesis of NPs of the required size and shape. As a result, keeping an eye on these variables might result in NPs with unique characteristics and applications that are simple to significantly increase and have a reduced synthesis time [87].

1.3.4.1. Time

During long-term storage, NPs can cluster, decrease, or increase, and they also have a shelf life that influences their total potential [88]. Mudunkotuwa et a. (2012) found out a difference in size and chemical behavior between new and old Cu NPs. For Cu NPs developed under ambient circumstances, the size of new Cu NPs grew from 10 ± 5 to 18 ± 10 nm. In comparison to new Cu NPs, the aged Cu NPs are found to be significantly oxidized [89].A significant correlation has been seen in the length of time of the green synthesis process. According to studies, the size of NPs is determined by the reaction time [90,91].

1.3.4.2. pH

One of the parameters determining the size of NPs in biosynthesis is the pH of the re-acting mixture NPs. The reaction medium's optimal pH varies from 7 to 9, indicating that an alkaline environment is the best for synthesizing NPs [92]. When NPs are biosynthesized in an alkaline environment pH >8, they are smaller sized NPs. The NPs produced in an acidic pH 7 environment are bigger, reaching up to 153.7 nm [93].

Rajesh and his co-researchers discovered that when the pH of the re-action mixture raised from 6 to 10, the size of the Cu NP reduced from 32 to 20 nm [94]. the other hand, reported the effect of pH on the structure of CuO NPs. At pH10 (alkaline), the formulation of CuO NPs generates loosely agglomerated NPs with sharp CuO NPs with such a particle size of 28.2 nm. At pH 7, however, more agglomerated CuO NPs with a size of 66.3 nm are formed neutral. Surprisingly, the genotoxicity of CuO NPs produced at pH10 is greater than that of CuO NPs synthesized at pH 7 [95].

1.3.5 Anti-microbial activity of copper nanoparticles

Pathogen antibacterial activity was determined by utilizing the very well diffusion technique. Copper nanoparticles' bactericidal effect is due to the higher surface-to-volume ratio and relatively small size, which allows interaction closely with microbial membranes. Two pathogenic bacteria, like *E. coli* and *Bacillus subtilis*, have been used in the antibacterial investigation of Cu NPs [96].

According to recent nanomaterials research, different metallic and metal-oxide nanoparticles NPs could have extremely promising and powerful roles as antibacterial agents. Because of their high surface to volume ratio and structure of crystalline, such (NPs) trigger biological reactions that vary from those elicited by the conventional ionic form of metals. Furthermore, metallic NPs have been discovered to have (a) 7 to 50 times less harmful impact on mammalian cells than their equivalent ionic forms and (b) a longer effect as a source of components in an organism. Copper and its complexes have been extensively used as inexpensive and efficient materials for sterilizing liquids and also human tissues for centuries among the various metals [97,98].

As a result, metallic Cu NPs might be a potential antibacterial agent in the future. The production of stable metallic Cu NPs is the most difficult job in this approach, because they quickly oxidize to Cu^{2+} ions in atmosphere or aqueous environments. Researchers have discovered a technique for synthesizing Cu NPs that is stable in ambient conditions for roughly a month , and whose bactericidal effect on both "Gram-negative and Gram-positive" bacteria is significantly higher than reported and prepared by others [99,100]. Yet, to employ them as a supernumerary for antibiotics or such as an antibacterial sterilizing agent, surveys on (a) the molecular instruments of bacterial cell-killing and (b) the manner of NP action in cell-killing is essential to be completed [96].

Although the processes behind metallic nanostructures' biocidal action are not fully understood, three plausible theories have been proposed and described in the literature:

1. The accumulation and breakdown of NPs in the bacterial membrane changes its permeability, allowing lipopolysaccharides, membrane proteins, and intracellular biomolecules to be released and the proton motive force to be dissipated across the plasma membrane [101,102].
2. Oxidative impairment to cellular constructions due to the manufacture of reactive oxygen species ROSs and their equivalent ions from NPs [103,104].
3. Absorption of metallic ions produced via NPs or NPs as a whole into cells, resulting in ATP depletion and DNA replication disruption [105,106].

1.4 Medicinal plants

Individuals and communities benefit greatly from the use of medicinal plants. The therapeutic significance of these plants is due to a number of chemical compounds that have a specific physiological effect on the human body. Alkaloids, tannins, flavonoids, and phenolic chemicals are the most significant bioactive components of plants [107]. Much of these local medicinal plants are also consumed as spices and food. They are also occasionally used for medicinal purposes as a supplement in meals for pregnant and nursing mothers [108].

Plants have a high degree in phytoconstituent biomolecules, which can be used as a medicine. Because of their capping and reducing characteristics, they have recently been used to make metal/metal oxide nanoparticles NPs. By manipulating parameters throughout the synthesis method, this green synthesis technique is ecologically safe and permits the creation of required NPs in various sizes and forms [109].

Any substance found in plants is referred to as phytochemicals ("the ancient Greek word phyton means plant"). Phytochemicals are non-nutritive plant compounds that have some disease preventive properties [110]. The word phytochemical, on the other hand, is frequently used to designate a wide spectrum of biologically active chemicals found in plants. The latter get their colour, taste, and natural insect defense from phytochemicals. A dietary that is rich in vegetables and fruits has been shown in several epidemiological studies to provide significant health advantages to people. Amongst these advantages are as follows; on the one hand, minimizing the probability of gaining a variety of cancer forms such as lung, pancreas, bladder, breast and prostate). Lessening the possibility of cardiovascular diseases on the other hand. The existence of phytochemicals in fruit and vegetables is responsible for a great deal of these positive benefits. Phytochemicals, in this context, are "non-nutrient" compounds present in plants that seem to have bioactivity against chronic diseases [111,112].

Plants generate a variety of secondary metabolites and phytochemicals, which can be used to synthesize Cu and other metals CuO NPs. Phenols and flavonoids are the most significant phytochemicals in plants, and they can be found in multiple parts of the plant, including shoots, leaves, stems, flowers, roots, and fruits. Such phenolic compounds have hydroxy and ketone groups, which help to chelate iron and, as a result, have a high antioxidant activity [113]. NPs produced using this green approach enhanced in stability, preventing NPs agglomeration and deformation, and allowing phytochemical adsorption on the interface of the NPs [114].

This study have the sort of plant Artemisia generally well-known as the desert wormwood ("Arabic name is Sheeh"), is a dwarf plant or semi-shrub growing extensively in Jordan and in the Middle-East. This plant is a recurrent, powerfully scented herb, with numerous basal, straight with leafy stems enclosed by woolen hairs. The heads of flower are around 0.5 cm in diameter, not attractive, and they are made merely of tubular florets. In Jordan, the plant is found in arbid mountains and desert regions such as, Tafila, Karak, Shaubak, Mafraq, Zarka, Ras an Naqab and the Eastern Desert [115]. Artemisia plant which was used in this study collected from Iraq and it is classified according to University of Babylon - College of Science - Biology department Kingdom: plant, Class: Angiosperm, Subclass: acotyledons, Order: Aster ales Tribe : Anthemideae, Genus :Artemisia ,Species :Artemisia sp [116]. as well as in local markets. The plant is mostly utilized by the local community as an anthelmintic and antispasmodic, as well as for various traditional medicinal purposes such as cough reduction, intestinal disturbances, runny noses, and pain reliever [117].

Artemisia absinthium (A. absinthium) is a wooden herbaceous permanent undershrub plant having fibrously roots originate to Central Europe; southern Siberia; North America; and Asia in which it is utilized as for medicine [118]. Artemisia has been named after Artemis, according to ancient Greek mythology, she is the goddess of the hunt and the wilderness, the protector of children, and the goddess of procreation, virginity, the earthly. The plant was often used to stimulate menstruation, and its name may come from its capacity to exhibit purity as a consequence. The recurrent root produces branching, strong, leaf stems that are often almost wooden at the base. The blooming stem is 2 to 2½ feet in height and whitish, with fine, silky hairs covering it [119].

A comprehensive literature review on phytochemical information of the type Artemisia shows that the Artemisia types mainly consist of coumarins, flavonoids, terpenoids, sterols, caffeoylquinic acids, and acetylenes. Among all different types of Artemisia, A. afra A. absinthium, A. annua, A. scoparia , and A. maritima (Waldst et Kit) are particularly rich in terpenoids[120].

1.5 Oxidative stress

When there is an overabundance of ROS and a shortage in enzymatic or non-enzymatic antioxidants, oxidative stress arises in a biological system [121]. The presence of oxygen is required for metabolic activities to take place. Free radicals, on the other hand, are mostly produced by oxygen. Molecules with electrons in their outer orbitals make up a large number of elements. During oxidation, such as oxidative phosphorylation in mitochondria, chemical bonds between these elements break, resulting in extremely re-active entities with unpaired electrons in their outermost orbitals. During oxidation, one electron is extracted from molecular oxygen, resulting in the formation of the superoxide radical ($O_2^{\bullet-}$) a precursor for most ROS. Hydrogen peroxide H_2O_2 is formed when the superoxide radical interacts with an electron [122].

Hydrogen peroxide can be a part of hydroxyl anion (OH^-) or totally compressed to water. ROS are, on the one hand, either free-radicals, For example hydroxyl radical OH, peroxyl radical, superoxide radical ($O_2^{\bullet-}$) ozone (O_3), or, on the other hand, non-radical reactive mixtures such as singlet oxygen (O_2) and hydrogen peroxide H_2O_2 as displayed in [123].

Free radical entities are extremely reactive and not stable. They acquire electrons from nucleic acids, lipids, proteins, carbohydrates, or any adjacent molecule to become stable, leading in a chain reaction that causes cellular damage and illness. Reactive oxygen species (ROS) and reactive nitrogen species (NOS) are the two main kinds of free radicals [124]. as displayed in **Figure1. 4 .**

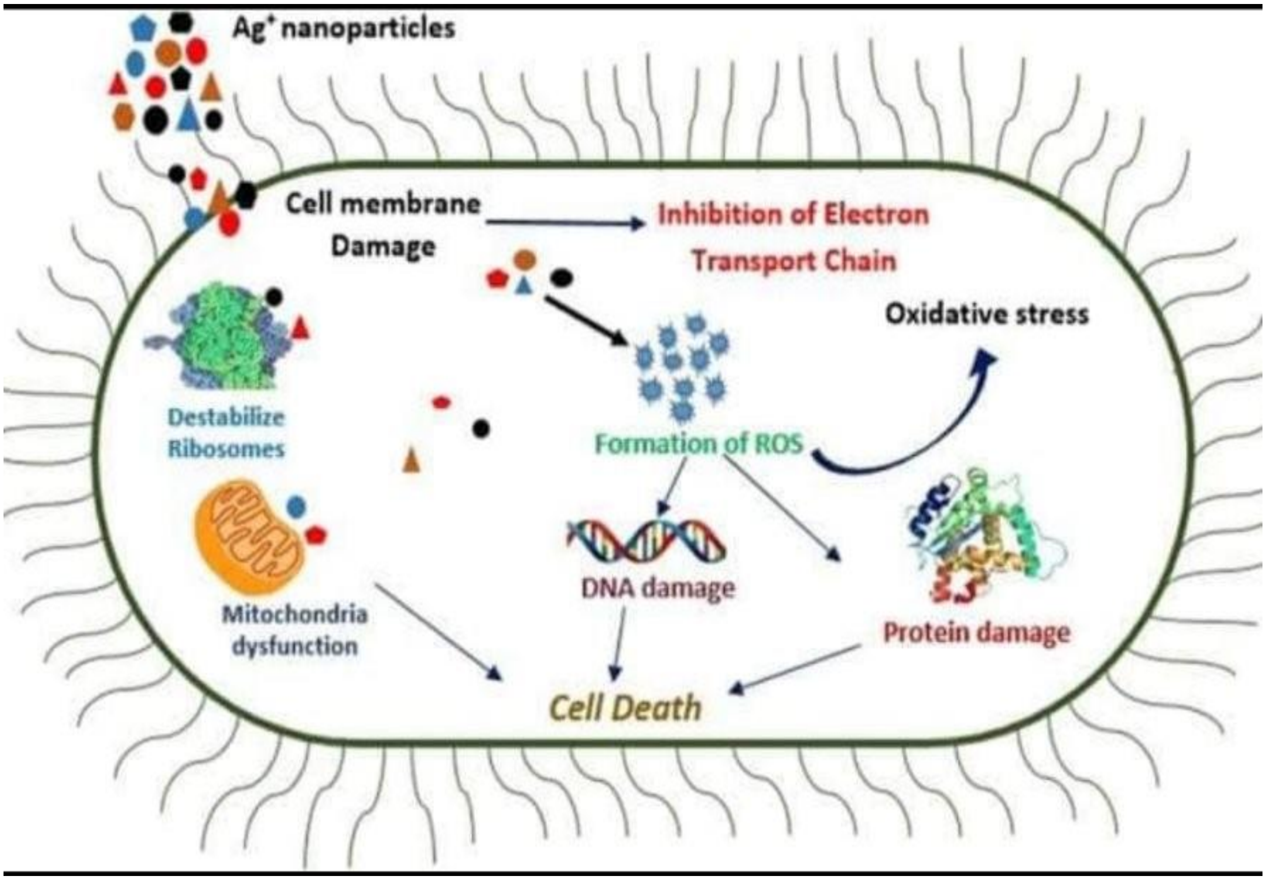


Figure 1.4: Instruments of oxidative stress induced cell damage[124].

They are crucial intermediates in natural processes including cytotoxicity and vascular tone control. Radiolysis is a strong technique for producing and measuring particular free radicals and their activity [122]. Endogenous cellular sources also generate free radicals during regular cell metabolism. Mitochondrial respiration is the major natural source of ROS. Increased generation of ROS can affect oxidation of proteins and lipids, changes in gene expression, and alterations in cell redox state [125].

Moreover, reactive oxygen type are made by substances including transitional metal-ions, petroleum pollutants, and pesticides [126].

Defines an oxidant as a material which can cause oxidative damage to a variety of biological targets, including nucleic acids (Such as base modification, single and double-strand breaks), lipids (for instance. Peroxidation, fatty acid loss), and proteins (e.g. Oxidation of particular amino-acid residues, creation of carbonyls)[122].

The most basic definition is that it is a molecule, ion, or atom with one or more single electrons in the outer shell. They are highly unstable particles that require an electron for becoming stable. They contain a lot of energy, which causes them to react quickly with stable compounds in order to achieve the most stable state [127].

Free radicals have had the capacity to oxidize biomolecules like as nucleic acids, lipids, proteins, and carbohydrates in their membranes, leading to changes in cell composition and programmed cell death [128]. Increased levels of free radicals promote the appearance and development of a variety of diseases, including cancer, lung disease, heart disease, infertility, high blood pressure, and thyroid disease, among others [129].

Living cells have produced a complicated defensive mechanism comprised of enzymatic and non-enzymatic antioxidants that transform these (ROS/RNS) species to harmless ones in order to counteract their attack. Enzymatic antioxidant defenses consist of Superoxide dismutase (SOD) detoxifies $O_2•^-$ to water and H_2O_2, catalase converts H_2O_2 to oxygen and water, and glutathione peroxidase detoxifies cellular peroxides to alcohols and water [130-132].

Non-enzymatic antioxidants are denoted by nutritive antioxidants such as ascorbic acid (vitamin C), α-tocopherol (vitamin E), polyphenolic, and carotenoids compounds. Antioxidants such as uric acid, bilirubin, glutathione, albumin, and further proteins (e.g., Transferrin, ceruloplasmin, myoglobin and ferritin) are regarded as endogenous antioxidants intended for defending vital biological targets against (ROS/RNS) action [133]. There is a suitable pro-oxidant/antioxidant balance in a typical biological system. When there is an overload of (ROS/RNS) or when antioxidant protection is reduced, the balance might shift towards pro-oxidant agents. This condition is also known as oxidative stress, and it may be induced by a variety of things, including diseases, nutrition, life activity, and environmental elements [134,135].

Extra (ROS/RNS) can damage cellular lipids, proteins, or nucleic acids, preventing them from performing their functions normally. As a result, oxidative stress has been linked to the development of a number of human diseases, which include atherosclerosis, cancer disease, cardiovascular disease, diabetes, inflammatory diseases, ischemia reperfusion damage, and neurodegenerative diseases (Alzheimer's and Parkinson's diseases), and also the age-related diseases [136].

The main processes for the synthesis of ROS and RNS in ***vivo*** are shown in **Figure 1.5**.

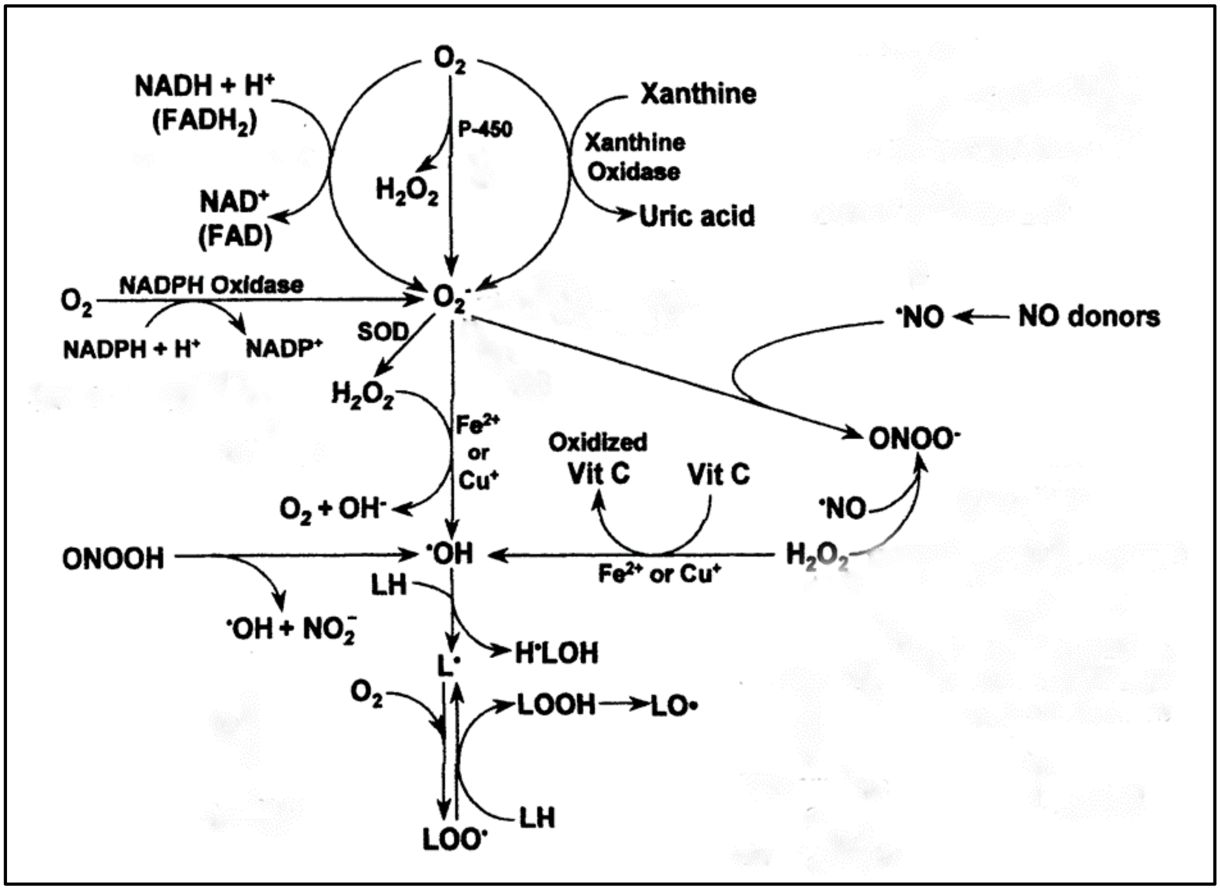


Figure 1.5: Synthesis of reactive oxygen and nitrogen types in mammalian cells [137].

