Republic of Iraq

Ministry of Higher Education

and Scientific Research

University of Misan

College of Science

Department of Chemistry

Biosynthesis of Zinc oxide Nanoparticles using *Capparis Spinosa* Plant extract and study of Biological activity

A Thesis Submitted to

The College of Science / University of Misan as Partial Fulfillment of the Requirements for the Master Degree of Science in Chemistry

By

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2023 A.D ***1445 H.D***

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الأنعام (99)

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We are the examination committee members, certify that we have read carefully this thesis entitled **(Biosynthesis of zinc oxide Nanoparticles using *Capparis spinosa* Plant extract and study of Biological activity),** examined the MSc. student **(Salma Aziz Neamah)** in its contents and in our opinion, it meets the standard of a thesis for the master degree in Chemistry with **(Excellent)** grade.

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Dedication

To The Sun and Moon Which Lighting my Life by Pave the Way to my Success…

My Father & My Mother

To Those who have Supported me and are Waiting for my Success…

My Brothers & My Close Friends

To Those who have Given me their Time and Knowledge…

My Supervisors

salma

Acknowledgements

Firstly, very much thanks for Allah the most merciful for his blessings, who gave me health, strength, and facilitated the ways for me to accomplish this work, and the prayer and peace of Allah be upon our Master and prophet Muhammad and his divine good family.

I would like to express my sincere gratitude to my supervisors, **Professor Dr. Salim Naamah Saleh** and **Assistant Professor Dr. Israa Qusai Falih** for highly inspiring guidance, patience, motivation, enthusiasm and immense knowledge, continuous support for completing this thesis.

Much appreciation is offered to the head of The Department of Chemistry,

Assistant Professor Dr.Tahsen Saddam Findi for help to complete my work.

I would like to express my sincere thanks to **Dr. Younus Jasim Abdullah**

Amara Medical technical Institute.

Also I would like to express my sincere thanks to **Prof. Dr Zahrah Adnan Alshammarri** Department of Biology/ University of Maysan .

finaly I express my sincere thanks and gratitude to my family for their help me in providing the appropriate ambiences for study .

Thanks to everyone who supported me.

Salma AL-musawi

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List of Abbreviations

Abbreviations	Key
ALT	Alanine transaminase
AST	Aspartate Aminotransferase
AKP	alkaline phenyl phosphatase
0D	Zero dimensional
1D	One dimensional
2D	Two dimensional
3D	Three dimensional
AFM	atomic force microscopy
ºC	Celsius degree
CYP	cytochrome P450 enzymes
CK	creatine kinase
DLS	Dynamic Light Scattering
DMEM	Dulbecco's Modified Eagle Medium
DRS	Diffuse Reflectance Spectroscopy
EDX	Energy Dispersion Analysis of X-ray
EDS	Energy Dispersion Analysis of X-ray
ELISA	The enzyme-linked immunosorbent assay
FESEM	Field Emission Scanning Electron Microscope
FTIR	Fourier Transform Infrared
GC-MS	Gas chromatography analysis spectroscopy
GST	glutathione S-transferase
KM	Michals constant
H	Hour
LDH	lactate dehydrogenase
IC50	the concentration of a particular drug
MHA	Müller-Hinton agar

nm	Nano meter
NPs	Nano Particles
NA	nutrient agar
NB	nutrient broth
PBS	Phosphate buffered saline
PDIs	polydispersity indexes
PLD	Pulsed laser ablative deposition
QR	Quinone reductase
RBCs	red blood cells
SEM	Scanning Electron Microscope
TEM	transmission electron microscopy
T	Temperature
t	Time
UDP	glucuronosyl transferase
UGT	UDP-glucuronosyl transferase
UV-Vis	Ultraviolet-Visible
VS	vapor-solid
VLS	vapor-liquid solid
V max	Velocity Maximum
γ-GT	γ-glutamyltransferase
XPS	X-ray photoelectron spectroscopy
XRD	X-Ray Diffraction
ZnO NPs	Zinc oxide nanoparticles
ppm	Part Per million
IU\L	International units per liter
µM	Micrometer
µg/mL	Microgram per milliliter
WHO	the World Health Organisation

Summary

In recent years, green chemistry has gained recognition as a viable approach for producing nanoparticles. This study aimed to explore the properties of zinc oxide nanoparticles (ZnO NPs) produced using *Capparis spinosa* L. (*C.spinosa* L.) fruit extract as a potent reducing agent. The investigation focused on various aspects, including cytotoxicity, antioxidant effect, hemocompatibility and antibacterial activities, as well as the potential inhibitory action on the Lactate dehydrogenase enzyme (LDH). The characterization of the aqueous extract of *Capparis Spinosa* L. and ZnO-NPs was confirmed using techniques such as Ultraviolet spectroscopy (UV-Vis), Fourier Transform Infrared Spectroscopy (FTIR), and Gas Chromatography-Mass Spectrometry Analysis (GC-MS). These analyses verified the presence of Bio active compounds, such as alkaloids, flavonoids, polyphenols, statins, and vitamins, in the aqueous extract. The ZnO-NPs were identified through X-Ray Diffraction (XRD), Dynamic Light Scattering (DLS), zeta potential (ζ), and Field Emission Scanning Electron Microscope (FESEM). The results indicated that ZnO-NPs were highly pure and crystalline, exhibiting agglomerated and spherical particles with an average size of approximately 37.49 nm. Additionally, UV-Vis maximum absorption wavelength 374 nm, while the ζ potential and DLS measurements for ZnO-NPs were -44.76 mV and 116 ± 8.0 nm, respectively. The study also assessed the antioxidant activity of ZnO NPs using the DPPH assay, as well as the hemocompatibility on erythrocytes and cell-cytotoxic properties through the MTT assay. ZnO NPs displayed good biocompatibility with red blood cells (RBCs) in the hemolytic activity experiment, with no observed hemolytic reaction at doses 7.5 to 120 g/mL. Furthermore, the extract and ZnO NPs exhibited antibacterial activity against *S.aureus* and *E.coli* . However, the concentrations of the aqueous extract demonstrated the ability to inhibit the

Lactate dehydrogenase enzyme at a concentration 0.13 mmol/L. In conclusion, the aqueous extract of *Capparis Spinosa* L. Fruit contains biologically active compounds that could be utilized as inhibitors of both antibacterial properties and the Lactate dehydrogenase enzyme (LDH). The greenly generated ZnO NPs displayed good inhibition processes in terms of antibacterial activity, antioxidant activity, and showed minimal cytotoxicity.