1.6 Antioxidants

An antioxidant is a component that inhibits the beginning or propagation of an oxidation chain reaction, consequently delaying or slowing the oxidation of other molecules [138,139].Antioxidants can function as a protective mechanism against free radical damage, neutralizing the negative impacts of ROS and RNS in a variety of ways and potentially protecting the body from a variety of diseases [140]. As a result, several antioxidant defense mechanisms in a typical adult cell can protect it from oxidative stress [141].

Antioxidants are vital components of the human body's defensive mechanism. They aid in the reduction of oxidative stress induced by reactive oxygen species. Antioxidants work by stabilizing or deactivating free radicals before they damage a biological cell's target [142].

Free radical deactivation is recognized to have a role in a variety of diseases, including diabetes, atherosclerosis, aging, immunological suppression, and neurodegeneration [143].

Antioxidants also work by donating electrons to free radicals, stabilizing them and stopping a chain reaction. They also have a protective impact on cells, reducing the damage caused by free radicals. Antioxidants defense primarily through four mechanisms: they inhibit the production of free radicals, scavenge newly generated radicals, and repair damage caused by oxidative stress [144].

They are also crucial for preserving overall good health and well-being since they are termed as the first barrier of defense against ROS negative impacts [145]. Endogenous (enzymatic) or exogenous (non-enzymatic) antioxidants are also possible [146]. **Figure 1.6**

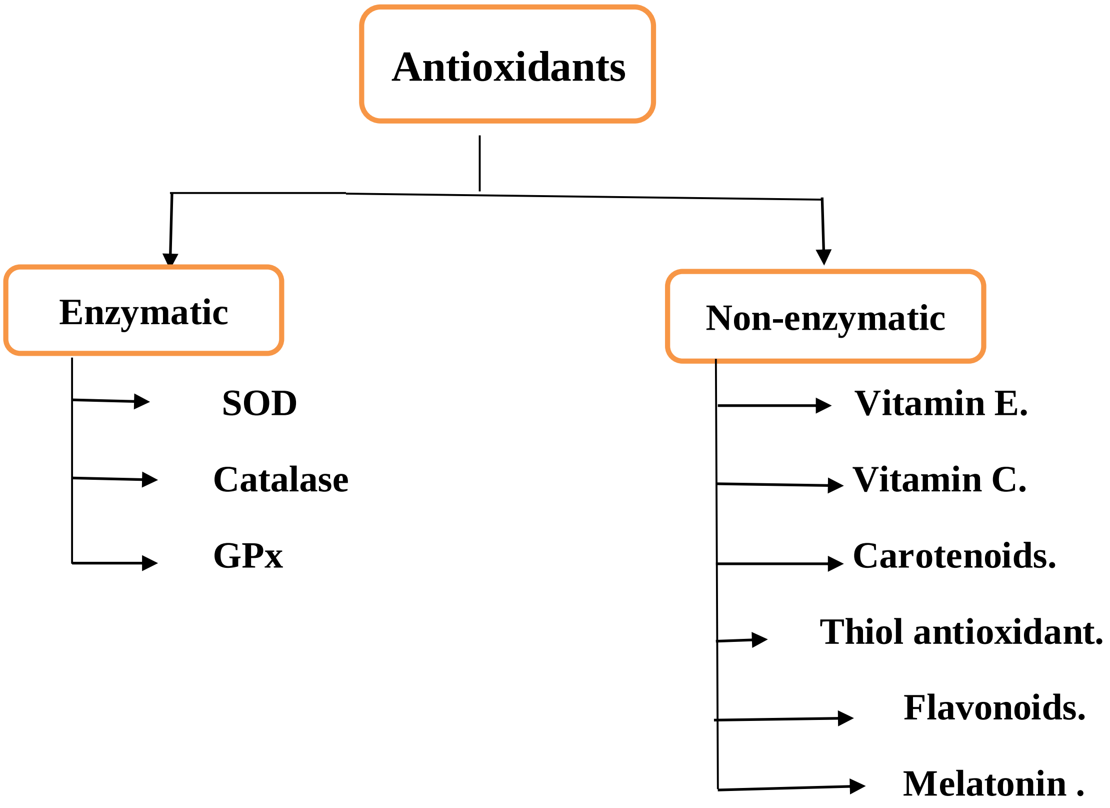


Figure1.6: Enzymatic and non-enzymatic taxonomy of antioxidants [147].

Antioxidants are found mainly in the human body and help to counteract the effects of oxidants. These can be divided in two groups for all practical purposes including enzymatic and non-enzymatic [148].

This study was focused on the enzymatic type.

1.6.1 Enzymatic Antioxidants

The most important enzymatic antioxidants of lungs are SODs (EC1.15.1.11), catalase (EC1.11.1.6), and (GSH-Px) (EC1.11.1.9) [149]. Other antioxidants exist in addition to these main enzymes including heme oxygenase-(EC 1.14.99.3), and redox proteins, such as thioredoxins (TRXs, EC 1.8.4.10),peroxiredoxins (PRXs, EC 1.11.1.15), and glutaredoxins, have been also realized to have crucial contributions in the pulmonary-antioxidant defenses as superoxide is the principal ROS made from a range of sources, [150]. Its dismutation by SOD is of major significance for each cell. All three forms of SOD, which are, Cu Zn-SOD, Mn-SOD, and EC-SOD, are commonly conveyed in human-lung. (Mn-SOD), thus, is confined in the mitochondria matrix. EC-SOD)is mainly confined in the extra-cellular matrix, particularly in parts covering extraordinary amounts of type one collagen fibers and nearby pulmonary and systemic vessels [151].

It has also been noticed that in the bronchial epithelium, alveolar macrophages, and alveolar epithelium. Overall, Cu Zn-SOD and Mn-SOD are, in general, believed to act as large scavengers of superoxide radicals. The comparatively high EC-SOD level in the lung with its exact binding to the extracellular matrix constituents can denote a central factor of lung matrix defense [152].

H_2O_2 that is shaped by the process of SODs or of the oxidases like xanthine-oxidase, is condensed to water by catalase and GSH-Px. Catalase occurs as a tetramer constituted of four equal monomers; each one comprises a heme group by the active site [153]. Degradation of H_2O_2 is made by means of the conversion between two conformations of ferri-catalase (iron coordinated to water) and compound **(I)** (iron complexed with an oxygen atom). Catalase, moreover, binds NADPH by means of a reducing corresponding to avoid oxidative inactivation of the enzyme (creation of compound **(II)** by H_2O_2 as it is condensed to water [154].

Additionally, from the oxidative impairment is intensified by the reduction in antioxidant enzyme acts, for instance, superoxide dismutase SOD, catalase glutathione S-transferase (GST), and glutathione peroxidase (GPx) which plays like a free radical scavengers in situations linked with oxidative stress [155,156]. **Table 1.1.**

Table 1.2.: Enzymatic Scavenger of Antioxidant Defense [157].

Name of Scavenger	Acronym	**Catalyzed Reaction**
Superoxide dismutase	**SOD**	$M^{(n+1)+}\text{-SOD} + O_2{}^{2} \longrightarrow Mn^{+}\text{-SOD} + O_2$ $Mn^{+}\text{-SOD} + O_2 + 2H^{+} \longrightarrow M^{(n+1)+}\text{-SOD} + H_2O_2$
Catalase	**CAT**	$2\,H_2O_2 \longrightarrow O_2 + 2\,H_2O$ $H_2O_2 + \text{Fe(III)-E} \longrightarrow H_2O + O = \text{Fe(IV)-E(.+)}$ $H_2O_2 + O = \text{Fe(IV)-E(.+)} \longrightarrow H_2O + \text{Fe(III)-E} + O_2$
Glutathione peroxidase	**GTPx**	$2GSH + H_2O_2 \longrightarrow GSSG + 2H_2O$ $2GSH + ROOH \longrightarrow GSSG + ROH + H_2O$
Thioredoxin	**TRX**	Adenosine monophosphate + sulfite + thioredoxin disulfide = 5'-adenylyl sulfate + thioredoxin Adenosine 3', 5' -bisphosphate + sulfite + thioredoxin disulfide = 3' -phosphoadenylyl sulfate + thioredoxin
Peroxiredoxin	**PRX**	$2\,R'\text{-SH} + ROOH \longrightarrow R'\text{-S-S-}R' + H_2O + ROH$
Glutathione transferase	**GST**	$RX + GSH \longrightarrow HX + R\text{-S-GSHOH}^{-}$

The three primary stages of defense in the cell form the foundation of the antioxidant system. SOD, GPx, catalase and metal-binding proteins from the first-level of defense through inhibition of free radical development. chain-breaking antioxidants (vitamin A,E,C, glutathione, carotenoids, uric acid… etc.) related to the second level of defense and dealing with inhibition and control chain creation and spread [149,158]. A third level of antioxidant defense is dealing with impaired molecules in the cell as a consequence of free-radical action and poisonous products of their metabolism and embraces several enzymatic organisms in control for healing or elimination of the impaired molecules [159].

Antioxidant enzymes are found in every cell in the body. Superoxide dismutase, catalases, and glutathione (GSH) peroxidases are the three primary families of antioxidant enzymes. There are also a variety of specific antioxidant enzymes that react with and detoxify oxidants [160,161].

1.6.1.1 Superoxide dismutase Superoxide

Dismutase turns the free radical superoxide to peroxide, which can then be eliminated by CAT or GPX processes. SOD is an enzyme that transforms the highly re-active superoxide radical to the less reactive H_2O_2[162].

$$O_2^- + O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Additional purpose of superoxide dismutase is to defend dehydratases (aconitase, dihydroxy acid dehydratase, fumarases (A and B), and phospho gluconate dehydratase) contrary to inactivation by the free radical superoxide [163].

Four categories of SOD have been acknowledged, holding either a dinuclear Cu, Zn or mononuclear (Fe), (Mn) or (Ni) cofactors [164]. Fe-SODs and Mn-SODs display homology and have equal metal chelating remains by the active site, distribution considerable order and three dimensional organizational homology, whereas the other superoxide dismutase are basically distinct. In humans, three forms of SOD are existed including; cytosolic (Cu-Zn-SOD), mitochondrial (Mn-SOD), and extracellular SOD (EC-SOD). SOD catalases the dismutation of ($O_2^{\cdot -}$) by sequential oxidation and lessening of the transition metal ion at the active-site in a Ping-Pong type mechanism with unusually high reaction degrees [165,166].

1- Manganese superoxide dismutase

Mn-SOD is a homesteader holding one manganese atom persubunit that sequences from Mn (III) to Mn (II) and back to Mn (III) throughout the two stages dismutation of superoxide. The respirational series in mitochondria is of a main basis of oxygen radicals. Mn-SOD is a nuclear-encoded principal antioxidant enzyme that functioning to eliminate these superoxide radicals [167].

2- Copper, zinc superoxide dismutase

Cu Zn-SOD is a kind of enzyme that has survived evolution and has two identical subunits of around 32 kDa, each carrying a metal group, the active site, made up of a copper and a zinc atom linked by a shared ligand [168,169].

3- Extracellular superoxide dismutase

EC-SOD is a secretory, tetrameric, copper and zinc comprising glycoprotein (with a great attraction for specific glycosaminoglycans like heparin and heparan sulfate that are found in the interstitial areas of tissues and besides in extracellular fluids; lymph, plasma, and synovial fluid contain the majority of the SOD activity. (EC-SOD) is not made by its substrate or further oxidants ("xanthine oxidase plus hypoxanthine, paraquat, pyrogallol, alpha-naphthoflavone, hydroquinone, catechol, (Fe^{2+} ions), (Cu_2^{+} ions), buthionine sulphoximine, diethyl maleate, t-butyl hydroperoxide, cumene hydroperoxide, selenite, citicoline and of high oxygen partial pressure") and Its control in mammalian tissues is largely mediated by cytokines, rather than by individual cells responding to oxidants [170-172].

4- Nickel superoxide dismutase

(Ni-SOD) has been isolated from Streptomyces sp. and Streptomyces coeli color's cytosolic fraction. It is made up of four similar 13.4 kDa subunits that are stable at (pH 4.0-8.0) and up to 70 degrees Celsius. Cyanide and H_2O_2 inhibit it, while azide has just a little effect. The composition of amino acids differs from that of iron, manganese, and zinc-copper SODs. The Apo enzyme deficient in nickel was unable to facilitate the transformation of superoxide anion into hydrogen peroxide, suggesting that (NiIII) is a key component of the activity [173].

1.6.1.2 Catalase

Catalase is a tetrameric haeminenzyme made up of four equivalent subunits arranged of 60 kDa . As a result, each molecule includes four ferriprotoporphyrin groups, and its molecular mass is around 240 kDa. One of the most effective enzymes discovered is catalase. It is so effective that H_2O_2 cannot saturate it at any concentration [174].

$$2H_2O_2 \xrightarrow{\text{CAT}} 2\ H_2O + O_2$$

$$ROOH + AH_2 \xrightarrow{\text{CAT}} H_2O + ROH + A$$

H_2O_2 is enzymically catabolized in aerobic organism by catalase and numerous peroxidases. (H_2O_2) is detoxified by CAT and GPX in animals. Catalase protects the cells against internal hydrogen peroxide production. Even though CAT is not required for such cell types in normal settings, it is critical for the acquisition of oxidative stress tolerance in the adaptive response of cells [155,175].

1.6.1.3 Glutathione peroxidase

The more significant of the selenium-containing peroxidases, glutathione peroxidase, catalyzes the reduction of a range of hydroperoxides (ROOH and H_2O_2) utilizing (GSH), preserving human cells from oxidative stress[176].

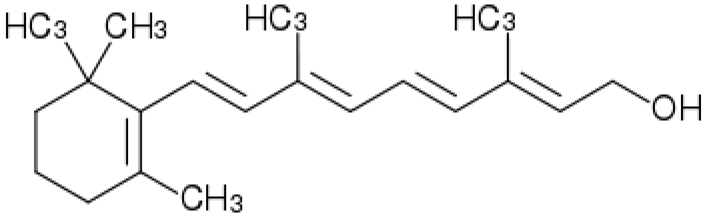
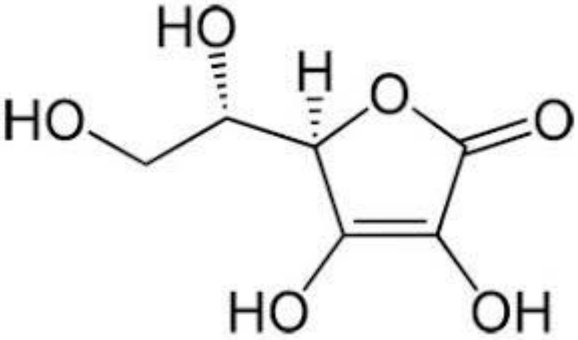
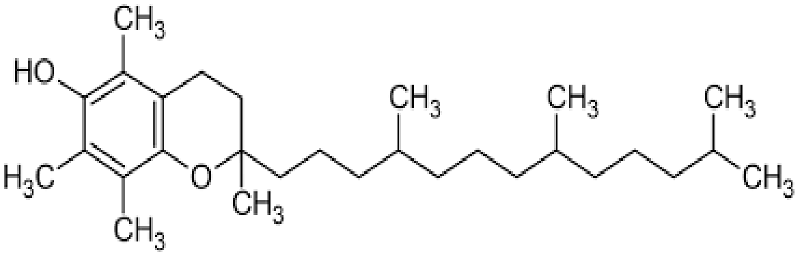
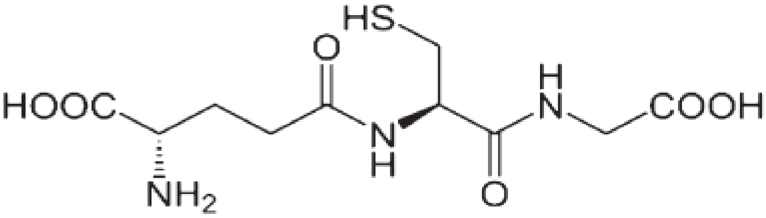
$$ROOH + 2GSH \xrightarrow{GP_X} ROH + GSSG + H_2O$$

In mammals, there are at least five (GP_X) isoenzymes. Despite their widespread expression, the levels of each isoform differ depending on the type of tissue. At the expense of glutathione, cytosolic and mitochondrial glutathione peroxidase (cGP_X or GPx_1) lowers fatty acid hydroperoxides and (H_2O_2). Most tissues contain glutathione peroxidase (GPx_4) and phospholipid hydroperoxide glutathione peroxidase (GPx_4) (or $PHGP_X$). (GPx_4) can be found in the cytosol as well as the membrane fraction. Phospholipid hydroperoxides, fatty acid hydroperoxides, and cholesterol hydroperoxides are all generated in peroxidized membranes and oxidized lipoproteins, and (PHGPx) may directly decrease them [177,178].

1.6.2 non-Enzymatic antioxidants

Non-enzymatic antioxidants are denoted by nutritive antioxidants such as ascorbic-acid (vitamin C), α-tocopherol (vitamin E), polyphenolic, and carotenoids compounds. Antioxidants such as uric acid, bilirubin, glutathione, albumin, and further proteins (e.g. Transferrin, ceruloplasmin, myoglobin and ferritin) are regarded as endogenous antioxidants intended for defending vital biological targets against (ROS/RNS) action [133,179]. as shown in **Table 1.2:**

Table 1.3: Non-enzymatic Scavenger of Antioxidant-Defenses [148].

Chemical Name of Scavenger	Name of Scavenger	Structure
All trans retinol 2	Vitamin A	
Ascorbic acid	Vitamin C	
α-Tocopherol	Vitamin E	
Glutathione		

The aims of study are:

1- Using Artemisia, namely Artemisia Judaica for Cu NPs preparation.

2- Test the activity as antibacterial agent using two different types of bacteria (gram positive and gram negative) in *vitro* study.

3- Estimation the activity of some antioxidants enzymes (SOD, CAT and GPx) before and after using treatment using Cu NPs in compared veterinary drug in *vitro* study.

4- Estimation the effect of synthesized NPs Cu NPs on fish induced with inflammation skin.

5- Estimation the activity of SOD, CAT, and GPx enzymes before and after treatments using fish skin tissue in comparison non-infected once .

CHAPTER
TWO
EXPERIMENTAL
PART

2.Experimental part

2.1 Reagents

The chemicals used in the present study are listed in **Table (2.1)** .

Table (2-1) Chemicals and Reagents.

Chemicals	Symbol	Purity%	Supplied company
5,5-Dithiobis (2-nitrobenzoicacid)	(DTNB)	99	Sigma-Aldrich
Ammonia	NH_3	99.0	Laboratory Chemicals
Cupper Sulphate	$CuSO_4.5H_2O$	99	Sigma-Aldrich
Di sodium hydrogen orthophosphate	(Na_2HPO_4)	98	Fluka
Ethanol	EtOH	99.98	Sigma-Aldrich
Ethylenediaminetetra aceticacid	$(EDTA\text{- }Na_2)$	99	BDH
Hydrochloric acid	(HCl)	37	Laboratory Chemicals
Methanol	(MeOH)	99	Fluka
Potassium dihydrogenortho phosphate	(KH_2PO_4)	99	BDH
Potassiumhydrogenorthophosphate	(K_2HPO_4)	98	Fluka
Pyrogallol	$C_6H_3(OH)_3$	98	Fluka
Reduced glutathione	(GSH)	99	Biochemical
Sodium azide	(NaN_3)	98	Sigma-Aldrich
Sodium hydroxide	NaOH	99	Laboratory Chemicals
Sulphuric acid	H_2SO_4	99	Sigma-Aldrich
Tri chloroaceticacid	(TCA)	98	Fluka
Tris-(hydroxyl methyl) aminomethane	(Tris)	98	BDH

2.2 Instrument

The instruments and equipment used in this study are listed in **Table (2.2).**

Table (2-2) Instruments and Equipments.

Instrument	Supplied company
Balance Sartorlus	**Welghing Technology-Germany**
Centerifuge Laboratory Retirezle	**Cordon-Germany**
Heater/hot plate	**IKA (Germany)**
Incubator	**Memert Edelsta (Germany)**
Magnetic stirrer VF2	**Funkentstort - Germany**
Oven	**Memmert 854 schwabach (Germany)**
pH meter 7110 WTW 82362	**Wellheim - Germany**
Shaker water bath	**BS-11 JEIO TECH -Korea**
UV-visible spectrophotometer 80	**Bioteh engireer ingmanagement CO. LTD, UK.**
Water Bath	**Memmert WNB (Germany)**

2.3 Preparation of Buffers

2.3.1 Phosphate Buffer of potassium pH 7.8.

The K_2HPO_4 3.485g, 0.02M and KH_2PO_4 0.681g, 0.005M solution were produced and autoclaved in double distilled water 500 ml prior to mixing.

$K_2HPO_4$449.8 ml and KH_2PO_4 50.2 ml solutions were added to double distilled water 500 ml for preparing 0.05 M buffer solution pH 7.8 [180].

2.3.2 Buffer of potassium phosphate 10 mM, pH 7.4.

This buffer is ready to dissolve 2.307 g of K_2HPO_4 in 151.78 ml distilled water and 0.102 g of KH_2PO_4 in 98.11 ml distilled water containing [181].

2.3.3 Buffer of phosphate 0.4 M, pH 7.

This buffer is dissolved by 10.755 g of K_2HPO_4 in (154.5 ml) distilled water and 12.96 g of KH_2PO_4 in 95.5 ml distilled water containing [180].

2.3.4 Tris Buffer

1. Tris - HCI dissolved in double distilling water 250 ml and Tris-base 0.33 g, 0.11 M buffer solution 3.467 g, 0.08 M. Dissolved in 250 ml of twin distilled water, solutions were put into an autoclave and a volume of 27.9ml was added. The base of Tris was obtained and combined with the volume of 2222,1ml Tris-HCI [182].

2. Tris-EDTA buffer 50mM 0.258 gm of Tris base were dissolved in 90 ml of D.W. Then 0.111 gm of EDTA solution 1mM was added and completed to the final volume of 100 ml with D.W. By adding 1N of HCl the pH was adjusted to 8.2 [183,184].

2.4 Preparation of aqueous extracts of Artemisia

Fresh Artemisia leaves were collected from local markets in Babil Governorate, Iraq. The leaves were washed several times using firstly tap water and then distilled water in order to remove scums. The leaves dried after cleaning under a sunscreen to remove moisture completely, then ground with a mechanical grinder and stored. 5 gm of crashed plant leaf was taken with 100 ml distilled water and left to boil at 80 C for 30 min.then cooled at room temperature. The solution was initially filtered through normal filter paper and stored at room temperature and dark palace for future work [185].

2.5 Green Synthesis of Copper Nanoparticles

Nano copper was synthesised flowing the published procedure[11], with some modification briefly, A 60 ml solution of plant extract was introduced into 200 ml of 0.005 M copper sulphate solution with stirring at room temperature until the colour changing to plane yellow. Then, the mixture was kept for 24 h in dark place, after that the solution was centrifuged for 15 min. at 11180Xg. The supernatant purified using Millipore Filter 0.22 µM. Finally, nanoparticles pallets collected and dried at 80 °C to get brown precipitate and stored at 4 ° C for the next day work [186,187].

In this study**,** copper nanoparticles have been prepared as shown in **Figure 2.1** and were mainly known by the color change from light brown to green in the plant extract. The progress in the reduction of copper ions to copper nanoparticles using a plant extract was indicating an increase in the intensity of the surface adsorption peak observed within 400 nm to 500 nm [188].

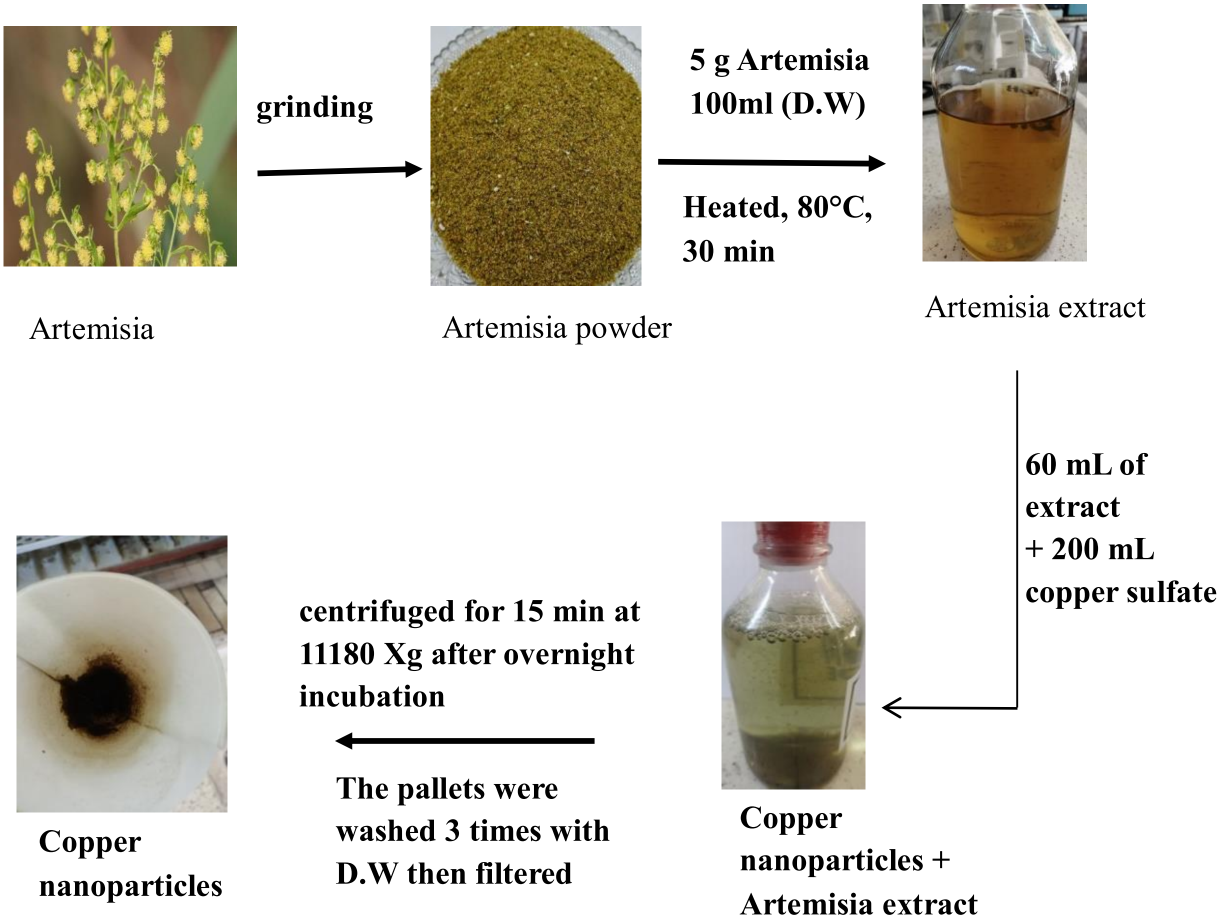


Figure 2.1: Copper nanoparticles preparation using Artemisia plant.

2.6 Healthy and Infected Tissue

40 fish have been collected to this study 10 of these have been chosen as a control. 10 fish were used to estimate the effectiveness while they were infected. Then 20 fish were punched with syringe and left to get infection for two weeks. After infection 10 fish were treated with Cu NPs and the rest were treated with commercial drug ((Fungus Guard).

2.7 Samples Collection and preparation

Bunni (Barbus sharpeyi) fish were collected from fish lakes located in Babil province in February, with a weight of 500 g and a length of 15 cm, they were placed in fish tanks with dimensions of (70 x 60 x 50) cm specialized for fish farming in the laboratories of the Department of Chemistry. Then a piece of tissue was removed at the laboratory site. All samples were homogenized by means of the glass homogenizer submerged in an icy water bath in-Ice cold buffer 100 mM Tris-HCl, pH 7.1. At 905.58Xg for 15min at 4°C, homogenized samples were centrifuged. The supernatant was collected and maintained at -20° C until completion of the study [189].

2.8 Characterizations of Copper Nanoparticles

UV-Visible Spectrophotometer, FTIR (Fourier Transform Infrared spectroscopy analysis, X- Ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and EDX have been used for Nano copper characterization.

2.8.1 UV-Visible Analysis.

The UV-visible absorbance spectrum recorded for Cu NPs exhibited λ max of 410 nm

2.8.2 X- Ray Diffraction

X-ray diffractometer were employed to determine the particles size of the prepared nano materials and the parameters of measurement are: an Anode Material Cu, k-alpha K=1.54 A° source. Step Size [°2Th.] 0.0500, Measurement temperature [°C] 25.00 and scan step time [s] 1.0000. The diffract grams of nanomaterials provide information about crystallite size phase composition, crystallographic orientation and lattice strain, The lattice planes in a crystal are separated by a distance) d. Braggs' law) relates the wave length (λ) of the reflected X-ray, the spacing between the atomic planes (d) and the angle of diffraction (θ) as follows:

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

To determine the crystal structure and crystallite size of the synthesized materials at room temperature by the Debye - Scherer's formula in the following equation:

$$\mathbf{D = \frac{k\lambda}{\beta\cos\theta}}$$

Where, The average size of crystal is the unifying value of the form factor for heterogeneous shape and 0.89 for homogeneous shape,

λ = 0.154 nms the X is the total width at the maximum diffraction peaks (FWHM), β is the full width and the Bragg peak angle is the wavelength of ($Cu_{k\alpha,}$ β) [190,191].

2.8.3 Scanning Electron Microscopy (SEM)

Scanning electron microscopy technique is used revealed the surface morphology properties of synthesized particle the specimens were analyzed by a Scanning Electron Microscope (FE-SEM, Zeiss, Germany) at electron high tension EHT10 Kv and 60 kx magnification [192].

2.8.4 Fourier Transform Infrared Spectroscopy(FTIR)

For characterization of the functional groups on the surface of the prepared materials under study Fourier transform infrared spectroscopy spectra were recorded on a SpactraIR-2, Perkin Elmer Instrument and spectrometer by using the potassium bromide KBr pellets method at room temperature. The FTIR spectrum was collected between the waves number of (400 - 4000 cm^{-1})[193].

2.9 Anti- bacterial Study

Agar disc-diffusion method has been used to identify the activity of Cu NPs as anti- bacterial agent against two different types of bacteria grams positive (*Bacillus - subtilis*) and gram negative bacteria *E. coli*). The bacterial strains were cultured in nutrient broth for 24 hours to get a good growth phase of the chosen bacteria flowing published producer [194,195]. During the preparation of samples Cu NPs,0.01mg.ml^{-1} and 0.02mg.ml^{-1} were taken 0.2 % Dimethyl Sulfoxide as a solvent. The agar disk was stuck at a diameter of 6 mm and the fluid was saturated with Cu NPs. Ampicillin was employed as a positive control, whereas negative 0.2% DMSO was chosen. Disks for 24h at 37°C have been incubated. Finally, antibacterial activity was measured in millimetres using a ruler in inhibitory zone [196,197].

2.10 Determination of Superoxide dismutase activity

2.10.1 Principle

Superoxide dismutase (SOD) activity was determined according to the method Markland [198].

2.10.2 Reagents

The using reagents are:

1-Tris-EDTA buffer 50mM. (Section 2.4.1)

2- 0.2 mM pyrogallol in 50 mM Tris- buffer, pH 8.2.

2.10.3 procedure

Reagent	Sample (µl)	STD (µl)	Blank (µl)
Tris-EDTA buffer	1000	1000	1000
Tissue	50	--------	---------
Pyragallol	1000	1000	
DW	--------	50	50

2.10.4 Calculation

% inhibition = $(A_{test}/A_{control})$ *100%

SOD activity (U/mg) = % inhibition / 50%

A_{test}: Absorbance of test solution.

$A_{control}$: Absorbance of standard solution.

2.11 Determination of Catalase activity

The activity of tissue catalase was based on the approach of Beer and Sizer (1952) [199].

2.11.1 Principle

The degradation of H_2O_2 is catalyzed by catalases. Absorption lowering wavelength was steadily enhanced in the UV H_2O_2 region. The deterioration of H_2O_2 can be followed immediately by a 240 nm decrease in extinction [175].

$$2H_2O_2 \longrightarrow 2\,H_2O + O_2$$

2.11.2 Reagents

The using reagents are:

1- buffer of potassium 10 mM, pH 7.4. (section 2.3.2).

2- Dichromate/acetic acid: Mix 50ml aqueous potassium dichromate solution with 20ml of glacial acetic acid, mixing 50 ml.

3- In a potassium phosphate buffer 50 mmol/l Freshly H_2O_2 200 mM.

2.11.3 Procedure

<table>
<tr><th>Reagent</th><th>Sample µL</th><th>Standard µL</th><th>Blank µL</th></tr>
<tr><td>phosphate buffer</td><td>1000</td><td>1000</td><td>1000</td></tr>
<tr><td>tissue</td><td>50</td><td>-------</td><td>-------</td></tr>
<tr><td>D.W</td><td>------</td><td>50</td><td>50</td></tr>
<tr><td colspan="4">Mix with vortex and incubate at 37 °C for 3 min, after that, add:</td></tr>
<tr><td>Hydrogen peroxide</td><td>1000</td><td>1000</td><td>-------</td></tr>
<tr><td colspan="4">The reaction was then halted in the water bath by the addition of the suspension solution (0.5ml). After refroiding, the precipitated protein (69.875Xg /5 min) has been removed by centrifugating the absorbent at (570 nm) against empty detector. Changes have been recorded.</td></tr>
<tr><td>Dichromate</td><td>500</td><td>500</td><td>500</td></tr>
</table>

2.11.4 Calculation

Activity Enzyme (U/mg) = $1/ t \text{ Log } S^o / S$

2.12 Determination of glutathione peroxidase activity (GPx)

2.12.1 Principle

The activity of GPx was measured in line with the published Hafemann et al (1974) [200].

The activity of GPx was assessed following incubation of the sample, with H_2O_2 and NaN3 being measured by a reduction in GSH level.

$$H_2O_2 + 2\ GSH \longrightarrow 2H_2O + 2\ GSSG$$

2.12.2 Reagents

The using reagents are:

1- buffer of phosphate 0.4 M, pH 7: (section 2.3.3)
2-NaN_3 1 mM, prepared by dissolving 0.0065 gm of NaN_3 in 10 ml of D.W.

3- GSH 2mM 0,015gm. of GSH in 25ml of D.W. was produced by dissolving.

4- 1.65 % HPO_3^{2-}

5-0.4 M Na_2HPO_4.

6- DTNB was produced for the DTNB in100ml of $NaNO_3$ by dissolving 0.0198g 0.1 %.

2.12.3 Procedure

Reagent	Sample µL	Standard µL	Blank µL
Tissue	100	-------	------
GsH	-------	100	------
Working reagent	2500	2500	2500
Mixed and vortex mixture, then incubate in water bath at $37C^0$ for 3 minutes.			
Supernatant	2000	2000	2000
$m\text{-}HPO_3^{2-}$	2000	2000	2000
Mixed in vortex mixture, then centrifuged at 25.16Xg for 10 minutes			
Na_2HPO_4	2000	2000	2000
DTNB	1000	1000	1000
D.W	--------	100	100

The samples were let to stand for 10 minutes; the absorbance of each reaction tube was measured at 412 nm.

2.12.4 Calculation:

$$C_T = \frac{A_T}{A_S} \times C_S$$

GRx (U/mg) = $(C_S - C_T) \times D.F / Time$

D.F = dilution factor =1

CHAPTER THREE

RESULTS AND DISCUSSION

Results and Discussion

3.1 Green synthesis of Cu NPs.

Stomach aches, heart difficulties, sexual weakness, diabetes, gastro-intestinal problems, and external wounds have all been treated with Artemisia as a medicinal plant. Inflammatory problems such as fungal infections, diabetes, atherosclerosis, cancer, and arthritis are all treated with this aromatic herb in numerous Arabic traditional treatments [201]. For all of these reasons, it is being used in this study to figure out how to solve livestock related difficulties. We believe we are the first to employ it as a copper ion reduction agent to produce Cu NPs.

In order to synthesize nanoparticles using a biological system, three critical conditions must be achieved: solvent medium, environmentally friendly reducing agent, and non-hazardous substrates as a capping agent or stabilizing agent [202].

3.2 UV-Vis's spectra of Cu NPs .

The interaction between conduction electrons of metal NPs and incident photons was responsible for colour change. Further, Cu NPs synthesis confirmed by a characteristic peak obtained at 410 nm. **Figure 3.2**.

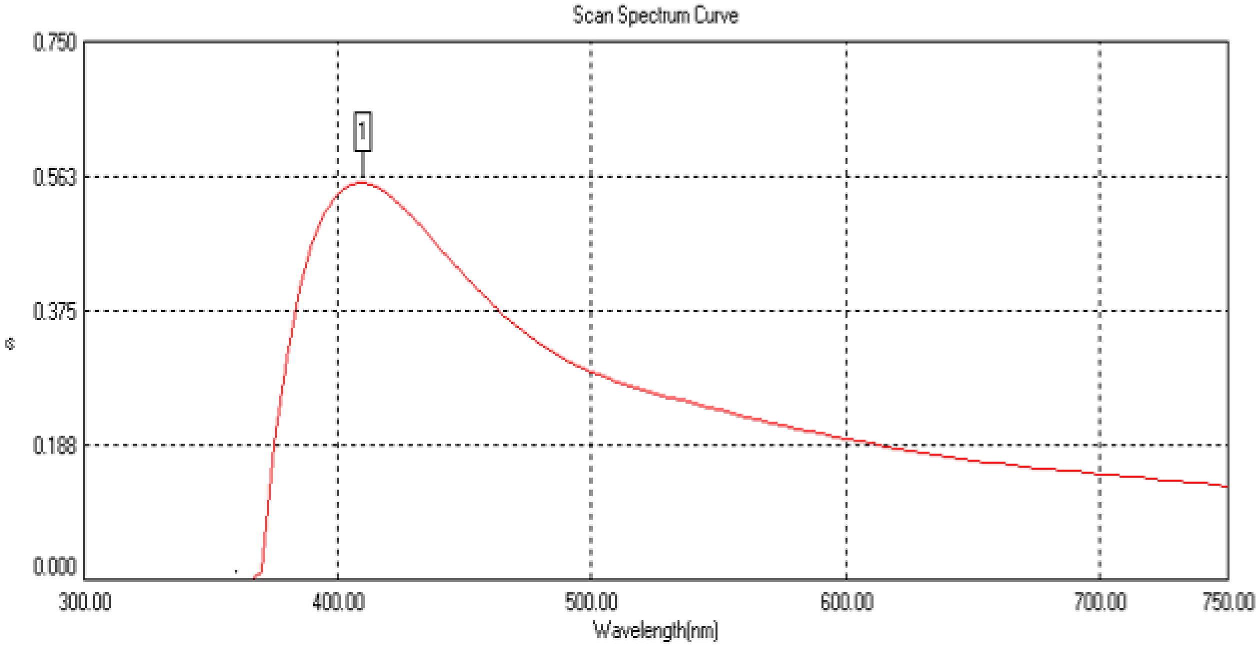


Figure 3.2 UV-visible spectrum of Cu NPs at room temperature after 22 h.

3.3 FTIR spectra analysis

In order to investigate the surface adsorption of functional groups on nanoparticles, FTIR analysis has been used.