CHAPTER ONE

Introduction and literature review

1. Introduction

Nanotechnology is a recent development in scientific research. This field was founded by American physicist Richard P. Feynman at Caltech when he presented his lecture titled "There is plenty of room at the bottom," in 1959, he had suggested Scaling down to the nanoscale was the way of the future for technology and advancement [1]. The science of nanotechnology deals with understanding and manipulating materials at the nanoscale size, which has dimensions between 1 and 100 nanometers. Nanoparticles are materials having a diameter of less than 100 nm. They have high surface/volume ratios and are smaller than their bulk materials [2]. As the particle size decreases, the number of constituent atoms surrounding the surface of the particles increases. Highly reactive particles with unique chemical, optical, physical , and electronic properties develop as a result. Nanotechnology showed a rapidly expanding branch of technology that holds enormous potential for the chemical, medicinal, engineering, and food-processing industries [3-5]. This rapid development of nanotechnologies suggests that nanoscale production will soon be used in almost every area of science and technology. . Metal oxide nanoparticles such as Fe_3O_4 , AgO, Al_2O_3, MgO, ZrO_2, CeO_2, TiO_2, ZnO, Fe_2O_3, and SnO; are the most adaptable materials because of their wide range of characteristics and uses. Zinc oxide (ZnO), Nanoparticles has received a lot of interest from scientists as a 'future material' [6-10]. ZnO nanoparticles are an important class of metal oxide nanoparticles exhibiting exciting biological and photocatalytic properties due to their small size and enhanced surface chemical reactivity [11], However, the majority of conventional techniques for producing ZnO NPs are based on wet chemical route options, which demand the use of numerous hazardous chemicals during laborious, prolonged multistep processes that result in the production of vast quantities of hazardous by

products and dangerous chemical waste [12]. In recent years, the use of more sustainable green techniques for preparation has increased focus on replacing harmful traditional chemicals with more environmentally friendly extracts from various natural materials, such as plants, fungi, bacteria, and algae, in order to prepare ZnO NPs, which demonstrated higher and comparable activities compared to the conventional ones. For instance, *Eucalyptus, Phlomis, pomegranate, Syzygium cumini, Ziziphus,* etc. have all been used as plant part extracts for the green biosynthesis of ZnO NPs. [13-17], whereby plant extracts are utilized as capping agents and stabilizers to both maintain the generated nanoparticles' stability and prevent their aggregation. [18]. besides, ZnO-NPs produced in green methods is safe for the environment and have also been used for biomedical purposes such as antioxidant, antibacterial, and anticancer properties [19,20]. It is important to note that *C.spinosa L.*is one of the middle east region's most widely-used plant and is distinguished by a variety of medicinal properties and that there are numerous long chains of natural compounds present that could serve as capping and stabilizing agents and avoid the nanoparticles from aggregating [21,22].

1.1 Nanoscience

The prefix "nano," which derives from a Greek word meaning "dwarf" or "something very small," represents one billion of a meter (10^{-9}m). It is also a reference to a Greek prefix. There is a clear need to differentiate between nanotechnology and nanoscience. Nanoscience is the study of structures and molecules on the sizes of nanometers ranging from 1 to 100 nm, and the technology that utilises it in practical applications such as electronics, Nanomedicine etc is termed nanotechnology [23]. Nanoscience is an interdisciplinary field of research that focuses on the atomic, molecular, and subatomic levels of matter. Nanotechnology refers to the focused research and development that is done for the purpose of understanding, manipulating,

and measuring materials at the atomic, molecular, and supermolecular levels. The term "nanotechnology" refers to materials, systems, and processes that function at a size of one hundred nanometers (nm) or less, according to a tentative definition of the term. One billionth of a metre is equal to one nanometer[24] .As a point of reference, the visible spectrum of light has a wavelength that ranges from 400 to 700 nanometers. The size of a leukocyte is 10000 nm, the size of a bacterium is 1000-10000 nm, the size of a virus is 75-100 nm, the size of a protein is 5-50 nm, the width of deoxyribonucleic acid (DNA) is 2 nm, and the size of an atom is 0.1 nm. On this scale, as seen in Figure (1-1), the physical, biological, and chemical properties of materials have fundamentally distinct relationships to one another, and as a result, the activities of these materials are often surprising. The size of viruses and other infectious agents is taken into consideration by nanotechnology. Therefore, it has a significant potential to detect and eradicate infections [25].

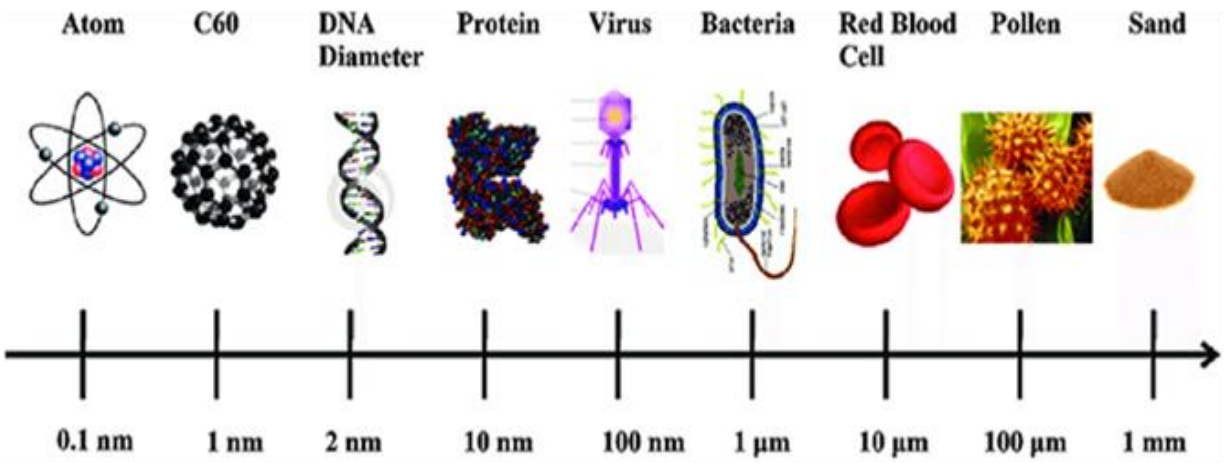


Figure (1-1) A basic concept on length scale that is showing size of nanomaterial's and their comparison to biological components and the definition of 'micro' and 'Nano' sizes[26].