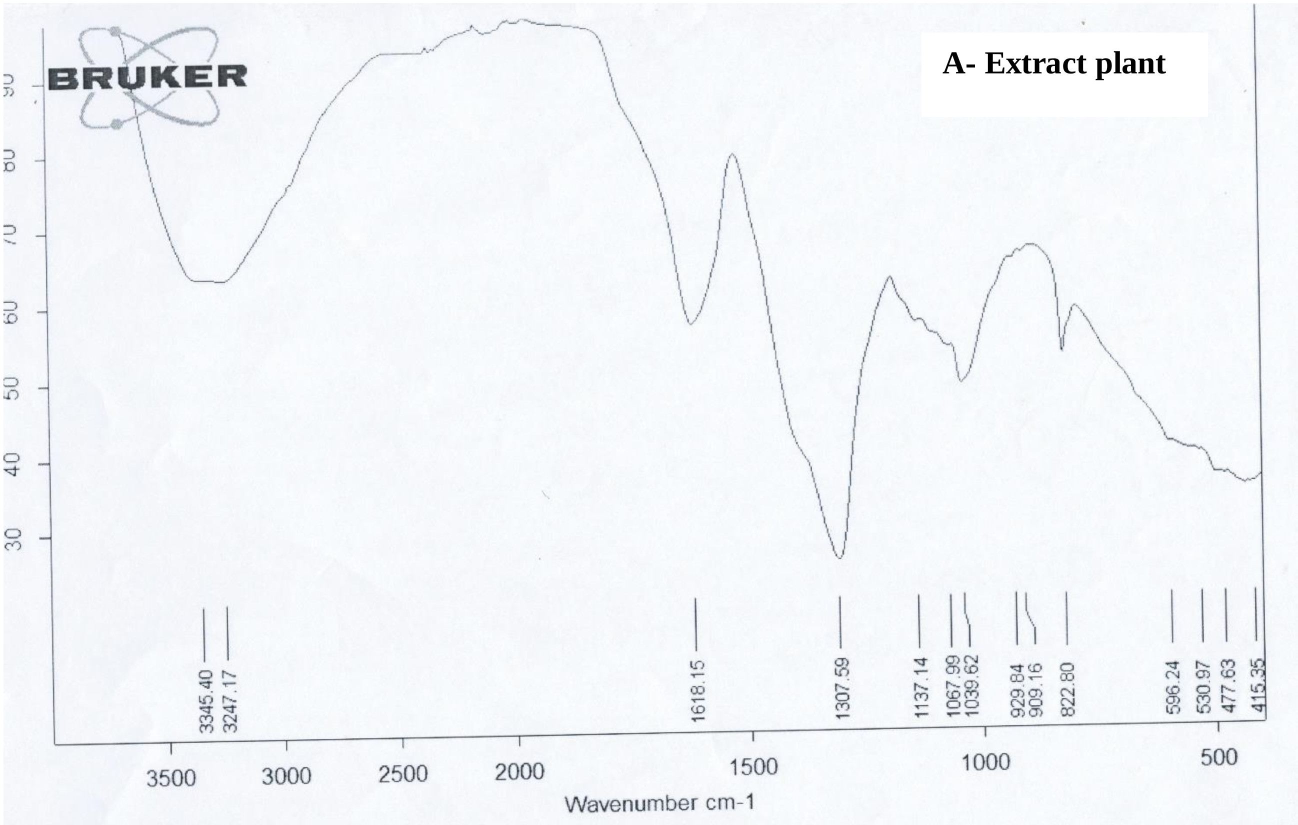
Samples were crashed with KBr and studied by Shimazu Fourier transform infrared 8400S spectrophotometer at a range of 4000–400 cm^{-1}.

Amine and carboxylate observation peaks at 3345.4 and 3247.17cm^{-1} found in Artemisia leaf extract and is responsible for the binding of phenols to the copper surface, resulting in the stability of biosynthesized nanoparticles.

The medium peak at 1067.99 cm^{-1} corresponds to C-O stretching of phenolic compounds. Most of the absorption peaks of plant extract also exist in the FTIR spectrum of Cu NPs [203].

Cu NPs spectra displays two clearly shafted peaks at 3367.82 and 3290.67 cm $^{-1}$ which indicated clearly O-H stretches of phenols and N-H amines respectively, while maintaining the peak of C=O was indicated at 1730.21 cm $^{-1}$. **Figure 3.3.**

The major absorption bands of carboxylate coordination and metals placed at 1623 cm^{-1}, which is confirmed a typical peak responsible for the interaction between copper and capping agent of extract **Figure 3.3B**.



A- Extract plant

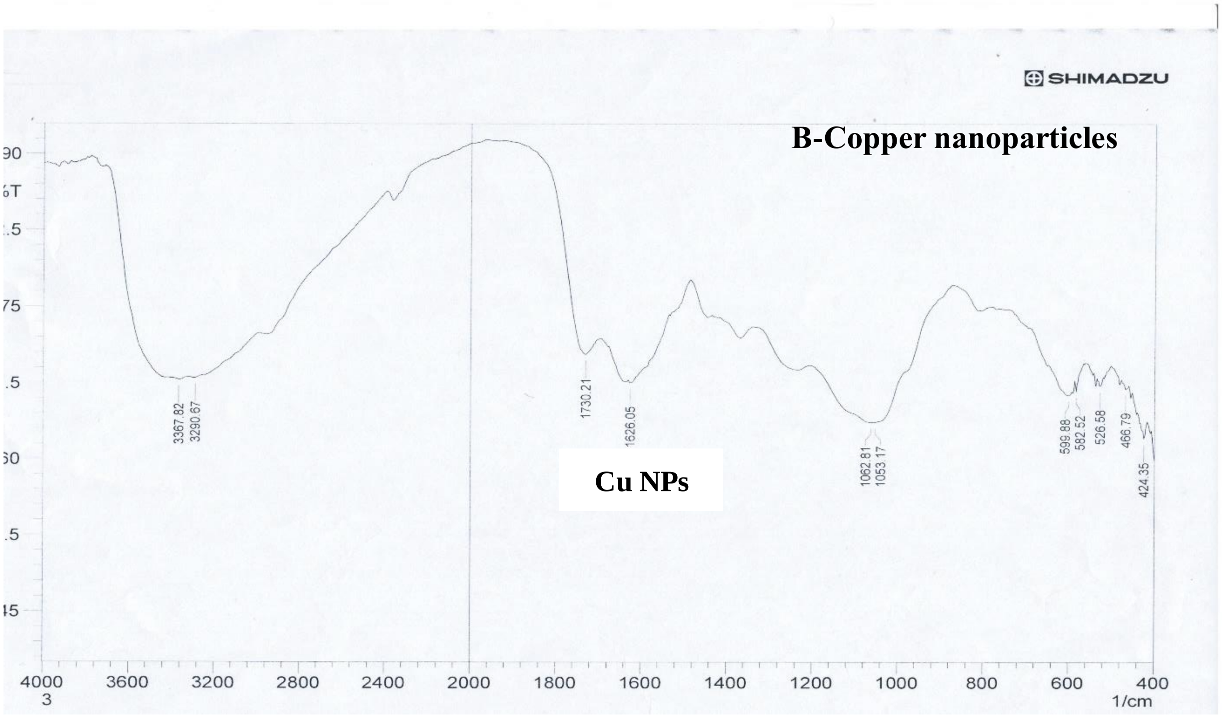


Figure 3.3 FTIR spectra of, A. plant extract and B. Cu NPs.

3.4. SEM analysis

The formation of Cu NPs was confirmed using SEM analysis within average size of 54.57 nm. All the possible spherical and irregular shapes of Cu NPs were found in the micrograph with no agglomeration as presented in **Figure 3.4**.

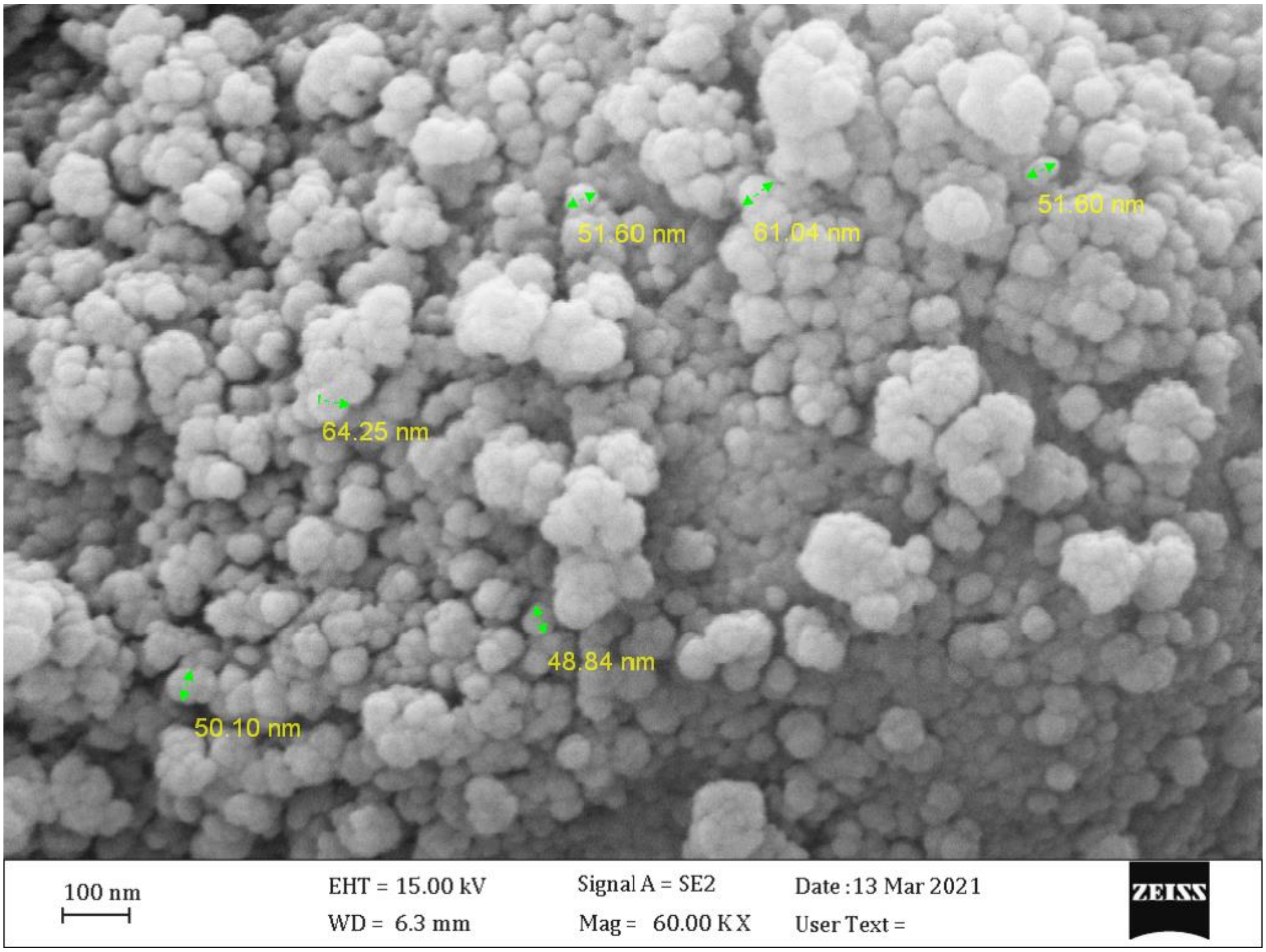


Figure 3.4: **The characterisation of Cu NPs using scanning electron microscopy.**

3.5. XRD analysis

X- Ray diffraction (XRD) pattern, of Cu NPs which is obtainable in **Figure 3.5** shows observing the pattern at 2 θ includes three main peaks at 35.86°, 44.58°, and 64.57°, which correspond to (111), (200), and (220), respectively, confirming the structure of Cu NPs, and the diffraction measurements were in expected agreement with recent published work [204,205].

While, it can be concluded from other revealed patterns that a few copper atoms on the surface of NPs may have oxidized to generate copper oxides when exposed to air.

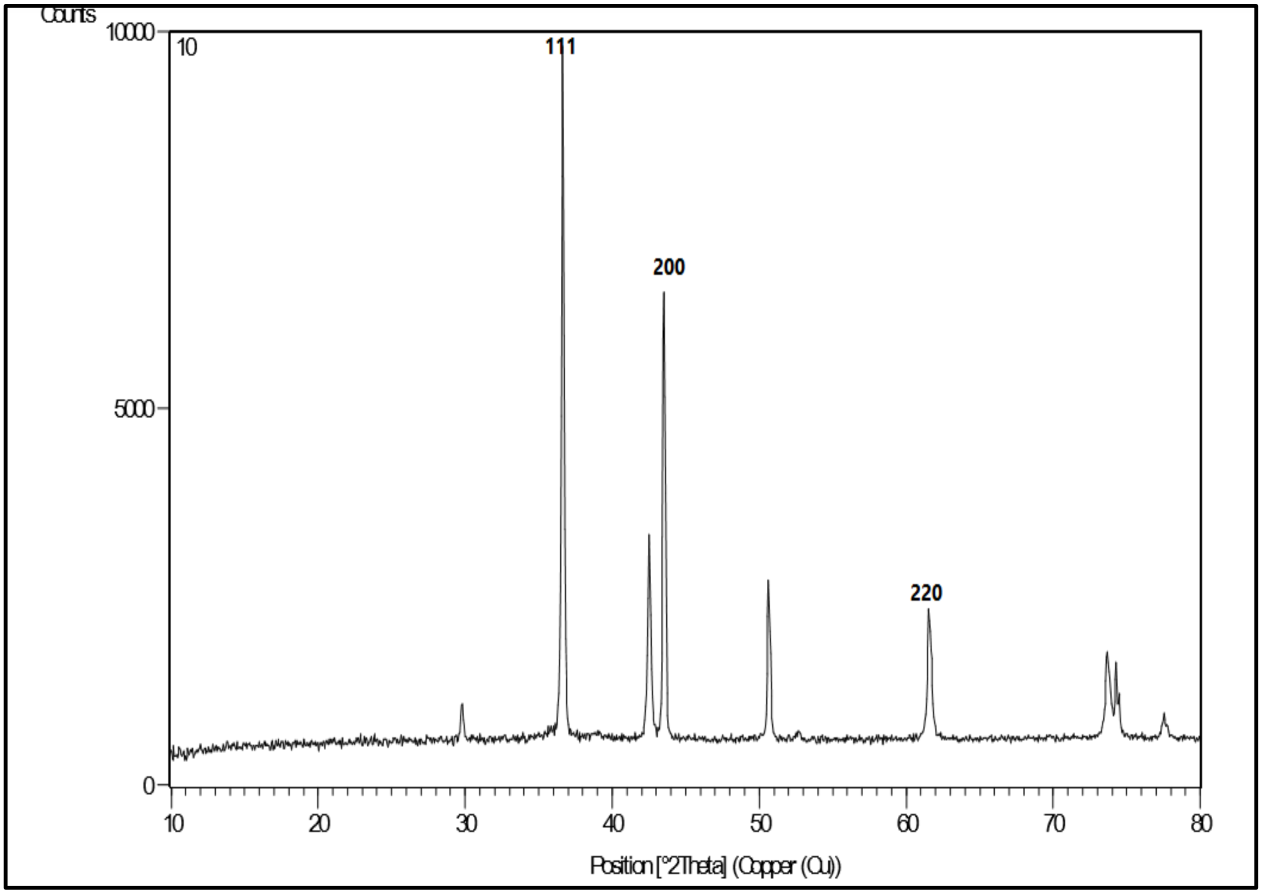


Figure 3.5: XRD analysis of Cu NPs.

3.6. EDX analysis

In order to confirm the composition and distribution of NPs, EDX analysis can be applied through spectrum and elemental mapping . The spectrum of EDX Cu NPs was shown in **Figure 3.6**. The peak of Cu , C , and O were readily identified, and no extra peaks were found, indicating that the synthesised material Cu NPs was more pure. The abundance of C and O could be due to the presence of capped bioactive compounds. It's also possible that a few copper atoms on the NPs' surface will be oxidized to minor amounts of CuO and Cu2O.[206]

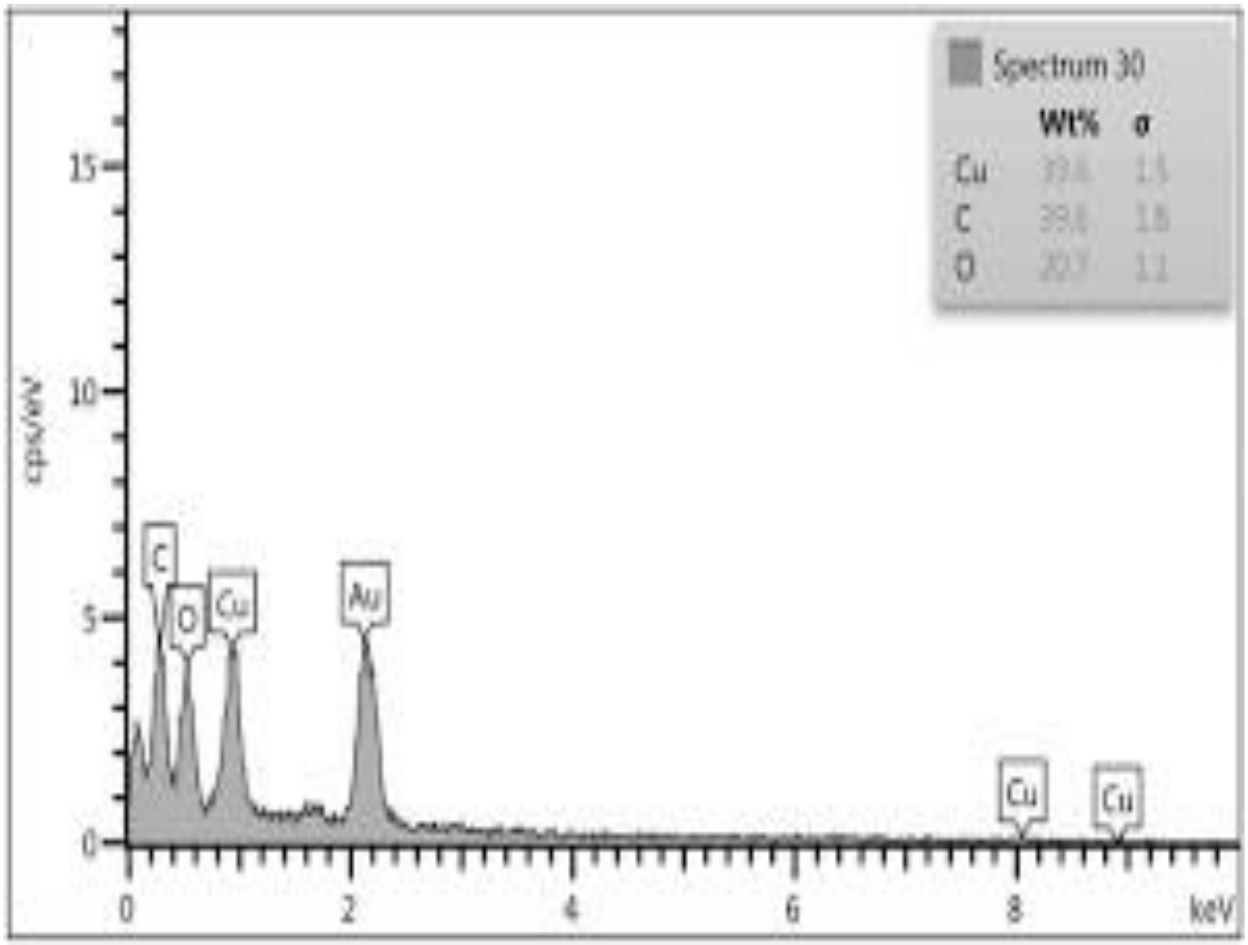


Figure 3.6: The characterisation of Cu NPs using EDX analysis.

3.7 The stability of copper nanoparticles

Many factors can be affected stability of Cu NPs such as pH, solute concentration, and the purification steps**,** all these are responsible for the physical characteristics and the stability of NPs formation [207].

In this study, the stability of copper nanoparticles has been studied according to the time from immediate preparation time to six weeks. The results were proven that the identification peak at 410 nm remains unaffected even after 6 weeks, indicating that Cu NPs are stable throughout this time. **Figure 3.7**

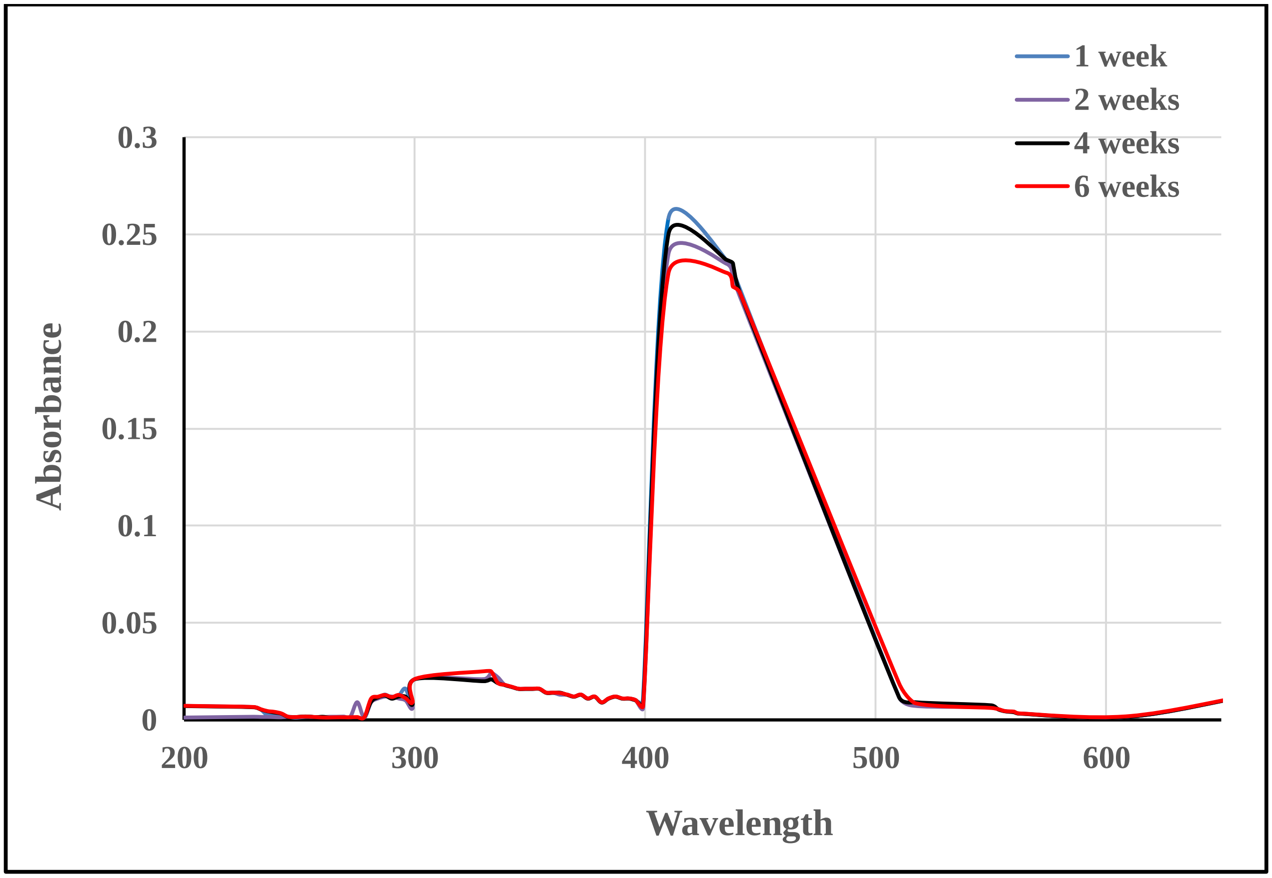


Figure 3.7: The effect of time on the stability of copper nanoparticles.

The effect of pH has been studied as well, the Nano copper was shown the highest absorption in a pH medium between 7-9. While, absorption peak at 410 nm remain unaffected **Figure 3.8**, which means pH has unaffected the stability of Cu NPs.

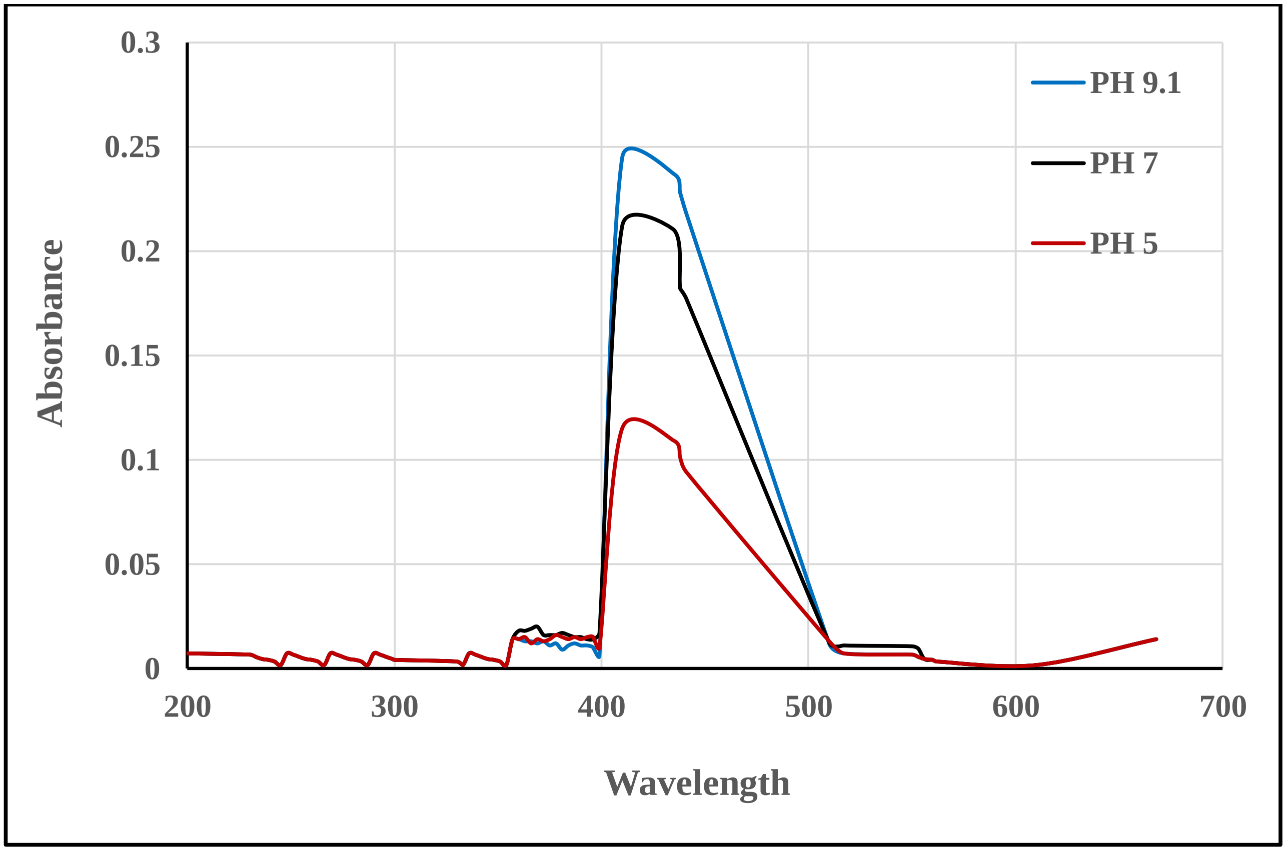


Figure 3.8: The effect of pH on the stability of copper nanoparticles.

3.8 Antibacterial activity

This study suggested that skin infection causes antioxidant deficiency. Therefore, tested whether copper nanoparticles treatment might be a mole of defense against oxidative stress have been investigated. The study was included two parts: in *vitro* and in *vivo* study.

3.8.1 In *Vitro* applications of Nano copper as antibacterial agent

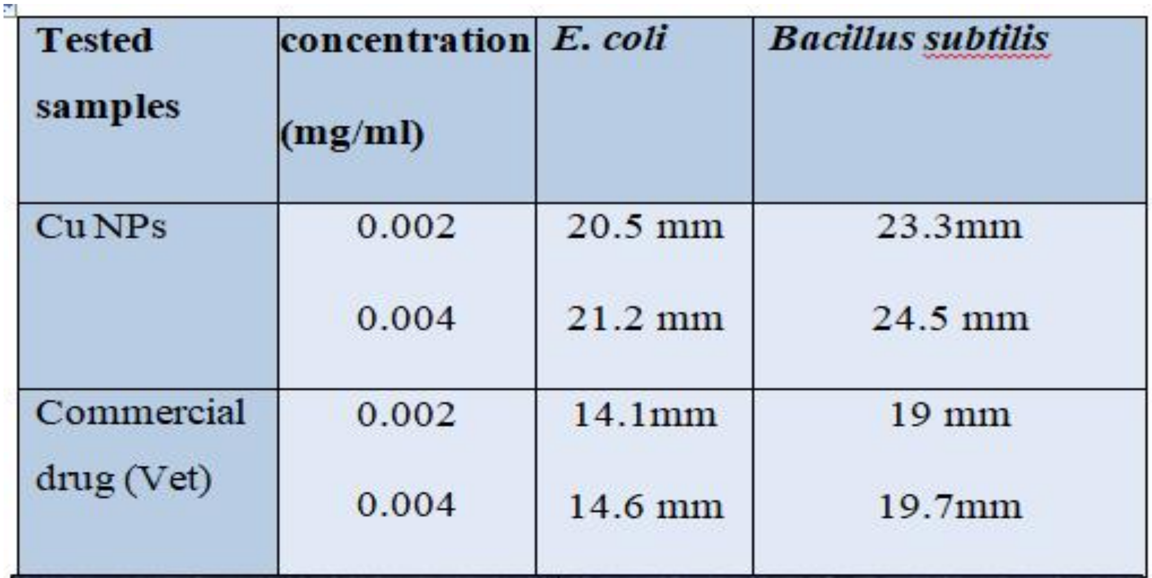
According to previous studies copper nanoparticles have been synthesized in order to investigate its ability as antibacterial agent using a lot of plants some describe in **Table 3.2** but Artemisia extract our results are good because of the size, shape, activity and treatment dosage of NPs.

Table 3.2: Antibacterial activity of copper nanoparticles using different sources of plants extract.

The name of plant	Type of Medication	NPs size	Zone inhibition	Tested concentration of Cu NPs	Aggregation	Ref.
Artemisia haussknechtii	Antibacterial E.coli	35.36 ± 44.4 nm	10 ± 1.73mm	10 mg/ml	+	[208]
Cissus arnotiana plant	Antibacterial *E. coli*	60–90 nm	22.20 ± 0.16 mm	0.075 mg/ml	+	[209]
Persea americana seeds	antifungal and antibacterial	42–90 nm	22.23 ± 0.15 mm	0.3 mg/ml	+	[210]
Allium saralicum leaves	antibacterial	65-100nm	45-90 mm	1–8 mg/ml	+	[211]
Present study Artemisia (Judaica)	Antibacterial *Bacillus subtilis*	57.53nm	23.3,24.5 mm	0.002,0.004 mg/ml	-	
	E. coli		20.5,21.2 mm			

There is no evidence of using Cu NPs which have synthesis by Artemisia Judaica extract as antibacterial agents therefore, it has been chosen to identify whether its efficient or not.

Two different concentrations of Cu NPs and (commercial drug) were put on the infected plates' surface. Discs were incubated for 24 hours at 37° C. Finally, the antibacterial activity was calculated as using the ruler measurements. **Figure 3.9.**



Tested samples	concentration (mg/ml)	*E. coli*	*Bacillus subtilis*
Cu NPs	0.002 0.004	20.5 mm 21.2 mm	23.3mm 24.5 mm
Commercial drug (Vet)	0.002 0.004	14.1mm 14.6 mm	19 mm 19.7mm

Figure 3.9: In *vitro* , antibacterial activity of Cu NPs on (*Bacillus subtilis* and *E. coli*) bacteria in comparison with commercial drug.

***1. commercial drug 2. Cu NPs 3. Solvent .**

The results were observed that Cu NPs have an excellent antimicrobial activity, due to their interaction with bacteria's cell membrane, the trans membrane electrochemical potential is reduced, affecting membrane integrity. Produced Cu NPs had a stronger antibacterial action against gram positive bacteria than gram negative bacteria [212].

Copper nanoparticles and copper ions have the ability to accumulate on the bacteria's cell surface and develop mines in the membrane, causing cellular component leakage both outside and inside the cell, resulting in oxidative stress and cell death [213].

3.8.2 In *Vivo* Applications

Some of antioxidant enzymes have been evaluated before and after treatment for: first for healthy and infected fish tissue, second treated and untreated tissue. Fish have been infected and then treated with two different types of treatment including (Vet) as commercial drug and Cu NPs. The results were shown excellent healing after treatment with nan- copper compare with commercial drug, **Figure 3.10**.

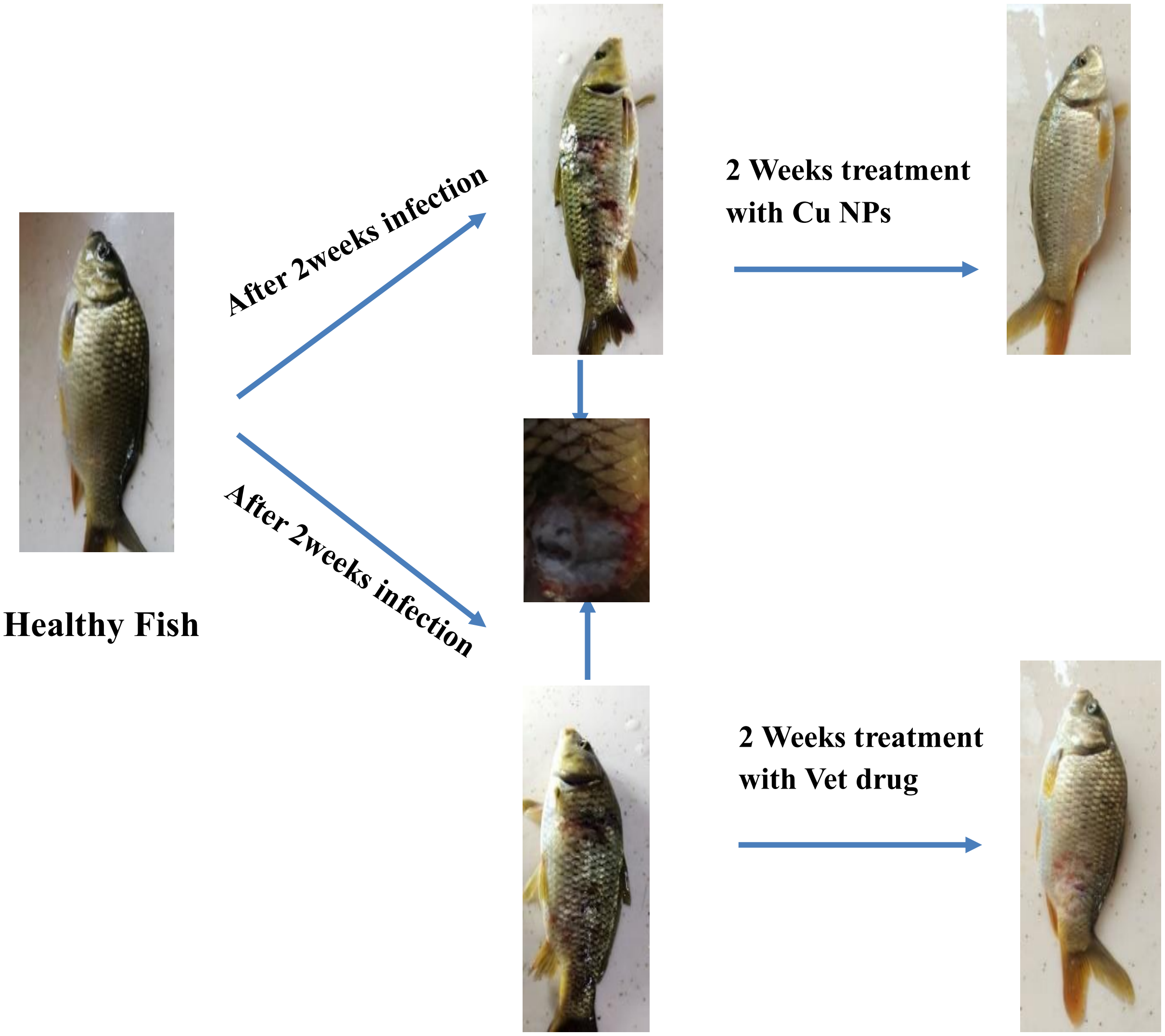


Figure 3.10: The comparing between fish treatment with (Vet) drug and Cu NPs.

Infection disease is one of the factors that may be affecting the balance between reactive oxygen species (ROS) and the antioxidant defense system. ROS and other oxidants can induce lipid, protein, and DNA oxidation, as well as tissue damage, resulting in membrane damage and lead into cell death [214]. Therefore, the levels of antioxidant enzymes such as SOD, CAT and GPx have been significantly decreased in infected fish tissue compared to healthy once **Table 3.3.**

Table 3. 3: The level of antioxidant enzymes (catalase, superoxide dismutase and glutathione peroxidase) in healthy and Infected fish.

Enzyme	Group	N.	Mean ± SD	95% Confidence Interval for Mean		Compared groups		P value
				Lower Bound	Upper Bound			
CAT (U/mg)	**Healthy Fish Tissue**	**10**	**0.0972±0.0022**	**0.0957**	**0.0988**	**1**	**2**	**0.000**
	Infected Fish Tissue	**10**	**0.0237±0.0049**	**0.0201**	**0.0272**			
SOD (U/mg)	**Healthy Fish Tissue**	**10**	**0.1473 ±0.0023**	**0.1456**	**0.149**	**1**	**2**	**0.000**
	Infected Fish Tissue	**10**	**0.0241± 0.0025**	**0.0223**	**0.025**			
GPx (U/mg)	**Healthy Fish Tissue**	**10**	**0.1384±0.0044**	**0.1352**	**0.1415**	**1**	**2**	**0.000**
	Infected Fish Tissue	**10**	**0.0550±0.0104**	**0.0475**	**0.0625**			

The results indicated a significant reduction in the level of antioxidant enzymes CAT, SOD and GPx in infected fish tissue compared to healthy tissue ($P < 0.05$).

Due to an important role of oxidative stress in the pathophysiology of a wide-ranging of diseases including inflammatory disease, however, looking for the developing of traditional treatment to avoid the oxidation damage becomes necessary. In this study, copper nanoparticles have been used to remove skin inflammatory damage from fish skin tissue in comparison with commercial drug.

With using a (0.05 mg/L) of Cu NPs, the activities of total superoxide dismutase and catalase as well as glutathione peroxidase increased remarkably compared to veterinary treatment using the same dosage. **Table 3.4, 3.5, 3.6 .**

Table 3.4: The level of CAT enzyme in treated fish tissue using Cu NPs and Veterinary treatment in comparison with healthy fish tissue

Group	N.	Mean ± SD	95% Confidence Interval for Mean		Compared groups		P value
			Lower	Upper			
Healthy Fish Tissue	10	0.0972±0.0022	0.0957	0.0988	1	2	0.000*
Cu NPs Treatment	10	0.0638±0.0033	0.0614	0.0663		3	0.000
Veterinary Treatment	10	0.0340±0.0073	0.0287	0.0393			

* The most significant change at the P = 3.79E-14 level.

Table 3.5: The level of SOD enzyme in treated fish tissue using Cu NPs and Veterinary treatment in comparison with Healthy fish tissue.