1.2 Classification of Nanomaterials

Nanomaterials have classified into 4 types including extremely small size which having at least one dimension 100 nm or less as shown in Figure (1-2) .

1.2.1 Zero dimensional nanostructures

Materials in all the dimensions are measured within the nanoscale. (no dimensions, or 0-D, are larger than 100 nm). The most common representation of zero-dimensional nanomaterials is nanoparticles[27].

1.2.2 One dimensional nanostructure

Materials in one dimension are outside the nanoscale. This leads to needle likeshaped nanomaterials.1-D materials include nanotubes, nanorods, and nanowires[27].

1.2.3 Two dimensional nanostructures

Materials that were located in Two dimensions may be considered to exist outside of the nanoscale. Plate-like forms may be seen in two-dimensional nanomaterials. Nanofilms, nanolayers, and nanocoatings are all included in this category[27].

1.2.4 Three dimensional nanostructures

As a direct consequence of this, these materials are distinguished by possessing three arbitrary dimensions that are more than 100 nm. Nanocrystalline structures or the existence of features on the nanoscale are characteristics of some materials. In terms of their nanocrystalline structure, bulk nanomaterials may be made up of a numerous arrangement of nanosize crystals, with many of these nanosize crystals often arranged in a variety of orientations. In terms of the existence of characteristics on the nanoscale, three-dimensional nanomaterials may include multinanolayers, dispersions of nanoparticles, bundles of nanowires and nanotubes, and other nanoscale structures[27].

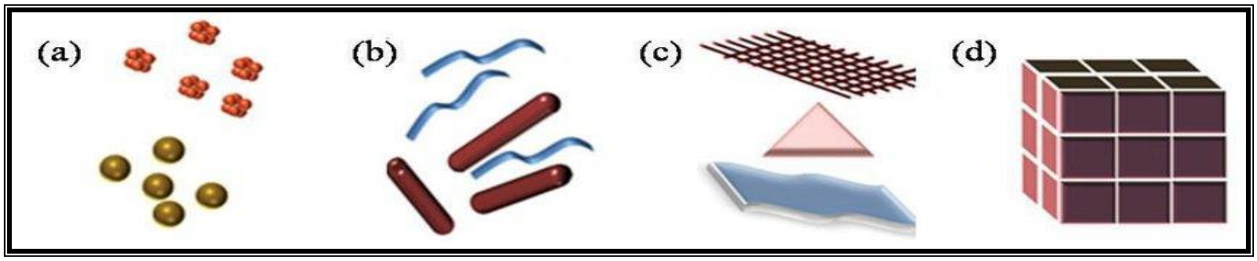


Figure (1-2) Classification of Nanomaterials (a) 0D clusters and spheres, (b) 1D nanofibers, rods, and wires, (c) 2D films, networks, and plates,(d) 3D Bulk Nanomaterial[27].

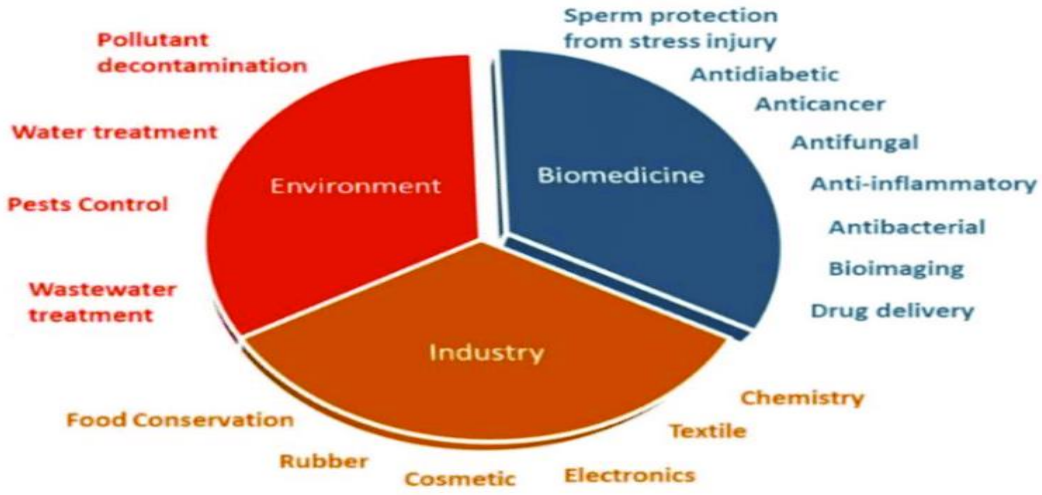
1.3 Zinc Oxide Nanoparticles (ZnO NPs)

ZnO is regarded as an inorganic material that is versatile, advantageous, helpful, and adaptable, and it has a wide range of potential uses. The other term for it is semiconductor [28]. On the periodic chart, zinc and oxygen are elements belong to group two and six, respectively. ZnO is a material that has a variety of unique features, including optical, chemical sensing, semiconducting, electric conductivity, and piezoelectricity [29]. Zinc oxide Nanoparticles ZnO NPs, possess exceptional chemical and physical characteristics, which explains why they utilized in a wide variety of sectors, among the most significant ones [30, 31]. Since ZnO NPs makes the rubber composite more water resistant, the rubber industry is the first sector to make use of these nanoparticles since they are the most significant. In addition to offering increased strength, ferocity, and anti-aging properties, it boosts the effectiveness of high polymer [32,33]. Because of its high and powerful UV absorption characteristics, zinc oxide (ZnO) is used in a variety of personal care and beauty products such as sunscreens and cosmetics [34]. In addition, ZnO NPs have good antibacterial and antimicrobial characteristics, It is generally believed that ZnO NPs are responsible for the remarkable functions of UV and visible light resistance, antibacterial and deodorant qualities in finished fabric in the textile sector [35]. Because of its little size, nano-ZnO is readily absorbed by the body; hence, ZnO is now

being added to a wide variety of foods. In comparison to other types of metal oxide, zinc oxide has a lower cost, more biocombinatoric potential, greater biocompatibility, and lower risk of harmful and toxic effects. These metal oxides are classified according to their capacity for photolysis and photooxidation in relation to various chemical and biological species [36]. A variety of analytical methods, such as ultraviolet visible spectroscopy, Fourier transform infrared spectroscopy (FT-IR),X-ray diffractometry (XRD), dynamic light scattering (DLS),transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), and atomic force microscopy (AFM), are utilised in the process of identifying the synthesised products [37][38].