Group	N.	Mean ± SD	95% Confidence Interval for Mean		Compared groups		P value
			Lower	Upper			
Healthy Fish Tissue	10	0.1473 ±0.0023	0.1456	0.149	1	2	0.000*
Cu NPs Treatment	10	0.1136±0.0049	0.110	0.117			
Veterinary Treatment	10	0.0810 ±0.0051	0.773	0.084		3	0.000

* The most is significant change at the P = 1.69E-13 level.

Table 3.6: The level of GPx enzyme in treated fish tissue using Cu NPs and Veterinary treatment in comparison with healthy fish tissue.

Group	N.	Mean ± SD	95% Confidence Interval for Mean		Compared groups		P value
			Lower	Upper			
Healthy Fish Tissue	10	0.1384±0.0044	0.1352	0.1415	1	2	0.000*
Cu NPs Treatment	10	0.1067±0.0334	0.0828	0.1306		3	0.000
Veterinary Treatment	10	0.0807±0.0034	0.0782	0.0832			

* The most is significant change at the P = 1.28E-13 level.

This could be due to its capacity to make touch with the skin in order to promote the health of the skin. It's also important for the synthesis and stability of skin proteins [215].

3.8.2.1 Catalase Activity in both healthy and infected fish tissue.

Reducing catalase activity may be prevented the converting of hydrogen peroxide to water and molecular oxygen, thus, causing inflammatory damage [216]. The levels of CAT activity in infected skin tissue compared to healthy fish skin significantly changed (P ≤ 0.05). **Figure 3.11.**

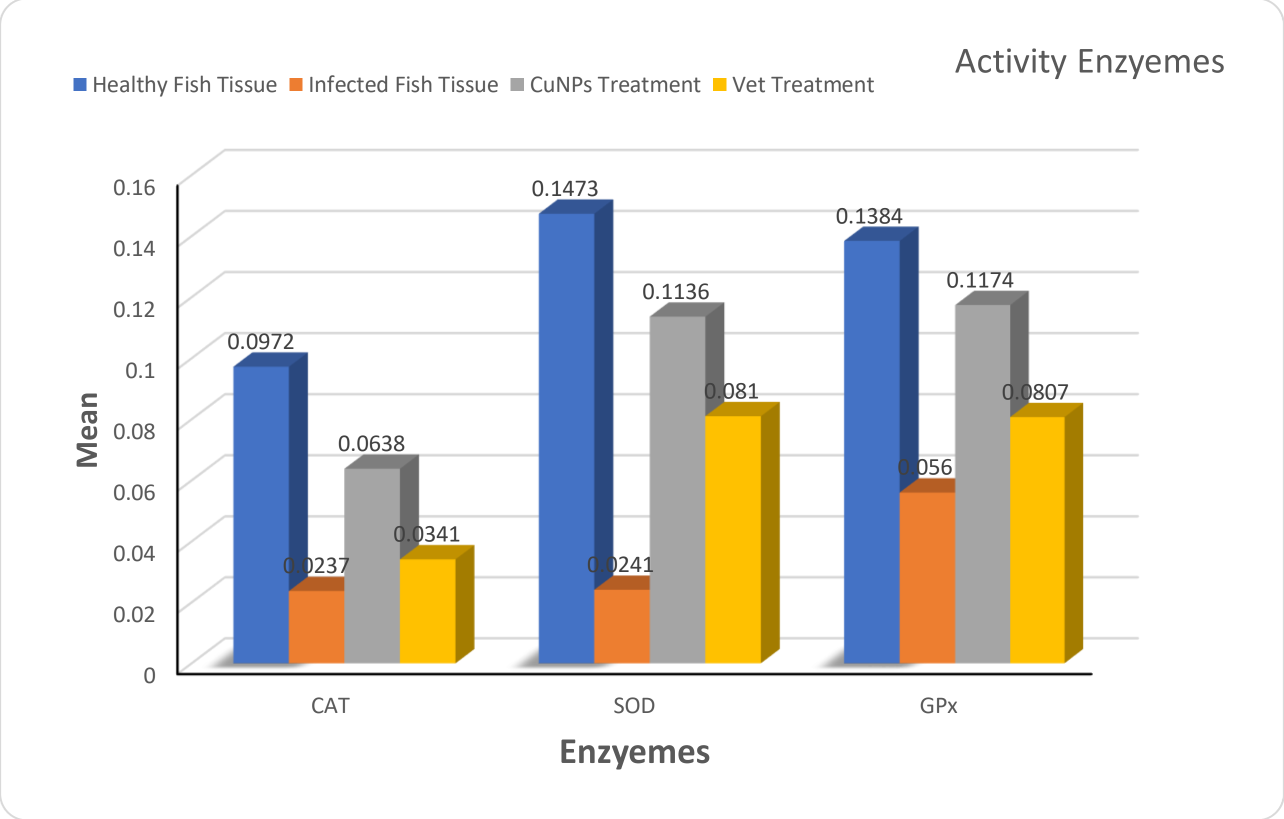


Figure 3.11: The effect of treatment on the activity of CAT, SOD and GPx Enzymes Activity in infected skin fish tissue in compressing with healthy once.

There was about 75-80 % inhibition in the activity of enzyme in infected samples compared to healthy ones.

On the other hand, CAT activity increases after Cu NPs treatment in infected samples compared to the healthy skin tissue because the ability of Cu NPs to reduce the level of oxidative stress resulting in, re- balance between oxidant and antioxidant system [217].

Oxidative stress caused by too much production of super oxidants and imbalances in antioxidant enzymes have been related to the development of inflammatory complications [218].

The results suggested that the accumulation of ROS can be avoided by using Cu NPs as a treated agent for maintaining the level of intracellular enzymatic antioxidant activities.

3.8.2.2 Superoxide Dismutase (SOD)

Superoxide dismutase is the initial line of defense against ROS-mediated cell damage by, stimulating the ratio of superoxide, and controlling oxygen metabolism. It has also important role to break down the molecular of oxygen and peroxide into less toxic compounds [219].

The results were presented a significant change ($P \leq 0.05$) in the activity of SOD in infected skin tissue in compare to the healthy ones. **Figure 3.11.**

The activity has been dropped more than 80% in infected group in comparison with healthy ones. This could be due to the enzyme's inefficiency or the antioxidant system's failure to overcome the influx of reactive oxygen species, resulting in an inflammatory effect [220].

Furthermore, decreased SOD activity in infected tissues could be due to an increased demand for this enzyme to deactivate the high influx of reactive oxygen species produced by inflammatory induction [221].

3.8.2.3 Glutathione peroxidase (GPX)

The results of determination of GP_X revealed a significant ($p \leq 0.05$) elevation in the healthy fish in comparison with those of the infected fish **Figure 3.11**.

3.9 The effect of treatment's types on the level of antioxidant enzymes

Fish inflammatory disease occurs when skin scratches become infected with the bacteria, this infection can get worse if left without treatment. Medicinal therapy may be used to monitor this disease; however antimicrobial treatments are not always effective when traditional drugs have been used due to development of resistance to the limited range of therapeutics [222].

For this reason, Cu NPs have been used as alternative type of drug for skin lesion treatment in order to maintain the level of antioxidants which help to neutralize oxidative stress, making a lack of production of excess free radicals toward protect cells, prevent illness, and reduce inflammatory damage.

3.9.1 Treatment's impact on SOD activity

In this study, veterinary medicine has been evaluated for their antioxidant properties in comparison with metal nanoparticles particularly, Cu NPs.

There is a confirmation from numerous studies about the relationship between copper nanoparticles and the increase in oxidative stress in organism systems.

The generation of ROS by copper nanoparticles may cause direct damage of bacterial cell membranes (bacterial protein functions). Nevertheless, this study focused on the relationship between the levels of some antioxidant enzymes (CAT, SOD, GPx) and Cu NPs treatment in infected fish tissue and healthy ones.

The results have shown a significant exhibition in the level of each antioxidant enzymes after copper nanoparticles treatment in comparison with higher level of oxidative enzymes in fish treated with Nano copper than veterinary treatment in infected tissue ($p \leq 0.05$).

In the case of SOD activity, the results indicated a good exhibited for the activity of SOD after Cu NPs treatment compared to veterinary treatment.

May be the treatment with copper nanoparticles suppresses ROS production in fish infected with inflammation better than vet treatment.

The results indicated excellent increase in the activity of SOD when compared with healthy skin tissue. The activity increased about 40% more after treatment with Cu NPs increasing in compared to vet treatment. **Figure 3.11.**

3.9.2 The effect of treatment on CAT activity

Antioxidant the most important defense mechanism against oxidative stress-induced cell damage. The activity of CAT was significantly increased after treatment and most significant with Cu NPs treatment when compared with the infected tissue ($p = 3.79*10^{-14}$, $p = 9.88*10^{-16}$), respectively) **Figure 3.11**.

So, the types of treatment play an important role on the activity of antioxidant enzymes and make healing skin faster.

3.9.3 The effect of treatment on GPx activity

This study suggested that the activity of GPx can increase by using copper nanoparticles as a medicinal agent to eliminate the fish skin infection. The results display that the activity of GPx increased efficiently when metal nanoparticles used as anti-infectious agent in comparison with vet treatment **Figures 3.11.**

Conclusions

1- Artemisia (Judaica) is used for first time in this study and improved its ability as reducing agent to synthesis pure and stable Cu NPs in comparison with other species.

2- The average particle size was determined using SEM image analysis with average size 54.57 nm.

3- The results obtained in the present study that Cu NPs can strongly increase the activity levels of (SOD, CAT and GPx) which we believe are the main cause of inflammatory disease.

4- Cu NPs can be used as anti-infectious disease perfectly without any toxicity or death to fish.

5- Both types of treatment shown good exhibited for antioxidant enzymes while, Cu NPs are the most significant and fastest healing.

6- Disc diffusion assay was used to investigate the activity of Cu NPs as antibacterial agent in *vitro* study with zone inhibition range 20.5 mm and 23.3mm against the tested bacteria *Bacillus subtilis* and *E-coli* respectively indicating the efficiency of nano - copper compared to other types of nanoparticles.

The current research project has the following implications for the future:

1. It may be possible to perform a detailed mechanistic analysis of the antibacterial action of nanoparticles.
2. In *vivo*, nanoparticles could be utilized to investigate the effect on industrially and therapeutically significant genes.

REFERENCE

Reference

[1] Tunçsoy Mustafa, et al. Effects of copper oxide nanoparticles on antioxidant enzyme activities and on tissue accumulation of Oreochromis niloticus. ***Bulletin of environmental contamination and toxicology***,(2017), 99(3): 360-364.

[2] Hong, Nguyen Hoa. "Introduction to nanomaterials: basic properties, synthesis, and characterization." ***Nano-Sized Multifunctional Materials. Elsevier***, (2019). 1-19.

[3] Lara, Sandra, and André Perez-Potti. "Applications of nanomaterials for immunosensing." ***Biosensors*** (2018) (8)4: 104.

[4] Saleh, Tawfik A. "Nanomaterials: Classification, properties, and environmental toxicities." ***Environmental Technology & Innovation*** (2020): 101067

[5] Ahmed Shakeel, et al. "A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. ***Journal of advanced research***, (2016), 7(1): 17-28.

[6] Huang Chien-Yu and Shyang Roeng Sheen.Synthesis of nanocrystalline and monodispersed copper particles of uniform spherical shape. ***Materials Letters***, (1997), 30(5-6): 357-361.

[7] Al-Khafaji, Mahmood Ali Abed, Rana AK Al-Refai'a, and Oda Mizil Yasser Al-Zamely. "Green synthesis of copper nanoparticles using artemisia plant extract." ***Materials Today: Proceedings*** (2021).10.067.

[8] Cheirmadurai K., et al. “Green synthesis of copper nanoparticles and conducting nanobiocomposites using plant and animal sources,” ***RSC Advances***, (2014.), 4(37):19507–19511.

Reference

[9] Rajesh K. M., et al. “Assisted green synthesis of copper nanoparticles using Syzygium aromaticum bud extract: Physical, optical and antimicrobial properties,” ***Optik***, (2018), 154: 593–600.

[10] Bummer Paul M. Physical chemical considerations of lipid-based oral drug delivery—solid lipid nanoparticles. ***Critical Reviews™ in Therapeutic Drug Carrier Systems,*** (2004), 21(1) 10.1615.

[11] Padma P. Naga, Syed Thanveer Banu, and S. Chaitanya Kumari. "Studies on green synthesis of copper nanoparticles using Punica granatum." Annual Research & Review in Biology (2018): 23(1): 1-10.

[12] Tamilvanan A., et al.. Copper nanoparticles: synthetic strategies, properties and multifunctional application. ***International Journal of Nanoscience***, (2014), 13(02): 1430001.

[13] Schaming Delphine and Hynd Remita. "Nanotechnology: from the ancient time to nowadays." ***Foundations of Chemistry*** (2015) 17(3): 187-205.

[14]Jeevanandam Jaison, et al. "Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations." ***Beilstein journal of nanotechnology*** (2018) 9(1): 1050-1074.

[15] Toumey Christopher P. Reading Feynman into nanotechnology: A text for a new science. Techné: ***Research in Philosophy and Technology***, (2008), 12(3): 133-168.

[16] Madkour Loutfy H. Introduction to nanotechnology (NT) and nanomaterials (NMs). In: Nanoelectronic Materials. ***Springer, Cham***, (2019). 116: 1-47.

[17] Varghese, R. Jose, et al. "Introduction to nanomaterials: synthesis and applications." ***Nanomaterials for Solar Cell Applications. Elsevier***, (2019). 75-95..

Reference

[18] Dolez Patricia I. Nanomaterials definitions, classifications, and applications. In: ***Nanoengineering. Elsevier***, (2015). 3-40.

[19] Lead Jamie R. and Kevin J Wilkinson. "Aquatic colloids and nanoparticles: current knowledge and future trends." ***Environmental Chemistry*** (2006) 3(3): 159-171.

[20] Hough R. M., R. R. P. Noble and M. Reich."Natural gold nanoparticles." ***Ore Geology Reviews*** (2011) 42(1): 55-61.

[21] Hough R. M. and Martin Reich. "Noble metal nanoparticles in ore systems." ***Ore Geology Reviews*** (2011) 42(1): 55-61.

[22] Colomban Philippe and Gwénael Gouadec. "The ideal ceramic fiber/oxide matrix composite: How to conciliate antagonist physical and chemical requirements." ***Ann. Chim. Sci. Matériaux*** (2005) 30(6): 673-688.

[23] Colomban Philippe. The use of metal nanoparticles to produce yellow, red and iridescent colour, from bronze age to present times in lustre pottery and glass: solid state chemistry, spectroscopy and nanostructure. ***Journal of Nano Research***, (2009), (8): 109-132.

[24] Sciau Philippe, et al. Double nanoparticle layer in a 12th century lustreware decoration: accident or technological mastery?. In: Journal of Nano Research. ***Trans Tech Publications Ltd***, (2009).(8): 133-139.

[25] Heiligtag Florian J. and Markus Niederberger. "The fascinating world of nanoparticle research." ***Materials Today*** (2013) 16(7- 8): 262-271.

[26] Matin Moujan, Michael Tite, and Oliver Watson. "On the origins of tin-opacified ceramic glazes: New evidence from early Islamic Egypt, the Levant, Mesopotamia, Iran, and Central Asia." ***Journal of Archaeological Science*** (2018) 97: 42-66.

Reference

[27] Krukemeyer Manfred Georg, et al. "History and possible uses of nanomedicine based on nanoparticles and nanotechnological progress." ***J Nanomed Nanotechnol*** (2015) 6(6): 336.

[28] Tian Konghu, et al. In situ synthesis of copper nanoparticles/polystyrene composite: ***Colloids and Surfaces A: Physicochem. Eng. Aspects*** (2012) 397; 12– 15.

[29] Saranyaadevi K., et al. Synthesis and characterization of copper nanoparticle using Capparis zeylanica leaf extract. ***Int J Chem Tech Res***, (2014), 6(10): 4533-4541.

[30] El-Batal Ahmed I., et al. "Biogenic synthesis of copper nanoparticles by natural polysaccharides and Pleurotus ostreatus fermented fenugreek using gamma rays with antioxidant and antimicrobial potential towards some wound pathogens." ***Microbial pathogenesis*** (2018) 118: 159-169.

[31] Mahmoudvand Hossein, et al. Antinociceptive effects of green synthesized copper nanoparticles alone or in combination with morphine. ***Annals of Medicine and Surgery***, (2020), 51: 31-36.

[32] Mali Suresh Chand, Shani Raj and Rohini Trivedi. Biosynthesis of copper oxide nanoparticles using Enicostemma axillare (Lam.) leaf extract. ***Biochemistry and biophysics reports***, (2019), 20: 100699.

[33] Mali Suresh Chand, et al. Green synthesis of copper nanoparticles using Celastrus paniculatus Willd. leaf extract and their photocatalytic and antifungal properties. ***Biotechnology Reports***, (2020), 27: e00518.

[34] Murthy H. C., et al. Synthesis of green copper nanoparticles using medicinal plant hagenia abyssinica (Brace) JF. Gmel. leaf extract: Antimicrobial properties. ***Journal of Nanomaterials,*** (2020), 2020. 3924081.

Reference

[35] Riggio Cristina, et al. Nano-oncology: clinical application for cancer therapy and future perspectives. ***Journal of Nanomaterials***, (2011), 2011. 164506.

[36] Aher H. R., et al. "Green synthesis of copper nanoparticles using syzygium cumin, leaf extract, characterization and antimicrobial activity." ***Chemical Science*** (2019) 8(1): 1-6..

[37] Khan Ibrahim, Khalid Saeed, and Idrees Khan. Nanoparticles: Properties, applications and toxicities. ***Arabian journal of chemistry***, (2019), 12(7): 908-931.

[38] Singh Prashant, et al. Copper nanoparticles in ionic liquid: An easy and efficient catalyst for the coupling of thiazolidine-2, 4-dione, aromatic aldehyde and ammonium acetate. ***Catalysis Communications***, (2008), 9(7): 1618-1623.

[39] Padma P. Naga Syed Thanveer Banu and S. Chaitanya Kumari. Studies on green synthesis of copper nanoparticles using Punica granatum. ***Annual Research & Review in Biology***, (2018), 1-10.

[40] Subbaiya R., et al. Biological synthesis of silver nanoparticles from Nerium oleander and its antibacterial and antioxidant property**. *Int. J. Curr. Microbiol. Appl. Sci***, (2014), 3(1): 83-87.

[41] Kim Young Hwan, et al. Synthesis of oleate capped Cu nanoparticles by thermal decomposition. ***Colloids Surf. A***. (2006);284:364-368.

[42] Mondal Amal Kumar, et al. Synthesis of ecofriendly silver nanoparticle from plant latex used as an important taxonomic tool for phylogenetic interrelationship advances in bioresearch vol. 2. ***Synthesis***, (2011), 31: 33.

[43] Eastman Jeffrey A., et al.. Anomalously increased effective thermal conductivities of ethylene glycol-based nanofluids containing copper nanoparticles. ***Applied physics letters***, (2001), 78(6): 718-720.

Reference

[44] Guduru Ramesh K., et al. Mechanical behavior of nanocrystalline copper**.** ***Materials Science and Engineering: A***, (2007), 463 (1-2): 14-21.

[45] Yuhas Benjamin D. and Peidong Yang. Nanowire-based all-oxide solar cells. ***Journal of the American Chemical Society***, (2009), 131(10): 3756-3761.

[46] Athanassiou Evagelos K., Robert N. Grass and Wendelin J. Stark. Large-scale production of carbon-coated copper nanoparticles for sensor applications. ***Nanotechnology***, (2006), 17(6): 1668.

[47] Ramyadevi Jeyaraman, et al.. Synthesis and antimicrobial activity of copper nanoparticles. ***Materials letters***, (2012), 71: 114-116.

[48] Rafique Muhammad, et al.. A review on synthesis, characterization and applications of copper nanoparticles using green method. ***Nano***, (2017), 12(04): 1750043.

[49] Letchumanan Devanthiran, et al. Plant-Based Biosynthesis of Copper/Copper Oxide Nanoparticles: An Update on Their Applications in Biomedicine, Mechanisms, and Toxicity. ***Biomolecules***, (2021), 11(4): 564.

[50] Tahvilian Reza, et al. Green synthesis and chemical characterization of copper nanoparticles using Allium saralicum leaves and assessment of their cytotoxicity, antioxidant, antimicrobial, and cutaneous wound healing properties. ***Applied organometallic chemistry***, (2019), 33(12): e5234.

[51] Velsankar K., et al. Green synthesis of CuO nanoparticles via Allium sativum extract and its characterizations on antimicrobial, antioxidant, antilarvicidal activities. ***Journal of Environmental Chemical Engineering***, (2020), 8(5): 104123.

[52] Jia Baoquan, et al.Preparation of copper nanoparticles coated cellulose films with antibacterial properties through one-step reduction. ***ACS applied materials & interfaces***, (2012), 4(6): 2897-2902.

Reference

[53] Duan Zhongyu, Guoli Ma and Wenjun Zhang. Preparation of copper nanoparticles and catalytic properties for the reduction of aromatic nitro compounds. ***Bulletin of the Korean Chemical Society***, (2012), 33(12): 4003-4006.

[54]Baig Nadeem, Irshad Kammakakam, and Wail Falath. "Nanomaterials: a review of synthesis methods, properties, recent progress, and challenges." ***Materials Advances*** (2021) 2(6): 1821-1871

[55]Shankar S. Shiv, et al. "Biological synthesis of triangular gold nanoprisms." ***Nature materials*** (2004) 3(7): 482-488.

[56]Firdhouse M. Jannathul and P. Lalitha. Biosynthesis of silver nanoparticles and its applications. Journal of ***Nanotechnology***, (2015), 2015. 829526.

[57]Songping Wu, et al. Preparation of ultra fine copper–nickel bimetallic powders for conductive thick film. ***Intermetallics***, (2007), 15(10): 1316-1321.

[58]Patra Jayanta Kumar and Kwang-Hyun Baek. Green nanobiotechnology: factors affecting synthesis and characterization techniques. ***Journal of Nanomaterials***,(2014), 2014. 219, 219.

[59]Jan, Hasnain, et al. "Biogenic synthesis and characterization of antimicrobial and anti-parasitic zinc oxide (ZnO) nanoparticles using aqueous extracts of the Himalayan columbine (Aquilegia pubiflora)." ***Frontiers in Materials*** (2020)7 : 249.

[60]Li Xiangqian, et al. Biosynthesis of nanoparticles by microorganisms and their applications. ***Journal of Nanomaterials***, (2011), 2011. 8, 1–16.

[61]Abou El-Nour Kholoud MM, et al. Synthesis and applications of silver nanoparticles. ***Arabian journal of chemistry***, (2010), 3(3): 135-140.

[62]Anastas Paul and Nicolas Eghbali. Green chemistry: principles and practice. **Chemical Society Reviews**, (2010), 39(1): 301-312.

Reference

[63]Bhardwaj Brahamdutt, et al. Eco-Friendly Greener Synthesis of Nanoparticles. ***Advanced Pharmaceutical Bulletin***, (2020), 10(4): 566.

[64]Narayanan Kannan Badri and Natarajan Sakthivel Natarajan. Green synthesis of biogenic metal nanoparticles by terrestrial and aquatic phototrophic and heterotrophic eukaryotes and biocompatible agents. ***Advances in colloid and interface science***, (2011), 169(2): 59-79.

[65]Singh, Akanksha, et al. "Synthesis and characterization of nanoparticles used in consumer products." Toxicology of nanoparticles: ***Insights from Drosophila. Springer, Singapore***, (2020). 1-27.

[66]Singh Manjit Ajay K. Sharma and R. S. Kaler . Investigations on optical timing jitter in dispersion managed higher order soliton system. ***Journal of Optics***, (2011), 40(1): 1-7.

[67]Thakkar Kaushik N. Snehit S. Mhatre and Rasesh Y. Parikh.; PARIKH, Rasesh Y. Biological synthesis of metallic nanoparticles. Nanomedicine: nanotechnology, ***biology and medicine***,(2010), 6(2): 257-262.

[68]Raveendran, Poovathinthodiyil, Jie Fu, and Scott L. Wallen. Completely “green” synthesis and stabilization of metal nanoparticles. ***Journal of the American Chemical Society***, (2003), 125(46): 13940-13941.

[69]Pedersen David B. Shiliang Wang and Septimus H. Liang. Charge-transfer-driven diffusion processes in Cu@ Cu-oxide core− shell nanoparticles: oxidation of 3.0±0.3 nm diameter copper nanoparticles. ***The Journal of Physical Chemistry C***, (2008), 112(24): 8819-8826.

[70]Crooks Richard M., et al.. Dendrimer-encapsulated metal nanoparticles: synthesis, characterization, and applications to catalysis. ***Accounts of chemical research***,(2001), 34(3): 181-190.

Reference

[71]Saranyaadevi K., et al.. Synthesis and characterization of copper nanoparticle using Capparis zeylanica leaf extract. ***Int J Chem Tech Res***, (2014), 6(10): 4533-4541.

[72]Tamilvanan A., et al. Copper nanoparticles: synthetic strategies, properties and multifunctional application. ***International Journal of Nanoscience***, (2014), 13(02): 1430001.

[73]Abskharon Romany NN, et al. The role of antioxidants enzymes of E. coli ASU3, a tolerant strain to heavy metals toxicity, in combating oxidative stress of copper. World Journal of Microbiology and Biotechnology, (2010), 26(2): 241-247.

[74] Qidwai Afifa, et al. Advances in biogenic nanoparticles and the mechanisms of antimicrobial effects. ***Indian Journal of Pharmaceutical Sciences***, (2018), 80(4): 592-603.

[75]Xin Lee K Shameli K, Miyake M, Kuwano N, Bt Ahmad Khairudin N B, Bt Mohamad S E and Yew Y P . Xin Lee, Kar, et al. "Green synthesis of gold nanoparticles using aqueous extract of Garcinia mangostana fruit peels." ***Journal of Nanomaterials*** 2016 (2016),1(7): 8489094.

[76]Ismail N A Shameli K, Wong M M, Teow S Y, Chew J and Sukri S. "Antibacterial and cytotoxic effect of honey mediated copper nanoparticles synthesized using ultrasonic assistance." ***Materials Science and Engineering***: (2019) 104: 109899.

[77]Rajeshkumar S., et al. "Antibacterial and antioxidant potential of biosynthesized copper nanoparticles mediated through Cissus arnotiana plant extract." ***Journal of Photochemistry and Photobiology B: Biology*** (2019) 197: 111531.

Reference

[78]Sharma P P S, Dave V, Tak K, Sadhu V, Reddy K R . "Green synthesis and characterization of copper nanoparticles by Tinospora cardifolia to produce nature-friendly copper nano-coated fabric and their antimicrobial evaluation." ***Journal of microbiological methods*** (2019) 160: 107-116.

[79]Mukhopadhyay Ria, Julekha Kazi and Mita Chatterjee Debnath. "Synthesis and characterization of copper nanoparticles stabilized with Quisqualis indica extract: evaluation of its cytotoxicity and apoptosis in B16F10 melanoma cells." ***Biomedicine & Pharmacotherapy*** (2018) 97: 1373-1385.

[80]Varghese Binu, et al. "Biochemical synthesis of copper nanoparticles using Zingiber officinalis and Curcuma longa: Characterization and antibacterial activity study." Materials Today: Proceedings (2020) 25: 302-306

[81]Batoool M. and B. Masood. "Green synthesis of copper nanoparticles using Solanum lycopersicum (tomato aqueous extract) and study characterization." ***J. Nanosci. Nanotechnol*** (2017) 1: 1-5.

[82]Arya A Gupta K, Chundawat T S and Vaya D. "Biogenic synthesis of copper and silver nanoparticles using green alga Botryococcus braunii and its antimicrobial activity." ***Bioinorganic Chemistry and Applications*** (2018) ,2018: 7879403.

[83]Noman M S M, Ahmed T, Niazi M B K, Hussain S, Song F, Manzoor. "Use of biogenic copper nanoparticles synthesized from a native Escherichia sp. as photocatalysts for azo dye degradation and treatment of textile effluents." ***Environmental Pollution*** (2020) 257: 113514.

[84]Noor S S Z, Javed A, Ali A, Hussain S B, Zafar S, Ali H, Muhammad S A . "A fungal based synthesis method for copper nanoparticles with the determination of anticancer, antidiabetic and antibacterial activities." ***Journal of Microbiological Methods*** (2020) 174: 105966.

[85]Olad A Alipour M and Nosrati R. The use of biodegradable polymers for the stabilization of copper nanoparticles synthesized by chemical reduction method. ***Bulletin of Materials Science***. (2017),40 1013-20.

[86]Upadhyay, Lata Sheo Bachan, and Nikhil Kumar. "Green synthesis of copper nanoparticle using glucose and polyvinylpyrrolidone (PVP)." ***Inorganic and Nano-Metal Chemistry*** (2017) 47(10): 1436-1440.

[87]Letchumanan Devanthiran, et al. Plant-Based Biosynthesis of Copper/Copper Oxide Nanoparticles: An Update on Their Applications in Biomedicine, Mechanisms, and Toxicity. ***Biomolecules***, (2021), 11(4): 564.

[88]Baer Donald R.. Surface Characterization of Nanoparticles: critical needs and significant challenges. ***J of Surface Anal***,(2011), 17: 163-9.

[89]Mudunkotuwa Imali A., John M. Pettibone and Vicki H. Grassian. Environmental implications of nanoparticle aging in the processing and fate of copper-based nanomaterials. ***Environmental science & technology***, (2012), 46(13): 7001-7010..

[90]Zhao Hongwei, et al. Biosynthesis of copper nanoparticles using Allium eriophyllum Boiss leaf aqueous extract; characterization and analysis of their antimicrobial and cutaneous wound-healing potentials. ***Applied Organometallic Chemistry***, (2020), e5587.

[91]Prakash Shanmugam, et al. Green synthesis of copper oxide nanoparticles and its effective applications in Biginelli reaction, BTB photodegradation and antibacterial activity. ***Advanced Powder Technology***,(2018), 29(12): 3315-3326.

Reference

[92]Akintelu S. A., et al. Green synthesis of copper oxide nanoparticles for biomedical application and environmental remediation. ***Heliyon***, (2020), 6(7): e04508.

[93]Sackey J., et al. Electrochemical properties of Euphorbia pulcherrima mediated copper oxide nanoparticles. Materials Chemistry and Physics, (2020), 244: 122714.

[94]Rajesh K. M., et al. Synthesis of copper nanoparticles and role of pH on particle size control. ***Materials Today: Proceedings***, (2016), 3(6): 1985-1991.

[95]Piri Fatemeh, Mahdi Shafiee Afarani and Amir Masoud Arabi Synthesis of copper oxide quantum dots: effect of surface modifiers. ***Materials Research Express***, (2019), 6(12): 125006.

[96]Chatterjee Arijit Kumar, Ruchira Chakraborty and Tarakdas Basu Tarakdas. Mechanism of antibacterial activity of copper nanoparticles. ***Nanotechnology***, (2014), 25(13): 135101.

[97]Rakhmetova A. A., et al. Wound-healing properties of copper nanoparticles as a function of physicochemical parameters. ***Nanotechnologies in Russia***, (2010), 5(3): 271-276.

[98]Borkow Gadi and Jeffrey Gabbay. Copper, an ancient remedy returning to fight microbial, fungal and viral infections. ***Current Chemical Biology***, (2009), 3(3): 272-278.

[99]Raffi Muhammad, et al. Investigations into the antibacterial behavior of copper nanoparticles against Escherichia coli. ***Annals of microbiology***, (2010), 60(1): 75-80.

[100]Yoon Ki-Young, et al. Susceptibility constants of Escherichia coli and Bacillus subtilis to silver and copper nanoparticles. ***Science of the Total Environment***, (2007), 373(2-3): 572-575..

Reference

[101]Azam Ameer, et al. Size-dependent antimicrobial properties of CuO nanoparticles against Gram-positive and-negative bacterial strains. International ***journal of nanomedicine***, (2012), 7: 3527.

[102]Amro Nabil A., et al. High-resolution atomic force microscopy studies of the Escherichia coli outer membrane: structural basis for permeability. Langmuir, (2000), 16(6): 2789-2796.

[103]Applerot Guy, et al.. Understanding the antibacterial mechanism of CuO nanoparticles: ***revealing the route of induced oxidative stress. Small***, (2012), 8.21: 3326-3337.

[104]Fang Jiasong, et al. Effect of a fullerene water suspension on bacterial phospholipids and membrane phase behavior. ***Environmental science & technology***, (2007), 41(7): 2636-2642.

[105]Kim Jun Sung, et al. Antimicrobial effects of silver nanoparticles. Nanomedicine: Nanotechnology, ***biology and medicine***, (2007), 3(1): 95-101.

[106]Sondi Ivan and Branka Salopek-Sondi. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. ***Journal of colloid and interface science***, (2004), 275(1): 177-182.

[107]Borokini Temitope Israel and Felix Oluwafemi Omotayo. Phytochemical and ethnobotanical study of some selected medicinal plants from Nigeria. ***Journal of Medicinal Plants Research***, (2012), 6(7): 1106-1118.

[108]Edeoga H. Okwu D. E. Okwu and B. O. Mbaebie. Phytochemical constituents of some Nigerian medicinal plants. ***African journal of biotechnology***, (2005), 4(7): 685-688.

[109]Ishak NAI Md S. K. Kamarudin and S. N. Timmiati. Green synthesis of metal and metal oxide nanoparticles via plant extracts: an overview. ***Materials Research Express***, (2019), 6(11): 112004.

Reference

[110]Harishchandra Baraiya Divyeksha, et al.Copper Nanoparticles: A Review on Synthesis, Characterization and Applications. ***Asian Pacific Journal of Cancer Biology***, 2020, 5(4): 201-210.

[111]Thakur Abhishek and Rakesh Sharma. Health promoting phytochemicals in vegetables: a mini review. ***International Journal of Food and fermentation Technology***, 2018, 8(2): 107-117

[112]Chede PANKAJA S, et al. Phytochemical analysis of Citrus sinensis peel. ***International Journal of Pharma and Bio Sciences***, (2013), 4(1): 339-343.

[113]Singh Suman, et al. Electrochemical sensing and remediation of 4-nitrophenol using bio-synthesized copper oxide nanoparticles. ***Chemical engineering journal***, (2017), 313: 283-292.

[114]Nasrollahzadeh Mahmoud, et al. Green synthesis of CuO nanoparticles using aqueous extract of Thymus vulgaris L. leaves and their catalytic performance for N-arylation of indoles and amines. ***Journal of colloid and interface science***, (2016), 466: 113-119..

[115]Nigam Manisha, et al. Bioactive compounds and health benefits of Artemisia species. Natural product communications, (2019), 14(7): 1934578X19850354.

[116]Reveal,J An outline of a classification scheme for extant flowering plants.***Phytoneuron,***(2012) **,** (37)1-221.

[117]Hudaib Mohammad M. and Talal A. Aburjai. Composition of the essential oil from Artemisia herba-alba grown in Jordan. ***Journal of Essential Oil Research***, (2006), 18(3): 301-304..

[118]Nin S. and A. Bennici.. Transgenic Artemisia (Wormwood). In: ***Transgenic Crops III. Springer, Berlin, Heidelberg***, (2001). 48: 27-42.

Reference

[119]Bhat Rahil Razzak, et al. Chemical composition and biological uses of Artemisia absinthium (wormwood). In: ***Plant and Human Health, Volume 3. Springer, Cham***, (2019). 37-63.

[120]Bora Kundan Singh and Anupam Sharma. The genus Artemisia: a comprehensive review. ***Pharmaceutical Biology***, (2011), 49(1): 101-109..

[121]Rahman Taibur, et al. Oxidative stress and human health, Advances in Bioscience and Biotechnology. ***Scientific Research Publishing***, (2012). 3 (7):25130,23

[122]Phaniendra Alugoju, Dinesh Babu Jestadi and Latha Periyasamy. Free radicals: properties, sources, targets, and their implication in various diseases. ***Indian journal of clinical biochemistry***, (2015), 30(1): 11-26.

[123]Halliwell Barry. Oxygen is a toxic gas-an introduction to oxygen toxicity and reactive oxygen species. ***Free radicals in biology and medicine***, (1999), 101(3), 272-285.

[124]Agarwal Ashok, Sajal Gupta and Rakesh K. Sharma. Role of oxidative stress in female reproduction. ***Reproductive biology and endocrinology***, (2005), 3(1): 1-21.

[125]Schieber Michael and Navdeep S. Chandel. ROS function in redox signaling and oxidative stress. ***Current biology***, (2014), 24(10): 453-462.

[126]Sevcikova M., et al. Metals as a cause of oxidative stress in fish: a review. ***Vet Med***, (2011), 56(11): 537-546.

[127]Yoshikawa Toshikazu and Yuji Naito. "What is oxidative stress?." ***Japan medical association journal*** (2002) 45(7): 271-276

[128]Halliwell Barry. Free radicals and other reactive species in disease. ***e LS***, (2001) .10.1038.

Reference

[129]Lobo, Vijaya, et al. "Free radicals, antioxidants and functional foods: Impact on human health." ***Pharmacognosy reviews*** (2010), 4(8): 118

[130]Pierce Janet D., Amanda B. Cackler and Melinda G. Arnett. Why should you care about free radicals? Because these molecules appear to play a role in the development of heart disease, cancer, diabetes, and other diseases. Understanding how free radicals form and what you can do to help keep them in balance is essential to maximizing the care you provide now--and in the future. ***Rn***, (2004), 67(1): 38-44.

[131]Attaran Marjan, et al. The effect of follicular fluid reactive oxygen species on the outcome of in vitro fertilization. ***International journal of fertility and women's medicine***, (2000), 45(5): 314-320..

[132]Kurutas Ergul Belge. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. ***Nutrition journal***, (2015), 15(1): 1-22..

[133]Krishnamurthy, Praveen, and Ashish Wadhwani. Antioxidant enzymes and human health. ***Antioxidant enzyme***, (2012), 3: 1-17.

[134]Poljsak Borut, Dušan Šuput and Irina Milisav. Achieving the balance between ROS and antioxidants: when to use the synthetic antioxidants. ***Oxidative medicine and cellular longevity***, (2013), 2013. 956792.

[135]Kohen Ron and Abraham Nyska. Invited review: Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. ***Toxicologic pathology***, (2002), 30(6): 620-650..

[136]Valko Marian, et al. Free radicals and antioxidants in normal physiological functions and human disease. ***The international journal of biochemistry & cell biology,*** (2007), 39(1): 44-84.

Reference

[137]Fang Yun-Zhong Sheng Yang and Guoyao Wu. Free radicals, antioxidants, and nutrition. ***Nutrition***, (2002), 18(10): 872-879.

[138]Kawamura F., et al. Study on antioxidant activities, total phenolic compound and antifungal properties of some Malaysian timbers from selected hardwoods species. In: ***Proceedings International Conference on Environmental Research and Technology***. Universiti Sains Malaysia, (2008). 472- 475.

[139]Sarker Umakanta, et al. Genotypic diversity in vegetable amaranth for antioxidant, nutrient and agronomic traits. ***Indian J. Genet. Pl. Breed***, (2017), 77: 173-176.

[140]Shakila P. Baby and K. Usha.. Antioxidant status in effluent treated grains of Vigna radiata. ***Journal of Agriculture and Environment***, (2011), 12: 22-26.

[141]Ahmad Riaz, et al.. Oxidative stress and antioxidant defense mechanisms in plants under salt stress. In: ***Plant abiotic stress tolerance. Springer, Cham***, (2019). 191-205.