1.3.1 Application of ZnO Nanoparticles

Zinc oxide has a wide variety of characteristics, both chemical and physical. It has widespread use across a variety of domains (Figures 1-3). ZnO is an essential component in a wide variety of features, including the following[39].



Figure(1-3) Applications of ZnO nanoparticles in biomedicine, industry, and environment [40].

1.3.2 Synthesis of ZnO Nanoparticle Methods

Prior to the development of nanoscience, it was challenging to synthesise nanomaterials using an easy, affordable, and highly effective process. Three procedures, known as the solid-phase, liquid-phase, and gas-phase processes, are used to create nanoparticles. Solid-phase processes include mechanical ball milling and mechanochemical methods; liquid-phase processes include laser ablation, exploding wire, solution reduction, and decomposition; and gas-phase processes include gas evaporation, exploding wire, laser ablation, and green synthesis. Additionally, chemical, physical, and biological methods may be used to create ZnO NPs[41,42].

1.3.2.1 Physical Methods

Vapour deposition, plasma, and ultrasonic irradiation are the physical processes that may be used to create ZnO NPs. These procedures are thought to be expensive and aren't widely employed in manufacturing since they demand heavy machinery and a lot of energy[43].

1.3.2.1.1 Laser Ablation Method

Laser cutting Pulsed laser ablative deposition (PLD), which produces nanoparticles with a limited size distribution and low levels of contaminants, is a clear synthetic technique. Nanoparticle production by an objective submerged in liquid and laser ablation synthesis both include three key processes[44]. Yousitake Masuda and colleagues discovered that a simple aqueous solution approach may be used to modify the shape of ZnO crystals in the liquid phase. At a temperature of 50 °C, ZnO nanowires with dimensions of 50 nm in width and 100 nm in length were successfully synthesised. The created nanowires lacked branching and aggregations [45].

1.3.2.1.2 Vapor Transport Method

The most prevalent technique for creating ZnO nanoparticles is the vapour transport approach. It may be ignited using either a catalyst-free vapor-solid (VS) technique or a catalyst-assisted vapor-liquid-solid (VLS) process, depending on how nanostructures are formed. A broad variety of nanostructures, including nanowire, nanorods, and nanobelts, may be produced naturally by the VS process. According to Kong etal. and colleagues, ZnO nanostructures such nanohelixes and nanobelts have a belt form with a width and thickness of 10-60nm and 5-20nm, respectively, and a length of several hundred micrometres [46]. In this intricate process, excited vapours of zinc, oxygen, and oxygen interact with one another to make ZnO nanoparticles. There are several methods for creating vaporised Zn and oxygen. Even though ZnO breakdown is limited to high temperatures, it is a straightforward process. In the VS technique, nanostructures are directly created by condensing from the vapour phase[47].

1.3.2.2 Chemical Methods

Chemical procedures include precipitation, microemulsion, chemical reduction, sol-gel, and hydrothermal processes. They may need a lot of energy if the pressure or temperature is high [48]. The most significant of the common chemical techniques is sol-gel synthesis, which was created by Spanhel and Anderson in 1991. Sol-gel synthesis makes use of a zinc precursor salt and a chemical reagent to control the pH of the solution and prevent the precipitation of $Zn(OH)_2$. After that, the solution is heated to 1000 °C to produce ZnO NP [49]. Chemical stabilisers such polymers or citrates like polyethylene glycols, polyvinyl pyrrolidone, and amphiphilic block copolymers are added to the ZnO NPs manufacturing practical to regulate nanoparticle size and prevent particle agglomeration [50]. Additionally, the concentration of chemicals used in chemical synthesis is an important factor

that has a significant impact on the size and shape of the particles produced. By varying the concentrations and ratios of the chemicals used, it is possible to produce particles with sizes ranging from nanometers (5–10 nm) to micrometers [51].

1.3.2.2.1 Sol Gel Method

The sol-gel synthesis technique was developed to create inorganic compounds using a particular solution's chemical reaction. Due to its good level of thermal stability, high mechanical stability, reasonable solution resistance, and propensity to agitate transformation, the sol-gel technique is beneficial. Agustinaa et al. successfully employed the sol-gel and calcination procedures for the production of ZnO NPs. The structure and particle size of the nano ZnO created in these conditions may be used to identify it[52]. The findings indicated that ultrasonic duration of 60 minutes and pH of 10 were realised as the ideal conditions for nanozinc oxide formation. The 45.35 nm-sized synthesised nano zinc oxide crystal displays a uniform shape. Nano zinc oxide has a size of roughly 50 nm and an 87.31% zinc content. The morphologically homogenous ZnO crystals made using these techniques have an approximate size of 45.53 nm. The pH for this manufacturing is around 10, and the production uses ultrasonic technology for 60 minutes [53].

1.3.2.2.2 Hydrothermal Method

The hydrothermal approach is simple, uncomplicated, and environmentally benign since it doesn't need the use of organic solvents or extra product processing (calcination and grinding). This synthesis method takes place in an autoclave, where a combination of substrates is slowly heated to a range of temperatures between 100 and 300 °C and let to cool over several days[54]. Crystal nuclei are created by progressively heating, cooling, and growing. This process is advantageous because it allows for the

synthesis to be done at low temperatures. The advantages of this process depend on the initial mixture, temperature, and pressure of the mixture, which will produce various distinct shapes and dimensions of the crystal. Nehal A. Salahuddin and colleagues created zinc oxide nanotubes using hydrothermal synthesis using zinc nitrate as a precursor. ZnO nanotubes were found to be 2.4 nm long and had an outer diameter of 200 nm on average [55,56].

1.3.2.2.3 Precipitation Method

Controlled precipitation is often employed to produce zinc oxide, usually in order to provide an output with predictable qualities. In order to stop the formation of particles larger than a specific size, a reducing agent is used to quickly and spontaneously reduce a zinc salt solution. This causes a precursor to zinc oxide to precipitate out of the solution. The precursor is thermally processed before being ground to remove contaminants[57]. It is challenging to swallow the calcined powders because of their high degree of agglomerates of particles. Temperature, pH, and the timing of the precipitation process are all factors that affect the precipitation process. The solution of zinc oxide is also precipitated from aqueous solutions of zinc chloride, zinc sulphate, and zinc acetate.The reagent concentration, the pace at which substrates are added, and the reaction temperature all play regulating roles in this procedure[58].

1.3.2.3 Biological Methods

The utilisation of biological approaches for synthesising ZnO NPs has received a lot of attention during the last ten years. The discovery of this novel technique is noteworthy and of primary interest since biological synthesis is both economical and environmentally beneficial due to the lack of harmful chemicals or high energy requirements [59]. According to the

literature, biological production of metallic and metal oxide nanoparticles is more environmentally or ecologically friendly than the usual chemical or physical procedures currently in use. As a result, the term "green synthesis" is often used to describe these biological synthetic processes[60].

1.3.2.3.1 Synthesis of ZnO Nanoparticles by Bacteria

The production of metal or metal oxide nanoparticles may take place in extracellular or intracellular environments by using microbial culture or biomass. Studies have shown that the enzymes and proteins that microorganisms make and release may decrease metal ions and stabilise the particles in the case of extracellular production[61]. ZnO nanoparticles may be stabilised by the enzyme secretions produced by Bacillus licheniformis bacterial cells. Zinc acetate and sodium bicarbonate react to make $Zn(OH)_2$, which is subsequently thermally decomposed to yield ZnO nuclei. The ZnO NPs are stabilised by enzymes provided by bacteria, preventing agglomeration and particle growth because of the metal oxide's tiny size[62]. Furthermore, it has been shown that microorganism-produced enzymes cause ZnO NPs to form. However, authors claim that the pH of the solution and the electrokinetic potential of the bacteria may influence the synthesis route by reducing the metal ions and subsequently activating the biosynthesis of the nanoparticles rather than forming $Zn(OH)_2$[63]. The same method was described in a similar work, in which Staphylococcus aurens produced ZnO NPs extracellularly. Additional research's has reported on the effective use of activated ammonia from Serratia ureilytica for the manufacture of ZnO NPs. According to the research, the production of $Zn(OH)_2$ and $[Zn(NH3)_4]_2$ caused by the interaction of Zinc (II) ions with rich culture medium of microorganisms and ammonia. The crystalline ZnO NPs powder is produced by thermal breakdown of these materials at a temperature of 50 C° [64]. Due

to the complexity of cell components and processes, the method of nanoparticle creation for intracellular synthesis is extremely demanding. However, several studies suggest that the metallic ions are incorporated into the cells and reduced by the proteins and enzymes to create the nanoparticles [65].

1.3.2.3.2 Synthesis of ZnO Nanoparticles by Fungi

Similar to the green synthesis, which uses bacteria to biosynthesize zinc oxide nanoparticles (ZnO NPs), the creation of metal and metal oxide nanoparticles by fungal biomass or culture follows a similar synthetic pathway [66]. However, in fungal biomass, Aspergillus fumigatus cell culture is employed to synthesise ZnO NPs. According to research, Aspergillus fumigatus proteins and enzymes are primarily responsible for the production and encapsulation of nanoparticles. When compared to bacterial cells, the fungus has a far better capability for secreting greater concentrations of metabolites into the medium culture, making it preferable to green synthesis. Additionally, fungal cells are more resistant to the subtleties and fluctuations of the synthesis process, such as pressure, flow rate, and stirring, which increases their potential for usage on a broad scale [67].

1.3.2.3.3 Synthesis of ZnO Nanoparticles by Algae

Despite being a very basic organism, algae's phytochemical makeup is exactly the same as that of plant extract. Numerous active chemicals with functional groups like hydroxyl and carboxyl groups are identified in different species of algae, and their antioxidant activity may also be calculated [68]. The existence of these active chemicals in algal extract is confirmed by Gas chromatography analysis spectroscopy (GC-Mass),Fourier Transform Infrared Analysis (FTIR), which uses them as a substrate to produce green ZnO NPs. Therefore, the biosynthesis method of ZnO NPs by

algal substrates is the same as the mechanism in plants, which uses active chemicals such flavonoids and polyphenols as reducing and chelating molecules or stabilising agents[69] .

1.3.2.3.4 Synthesis of ZnO Nanoparticles by Plants

They are employed for the synthesis of ZnO NPs due to the distinctive phytochemical production of plant parts such stem, leaf, fruit, root , and seed. Utilising natural extracts from plant parts is cost-effective, environmentally beneficial, and sustainable since no intermediary base groups are included. It saves time, doesn't need heavy gear or equipment, and produces a large amount of extremely pure, impurity-free output [70]. NPs plants are a highly preferred source of NPs production due to their extensive production and stable, diversified in form and size [71]. Phytochemicals released by plants, such as polysaccharides, ,amino acids, polyphenolic compounds, vitamins, alkaloids and terpenoids, may convert metal oxides or metal ions to Neutral valence metal nanoparticles in a process known as bioreduction [70, 71].

1.4 Important of Green Synthesis

The traditional techniques of making nanoparticles were inefficient in terms of cost and required the use of hazardous chemical compounds or organic solvents as reducing agents, which explain why green nanoparticle synthesis methods have became under scrutiny recently [72]. Green synthesis is also environmentally beneficial and reduces the danger of contamination at the source or begetter scale. It also produces no waste, thus there is no need to scavenge garbage after the synthesis. The use of environmentally and ecologically friendly reagents were given top priority in green synthesis. Despite the fact that physical and chemical techniques are quick and simple for the synthesis of nanoparticles, as illustrated in Figure(1-4), the biological

approach of synthesis has an advantage over them since it is environmentally safe and non-hazardous [73]. The production of new sources of dynamic materials that are nontoxic, stable, nonhazardous, affordable, eco- and environment-friendly occurs during the synthesis of nanoparticles employing biological organisms in green synthesis. Due to their smaller size, unique structure, and specific biological substrate features utilised in their green production, nanomaterials' properties are heightened[74].