[142]Lobo Vijaya, et al. Free radicals, antioxidants and functional foods: Impact on human health. ***Pharmacognosy reviews,*** (2010), 4(8): 118.

[143]Singh Randhir, Sushma Devi and Rakesh Gollen;. Role of free radical in atherosclerosis, diabetes and dyslipidaemia: larger-than-life. ***Diabetes/metabolism research and reviews***, (2015), 31(2): 113-126.

[144]Nimse Satish Balasaheb and Dilipkumar Pal. Free radicals, natural antioxidants, and their reaction mechanisms. ***RSC advances***, (2015), 5(35): 27986-28006...

[145]Kurutas, Ergul Belge. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. ***Nutrition journal***, (2015), 15(1): 1-22.

Reference

[146]Moussa Ziad, Zaher MA Judeh and Saleh A. Ahmed. Nonenzymatic exogenous and endogenous antioxidants. ***Free Radical Medicine and Biology***. (2019). 10(8): 1105.

[147]Flora Swaran JS. Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure. ***Oxidative medicine and cellular longevity***, (2009), 2(4): 191-206.

[148]Birben Esra, et al.. Oxidative stress and antioxidant defense. ***World Allergy Organization Journal***, (2012), 5(1): 9-19.

[149]Ighodaro O. M. and O. A. Akinloye. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. ***Alexandria journal of medicine***, (2018), 54(4): 287-293.

[150]Das Kaushik and Aryadeep Roychoudhury. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. ***Frontiers in environmental science,*** (2014), 2: 53.

[151]Fukai Tohru and Masuko Ushio-Fukai. Superoxide dismutases: role in redox signaling, vascular function, and diseases. ***Antioxidants & redox signaling***, (2011), 15(6): 1583-1606.

[152]Gao Fei, et al. Extracellular superoxide dismutase in pulmonary fibrosis. ***Antioxidants & redox signaling***, (2008), 10(2): 343-354.

[153]Vani M., et al. Glutathione-S-transferase, superoxide dismutase, xanthine oxidase, catalase, glutathione peroxidase and lipid peroxidation in the liver of exercised rats. ***Biochemistry international***, (1990), 21(1): 17-26..

[154]Heck Diane E., et al. Mechanisms of oxidant generation by catalase. ***Annals of the New York Academy of Sciences***, (2010), 1203: 120.

Reference

[155]Kurutas Ergul Belge. The importance of antioxidants which play the role in cellular response against oxidative/ nitrosative stress: ***current state. Nutrition journal***, (2015), 15(1): 1-22.

[156]Espinosa-Diez Cristina, et al. Antioxidant responses and cellular adjustments to oxidative stress. ***Redox biology***, (2015), 6: 183-197.

[157]Birben, Esra, et al. Oxidative stress and antioxidant defense. ***World Allergy Organization Journal***, (2012), 5(1): 9-19.

[158]Mehta Shalini Kapoor and Sivakumar Joghi Thatha Gowder. Members of antioxidant machinery and their functions. ***Basic principles and clinical significance of oxidative stress***, (2015), 60-85.

[159]Go Young-Mi and Dean P. Jones. Redox theory of aging: implications for health and disease. ***Clinical Science***, (2017), 131(14): 1669-1688.

[160]Kabel Ahmed M. Free radicals and antioxidants: role of enzymes and nutrition. ***World Journal of Nutrition and Health***, (2014), 2(3): 35-38..

[161]Valko Marian, et al. Free radicals and antioxidants in normal physiological functions and human disease. ***The international journal of biochemistry & cell biology***, (2007), 39(1): 44-84.

[162]R Buettner Garry. Superoxide dismutase in redox biology: the roles of superoxide and hydrogen peroxide. ***Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)***, (2011), 11(4): 341-346.

[163]Gottlieb Klaus, et al. β-Carotene decreases markers of lipid peroxidation in healthy volunteers. ***Journal of Nutritional & Environmental Medicine,*** (1993). 19: 207-212.

Reference

[164]Dhalla Naranjan S., Rana M. Temsah and Thomas Netticadan. Role of oxidative stress in cardiovascular diseases. ***Journal of hypertension***, (2000), 18(6): 655-673.

[165]Zelko Igor N., Thomas J. Mariani and Rodney J. Folz. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. ***Free Radical Biology and Medicine***, (2002), 33(3): 337-349.

[166]Tiwari Brahm Kumar, et al. Markers of oxidative stress during diabetes mellitus. ***Journal of biomarkers***, (2013), 2013 378790, 8 .

[167]Kinnula Vuokko L. and James D. Crapo. Superoxide dismutases in the lung and human lung diseases. ***American journal of respiratory and critical care medicine***, (2003), 167(12): 1600-1619..

[168]Holley Aaron K., et al. Manganese superoxide dismutase: guardian of the powerhouse. ***International journal of molecular sciences***, (2011), 12(10): 7114-7162.

[169]Mondola Paolo, et al. The Cu, Zn superoxide dismutase: not only a dismutase enzyme. ***Frontiers in physiology***, (2016), 7: 594.

[170]Asami Shinya, et al. Cigarette smoking induces an increase in oxidative DNA damage, 8-hydroxydeoxyguanosine, in a central site of the human lung. ***Carcinogenesis***, (1997), 18(9): 1763-1766.

[171]Oury Tim D., et al. Human extracellular superoxide dismutase is a tetramer composed of two disulphide-linked dimers: a simplified, high-yield purification of extracellular superoxide dismutase. ***Biochemical Journal***, (1996), 317(1): 51-57.

Reference

[172]Petersen Steen V., et al. Extracellular superoxide dismutase (EC-SOD) binds to type I collagen and protects against oxidative fragmentation. ***Journal of Biological Chemistry***, (2004), 279(14): 13705-13710.

[173]Fattman Cheryl L., Lisa M. Schaefer and Tim D. Oury. Extracellular superoxide dismutase in biology and medicine. ***Free Radical Biology and Medicine***, (2003), 35(3): 236-256.

[174]Kim Eun-Ja, et al. Differential expression of superoxide dismutases containing Ni and Fe/Zn in Streptomyces coelicolor. ***European Journal of Biochemistry***, (1996), 241(1): 178-185.

[175]Pakhomova Svetlana, et al. The structure and peroxidase activity of a 33-kDa catalase-related protein from Mycobacterium avium ssp. ***paratuberculosis. Protein Science***, (2009), 18(12): 2559-2568.

[176]Nandi Ankita, et al. Role of catalase in oxidative stress-and age-associated degenerative diseases. ***Oxidative medicine and cellular longevity***, (2019), 2019:613090, .

[177]Lubos Edith, Joseph Loscalzo and Diane E. Handy. Glutathione peroxidase-1 in health and disease: ***from molecular mechanisms to therapeutic opportunities***.(2011). 1; 15(7): 1957–1997.

[178]Sarıkaya Eren and Selami Doğan.. Glutathione peroxidase in health and diseases. Glutathione system and oxidative stress in health and disease, (2020). 10.5772.91009

[179]Wendel Albrecht. Glutathione peroxidase. ***Enzymatic basis of detoxication***, (1980), 1: 333-353.

[180] Gomori, G. "Preparation of buffers for use in enzyme studies." CRC Handbook of Biochemistry and Molecular Biology. CRC Press, (2018). 370-377.

Reference

[181]Gallagher Sean R. Recipes for Commonly Encountered Reagents. ***Current Protocols Essential Laboratory Techniques***, (2018), 17(1): e24.

[182]Green Michael R., and Joseph Sambrook. "Buffers." Cold Spring Harbor Protocols (2018) 2018(2): 098210.

[183]Fling Steven P. and Dale S. Gregerson. Peptide and protein molecular weight determination by electrophoresis using a high-molarity tris buffer system without urea. ***Analytical biochemistry,*** (1986), 155(1): 83-88.

[184]Durst Richard A. and Bert R. Staples. Tris/Tris· HCl: a standard buffer for use in the physiologic pH range. ***Clinical Chemistry***, (1972), 18(3): 206-208.

[185]Segel, Irwin H., et al. Biochemical calculations. ***Wiley***, (1975).

[186]Aher H. R., et al. Green synthesis of copper nanoparticles using syzygium cumin, leaf extract, characterization and antimicrobial activity. ***Chemical Science***, (2019), 8(1): 1-6.

[187]Bhavyasree P. G. and T. S. Xavier. Green synthesis of Copper Oxide/Carbon nanocomposites using the leaf extract of Adhatoda vasica Nees, their characterization and antimicrobial activity. ***Heliyon***, (2020), 6(2): e03323.

[188]Mali Suresh Chand, et al. Green synthesis of copper nanoparticles using Celastrus paniculatus Willd. leaf extract and their photocatalytic and antifungal properties. ***Biotechnology Reports***, (2020), 27: e00518.

[189] Squire, John M., and Carlo Knupp. "Analysis methods and quality criteria for investigating muscle physiology using x-ray diffraction." ***Journal of General Physiology*** (2021), (153)10: e202012778.

[190] Geng, Guoqing, et al. "Atomistic structure of alkali-silica reaction products refined from X-ray diffraction and micro X-ray absorption data." ***Cement and Concrete Research*** (2020) 129: 105958.

Reference

[191]Cargnello Matteo. Tailored nanoarchitectures based on transition metals for heterogeneous catalysis. 2012.

[192]Oseghale Charles Ijogbemeye. Gold Copper Based Catalysts in the Development of Direct Formic Acid Fuel Cells.. PhD Thesis. University of Sheffield.١٤١-١٤٩ :٣٤٧ ،(٢٠١٧) .

[193] Veerasingam, S., et al. "Contributions of Fourier transform infrared spectroscopy in microplastic pollution research: A review." ***Critical Reviews in Environmental Science and Technology*** (2021), (51)22: 2681-2743.

[194] Agarwal Happy, et al. "Mechanistic study on antibacterial action of zinc oxide nanoparticles synthesized using green route." ***Chemico-biological interactions*** (2018) 286: 60-70.

[195] Sánchez-López Elena, et al. "Metal-based nanoparticles as antimicrobial agents: an overview." ***Nanomaterials*** (2020) (10)2: 292.

[196]Sánchez-López Elena, et al. Metal-based nanoparticles as antimicrobial agents: an overview. Nanomaterials, (2020), 10(2): 292.

[197]Balouiri Mounyr, Moulay Sadik and Saad Koraichi Ibnsouda. Methods for in vitro evaluating antimicrobial activity: A review. Journal of pharmaceutical analysis, (2016), 6(2): 71-79.

[198]Marklund Stefan and Gudrun Marklund.Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European journal of biochemistry, (1974), 47(3): 469-474.

[199]Beers Roland F. and Irwin W. Sizer. "A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase." Journal of biological chemistry (1952) 195(1): 133-140.

Reference

[200]Hafeman D. G., R. A. Sunde and W. G. Hoekstra. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. The Journal of nutrition, (1974), 104(5): 580-587.

[201]Abu-Darwish M. S., et al. Chemical composition and biological activities of Artemisia judaica essential oil from southern desert of Jordan. ***Journal of ethnopharmacology***, (2016), 191: 161-168.

[202]Ismail N A Shameli K, Wong M M, Teow S Y, Chew J and Sukri S. "Antibacterial and cytotoxic effect of honey mediated copper nanoparticles synthesized using ultrasonic assistance." ***Materials Science and Engineering***: (2019) 104: 109899.

[203] Saranyaadevi, K., et al. "Synthesis and characterization of copper nanoparticle using Capparis zeylanica leaf extract." ***Int J Chem Tech Res*** (2014) 6.10: 4533-4541

[204]Theivasanthi T. and Marimuthu Alagar. X-ray diffraction studies of copper nanopowder. ***arXiv preprint arXiv:1003.6068***, (2010) ,318(1): 88-95..

[205]Gopalakrishnan V. and S. Muniraj. Neem flower extract assisted green synthesis of copper nanoparticles–Optimisation, characterisation and anti-bacterial study. ***Materials Today: Proceedings***, (2021), 36: 832-836.

[206]Alavi Mehran and Naser Karimi. 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. ***Artificial cells, nanomedicine, and biotechnology***, (2018), 46(8): 2066-2081.

[207]El-Saadony Mohamed T., et al. Ecofriendly synthesis and insecticidal application of copper nanoparticles against the storage pest Tribolium castaneum. ***Nanomaterials***, (2020), 10(3): 587.

Reference

[208]Alavi Mehran and Naser Karimi. "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." ***Artificial cells, nanomedicine, and biotechnology*** (2018) 46(8): 2066-2081.

[209]Rajeshkumar S., et al. "Antibacterial and antioxidant potential of biosynthesized copper nanoparticles mediated through Cissus arnotiana plant extract." ***Journal of Photochemistry and Photobiology B: Biology*** (2019) 197: 111531.

[210]Rajeshkumar S. and G. Rinitha. "Nanostructural characterization of antimicrobial and antioxidant copper nanoparticles synthesized using novel Persea americana seeds." ***OpenNano*** (2018) 3: 18-27.

[211]Tahvilian Reza, et al. "Green synthesis and chemical characterization of copper nanoparticles using Allium saralicum leaves and assessment of their cytotoxicity, antioxidant, antimicrobial, and cutaneous wound healing properties." ***Applied organometallic chemistry*** (2019) 33(12): e5234.

[212]Deryabin, D. G., et a. Investigation of copper nanoparticles antibacterial mechanisms tested by luminescent Escherichia coli strains. ***Nanotechnologies in Russia***, (2013), 8(5): 402-408.

[213]Saleem, Samia, et al. Inhibition of growth and biofilm formation of clinical bacterial isolates by NiO nanoparticles synthesized from Eucalyptus globulus plants. ***Microbial pathogenesis***, (2017), 111: 375-387.

[214]Kulbacka Julita, Jolanta Saczko and Agnieszka Chwiłkowska. Oxidative stress in cells damage processes. ***Polski merkuriusz lekarski***: ***organ Polskiego Towarzystwa Lekarskiego***, (2009), 27(157): 44-47.

Reference

[215] Borkow Gadi. Using copper to improve the well-being of the skin. ***Current chemical biology***, (2014), 8(2): 89-102.

[216] Gaetani, Gian Franco, et al. "Active involvement of catalase during hemolytic crises of favism." ***Journals in Medicine*** (1996) ,88(3): 1084-1088.

[217] Kakkar Rakesh, et al.. Lipid peroxidation and activity of antioxidant enzymes in diabetic rats. ***Molecular and cellular biochemistry***, (1995), 151(2): 113-119.

[218] Rg Ahmed. Is there a balance between oxidative stress and antioxidant defense system during development***?. Med. J. Islamic Academy of Sci,*** (2005), 15(2): 55-63.

[219] Tiwari Brahm Kumar, et al. Markers of oxidative stress during diabetes mellitus. ***Journal of biomarkers***, (2013), 318(1): 88-95..

[220] Kamesh Venkatakrishnan and Thangarajan Sumathi. Nephroprotective potential of Bacopa monniera on hypercholesterolemia induced nephropathy via the NO signaling pathway. ***Pharmaceutical biology***, (2014), 52(10): 1327-1334.

[221] Tørud Brit and Tore Håstein. Skin lesions in fish: causes and solutions. ***Acta Veterinaria Scandinavica***, (2008), 50(1): 1-3.

[222] Sánchez-López Elena, et al. Metal-based nanoparticles as antimicrobial agents: an overview. ***Nanomaterials***, (2020), 10(2): 292**.**

الخلاصة:

تقنيات النانو هي العلم الذي يهتم بدراسة معالجة المادة على المقياس الذري والجزيئي .تهتم تقنية النانو بابتكار تقنيات ووسائل جديدة تقاس أبعادها بالنانومتر وهو جزء من الألف من الميكرومتر أي جزء من المليون من الميليمتر .عادة تتعامل تقنية النانو مع قياسات بين ١ إلى ١٠٠.وتدخل تقنية النانو في الكثير من المجالات مثل الطب والصناعة ولها تطبيقات عديدة مثل التخليق البيولوجي للنحاس ودراسة تأثيرات النحاس النانوي على الكثير من المجالات لخصائصه المضادة للالتهابات. بالمقارنة مع العلاجات النموذجية، قد يكون للنحاس النانوي تأثير بيولوجي أفضل وله استخدامات متنوعة في الرعاية الصحية.

في هذه الدراسة، تم دراسة تخليق وتقدير جزيئات النحاس النانوية واستخدامها كعامل مضاد للالتهابات. كان الهدف من هذه الدراسة هو إنتاج جسيمات النحاس النانوية باستخدام مستخلص نباتي ثم تقييم فعاليتها في زيادة القدرة على الشفاء من خلال التأثير على مستويات الإنزيمات المضادة للأكسدة التي يمكن أن تعمل على التقليل من الالتهابات. تم الانتهاء من تنقية وتقدير المنتج النهائي. تم تحضير جزيئات النحاس النانوية بعملية تخليق خضراء من محلول كبريتات النحاس من خلال مستخلص نبات الشيح.

تم استخدام التحليل الطيفي المرئي للأشعة فوق البنفسجية ،وطيف الاشعة تحت الحمراء ،وحيود الاشعة السينية ،طيف تستت الطاقة بالاشعة السينية و المسح المجهري الإلكتروني. لتقدير تكوين (جزيئات النحاس النانوية). وجد أن متوسط حجم الجسيمات ٥٤,٥٧ نانومتر. تم اختبار الفعالية المضادة للبكتيريا لجزيئات النانو المحضرة ضد مسببات الأمراض (***Bacillus subtilis*** ،***E- coli***) ، وكذلك الفعالية العلاجية للمادة البيولوجية.

في حالة المادة البيولوجية بشكل خاص ، أنسجة جلد السمك ، تم تقييم نشاط بعض الإنزيمات المضادة للأكسدة (انزيم الكتليز ،انزيم سوبراوكسايد ديسموتيز ، والكلوتاثيون بيروكسيديز).

حيث يتضمن دارسة البحث تقسيم أربعين سمكة إلى مجموعتين لإجراء بحث مختبري لقياس فعالية بعض الإنزيمات المضادة للأكسدة. تم استخدام عشرة منها كأسماك سليمة (غير مصابة بالتهاب) ، بينما أصيب ثلاثون سمكة بالتهاب الجلدوأخذ عشره منها وتم تقدير فعالية الانزيمات المضادة للاكسدة اعتبرت كمجموعة السيطرة .

تمت دراسة آثار العلاج البيطري و (Cu NPs) على مستويات كل إنزيم مضاد للأكسدة بعد أسبوعين من العلاج. عولجت عشرة أسماك بالأدوية البيطرية ، بينما عولجت العشرة الأخرى بـ (Cu NPs).

أشارت النتائج إلى تغير معنوي فعالية الانزيمات SOD و CAT و GPx في أنسجة جلد الأسماك المصابة مقارنة مع تلك الموجودة في الأنسجة السليمة.

عندما تم استخدام جزيئات النحاس النانوية لعلاج الالتهابات في الأسماك المصابة ، أظهرت النتائج زيادة كبيرة في فعالية كل إنزيم من انزيمات مضاد للأكسدة وكان أكثر فعالية من العلاج البيطري ($p \leq 0.05$) مقارنة بالأسماك السليمة.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل/ كلية العلوم
قسم الكيمياء

تأثير النحاس النانوي على بعض مضادات انزيمات الاكسدة والتقليل من الالتهاب في الانسجة

أطروحة مقدمة إلى مجلس
كلية العلوم / جامعة بابل وهي جزء من متطلبات نيل درجة الماجستير فلسفة
في علوم الكيمياء

من قبل
محمود علي عبد حنوف
بكالوريوس علوم كيمياء /كلية العلوم /جامعة بابل ٢٠٠٣

بإشراف

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الأستاذ
د.عوده ياسر مزعل الزاملي
كلية العلوم / جامعة بابل

ربيع الأول ١٤٤٣ هـ

كانون الأول ٢٠٢١ م