Figure(1-4) Green Synthesis Advantages[75]

1.5 *Capparis Spinosa*

Caper (*Capparis spinosa L.*) is a perennial shrub of the family Capparaceae, endemic to circum-Mediterranean countries, Iran and in the south of Iraq And called in Iraq Shafallah plant [76]. *Capparis Spinosa L.* is a famous medicinal herb; it has traditional use, possesses a nutritional value and obvious benefit. It distinguished by containing vitamins and antioxidants compounds such as flavonoids and alkaloids [77], also has several properties: anti-bacterial, antifungal, anti-inflammatory and anti-hepatotoxic actions [78]. Report refer to that the (ether, methanol, ethanol, hexane, and aqueous) extracts of aerial parts have antifungal, antibacterial, and antiviral activities [79]. Besides, these extracts do not have an anti-environmental effect that gives them a great advantage to be used as insecticides against plant diseases, which has negatively; affect the environment and human health [80]. Added to this, essential oils in the composition of such plants act as antifungals and antiviral [81]. The literature indicates that most of the studies focus on the leaves and roots. In present study, we are highlighting on the fruits, so their phytochemical content and therapeutic potential were examined[82].

The methanol fruit extracts and leaf of capers are reported to have a considerable hepatoprotective effect [83]. Caper extracts contain anti-hepatotoxic action. Phase I detoxification enzymes like cytochrome P450 enzymes (CYP) and phase II detoxification enzymes like glutathione S-transferase (GST), quinone reductase (QR), UDP-glucuronosyl transferase (UGT), amino acid transferases, N-acetyl transferases, and methyl transferases may be increased, which may stop the progression of liver damage [84]. Other liver enzymes including ALT, AST, AKP, γ-glutamyltransferase (γ-GT), and lactate dehydrogenase (LDH) that released in response to injury or illness may also be decreased by caper extracts [85].

Medical plants have been used as therapeutic agents for the management of health and treatment of ailments since ancient times because they have health-promoting properties and contain bioactive components [86]. 80 percent of people worldwide utilise traditional medicine, according to the World Health Organisation (WHO). According to estimates [87], traditional Chinese medicine formulations make up 30–50% of all medications consumed in China. About 90% of people in Germany reported using natural remedies for a variety of health concerns [88].

As a consequence, traditional medicine is growing increasingly well-liked in both developing and non-developed nations, indicating that the traditional medicine market is still robust globally. The market for herbal medicines is worth more than $60 billion annually and is continuously expanding. Because of this, medicinal plants like *Capparis spinosa (C. spinosa*) continue to be crucial in the healthcare industry [89].One of the Capparidaceae family's most commercially significant species, which includes around 40–50 genera and 700–950 species, is *C. spinosa*. The Brassicaceae (Cruciferae) family, which is rich in glucosinolates and flavonoids, and the Capparidaceae family are closely related. In China, *C.spinosa* is also known as caper or wild watermelon [90].

C.spinosa possess therapeutic properties, it was used by the Sumerians, Greeks, and Romans throughout history. The fruit and root of the caper are employed as antirheumatics, diuretics, tonics, and astringent in Iranian traditional medicine, but the fresh aerial parts of the caper are widely used in the culinary world [91] . These portions are used as a condiment in Mediterranean countries after being preserved in vinegar or an acidic brine [92] , where the flower buds are one of the most popular spices due to their bitter and spicy flavors [93] . The existence of various chemical families with extremely interesting biological effects was discovered in phytochemical analyses of capers extracts. Alkaloids, fatty acids, flavonoids, phenolic acids,

aldehydes, esters, vitamins, and glucosinolates are examples of these compounds demonstrated that the aqueous extract of *Capparis spinosa* possesses antifungal properties[94] . The impact of polyphenol-rich foods, such as fruits and vegetables, on human health has received more attention in previous studies [95] , where Polyphenols have been demonstrated to offer some potential health benefits, and they are abundant in foods consumed by people all over the globe. *Capparis spinosa (C. spinosa*) is a significant source of a variety of secondary metabolites of human interest. *C. spinosa's* traditional medicinal uses have been documented from the time of the Ancient Romans. Several bioactive phytochemical compounds have been extracted and discovered from various portions (aerial parts, roots, and seeds) of *C. spinosa* that are responsible for its varied pharmacological effects either alone or in combination. As a result, this work offers a review of literature on *C.spinosa's* phytochemical and pharmacological qualities. There isn't enough proof.

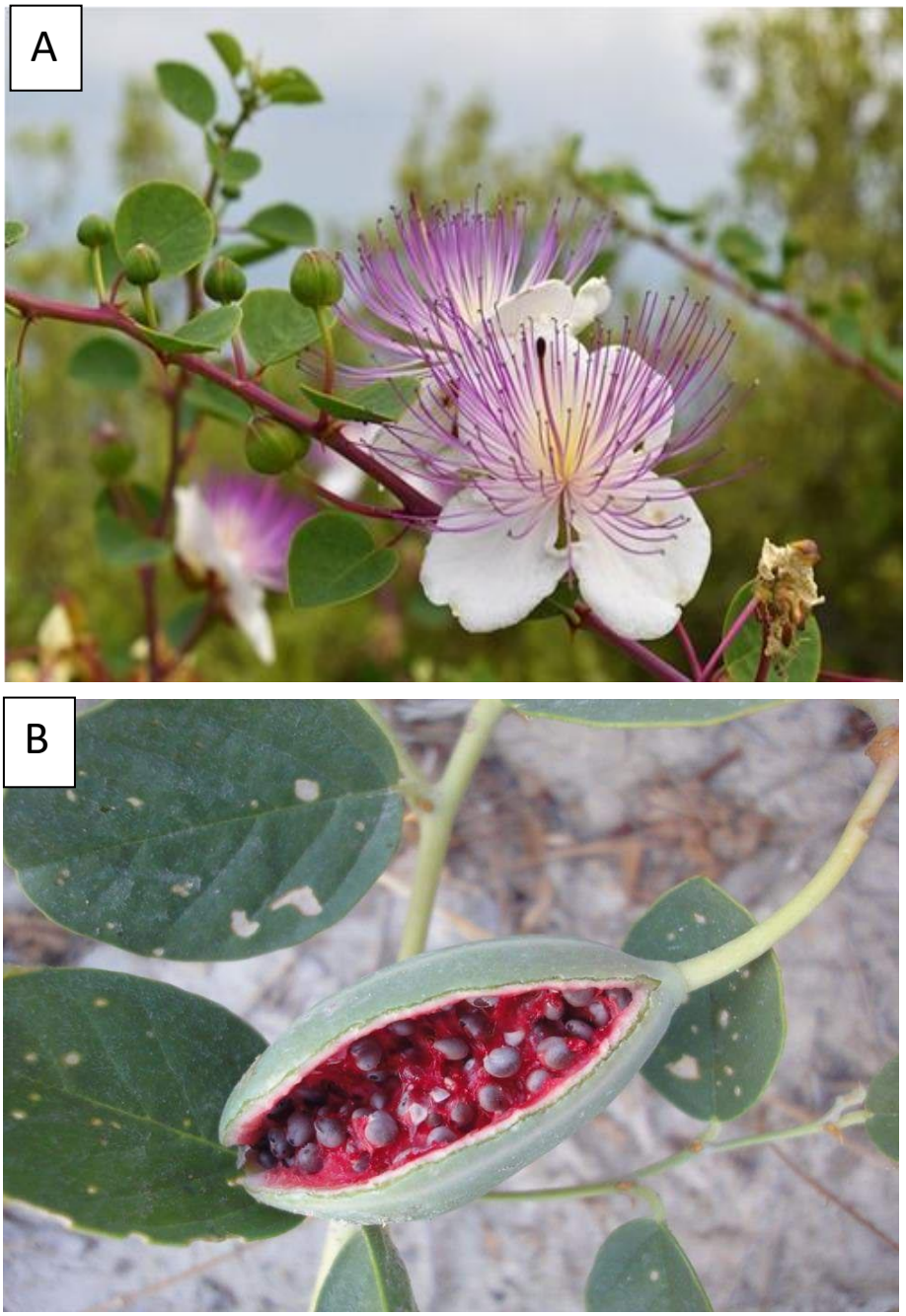


Figure (1-5) A- the plant and B- fruit part of *C. spinosa* [96].

1.6 Free Radicals and Antioxidant of *Capparis Spinosa*

Free radicals is an atom, a molecule, or an ion that contains one or more single electrons in its outer orbit, and they are very unstable Species , so they need an electron to become stable, and they have high and intense energy, which makes them interact at high speed with stable compounds to be a more stable form [97] . Free radicals are formed naturally in the body through the metabolic activities of cells through the chain of transmission of electrons in the mitochondria, increase in concentrations of free radicals than the normal limit causes the emergence and development of many diseases such as cancer, heart disease, infertility ,lung diseases, high blood pressure and thyroid diseases [98], and Free radicals have the ability to oxidize biomolecules in cell membranes such as nucleic acids, fats, proteins and carbohydrates, which causes change in the composition of the cell, which leads to the apoptosis as shown in Figure(1-6) [99].

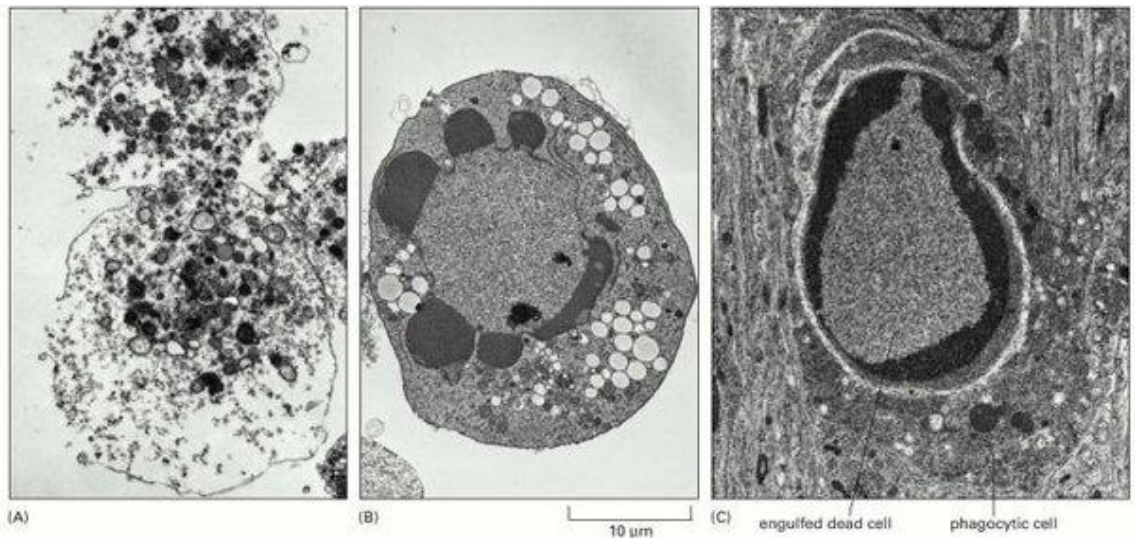


Figure (1-6) Programmed Cell Death (Apoptosis)[99]

On the other hand, some researchers found that the presence of free radicals in low concentrations is beneficial. The body has many mechanisms where

free radicals act as signals to stimulate several enzymes such as catalase as well as its role in immune defense mechanisms against germs and regulation of cell growth [100,101] .

There are some mechanisms and factors can inhibit the activity of free radicals (acts as a defense system against oxidative stress), among these factors are antioxidants [102]. The harmful effects result from the interactions of free radicals, as studies have shown, the use of antioxidants reduces the risks of free radicals. Oxidative stress increases in the case of a decrease in antioxidants resulting increase free radicals [103] .In natural cases, antioxidants have the capacity to reduce free radicals' efficacy and remove them in the event of their formation by giving them an electron when interacting with free radicals and transforming the most toxic unstable compounds into stable less toxic compounds [104] shown in the figure (1-7)

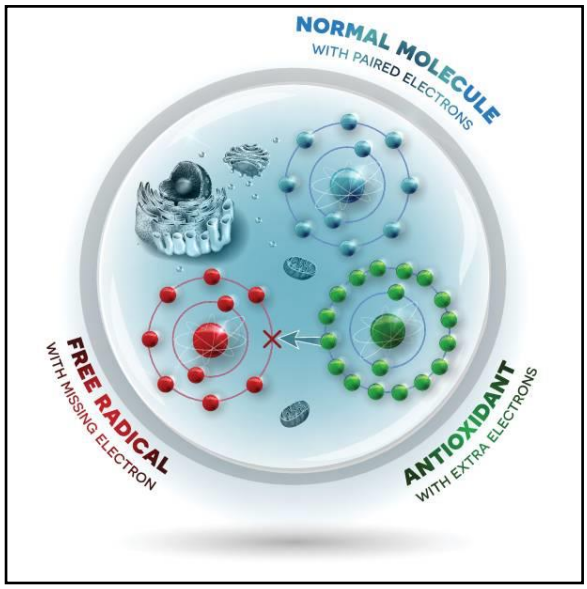


Figure (1-7) Antioxidants have the power to reduce the effect of free radicals[105]

Various extracts of *C. spinosa* have been demonstrated variety of health benefits including antidiabetic and hypolipidemic properties [106]. The ethanolic extract of *C. spinosa*, for example, has been suggested as a treatment for oxidative stress pathological disorders after showing notable antioxidant/free radical scavenging activity in diverse models, has been proposed as a therapy for oxidative stress-related pathologies [107] . Previous research on the chemical composition of several extracts from *C. spinosa* has revealed the existence of numerous beneficial chemicals known for their antioxidant characteristics, such as polyphenols, flavonoids, and vitamins [108][109] previous study have shown the use of the extracts of the *C. spinosa* plant as an antioxidant in several ways of extraction for the different parts of the plant. The results of these studies varied, because the plant parts contain varying amounts of components that are considered antioxidants, the most important are vitamin E and its derivatives, as well as vitamin C [110].

1.7 Enzyme Inhibitor and Anti-bacterial of *C.spinosa*

Enzymes are proteins that speed up chemical reactions necessary for life, in which substrate molecules are converted into products. An enzyme facilitates a specific chemical reaction by binding the substrate to its active site, a specialized area on the enzyme that accelerates the most difficult step of the reaction. Enzyme inhibitors are molecules that interact with enzymes (temporary or permanent) in some way and reduce the rate of an enzyme-catalyzed reaction or prevent enzymes to work in a normal manner. [111]. Reversible and irreversible inhibitors are chemicals which bind to an enzyme to suppress its activity. One method to accomplish this is to almost permanently bind to an enzyme. These types of inhibitors are called irreversible. However, other chemicals can transiently bind to an enzyme, these are called reversible. Reversible inhibitors either bind to an

active site (competitive inhibitors), or to another site on the enzyme (non-competitive inhibitors)As demonstrated in Figure (1–8) [112,113].

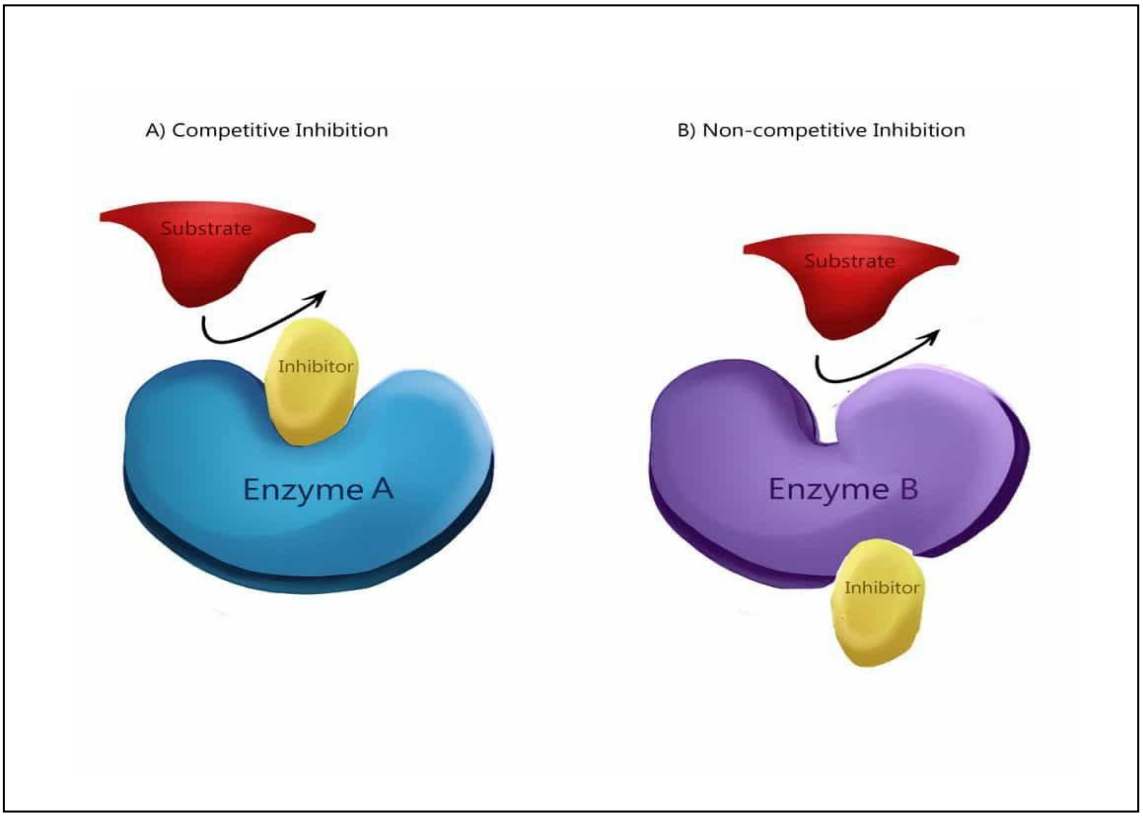


Figure (1-8)Diagram illustrating the impact of enzyme inhibitors that are competitive and non-competitive[113].

Anti-bacterial medications are used to prevent the spread of disease-causing germs. It is able to ward against the sickness and eliminate microorganisms. In order to treat the condition, several antibiotics derived from plants and other chemicals have been produced. Bacteria are the infectious agents that contribute to the development of toxicity [114,115].

It was discovered in earlier investigations that an extract of *C.spinosa* L. with ethyl acetate demonstrated antioxidant activity. *C.spinosa* L. is responsible for a number of pharmacological actions. The lactate dehydrogenase (LDH) and creatine kinase (CK) activities were reduced

when the ethyl acetate extract of *C.spinosa* L was used. This extract was shown to be protective against the harmful effects of doxorubicin on the heart. adjusted the energy metabolism of myocardial tissue, inhibited the generation of a large number of reactive oxygen species in the cells, increased the recovery activity of antioxidant enzymes, increased the ability of myocardial tissue to scavenge free radicals, and improved the metabolism of free radicals [116]. *C.spinosa* has been extensively studied in the control of blood glucose [117]. Several clinical studies have evaluated the chronic effects of *C. spinosa* and *C. spinosa* extracts in subjects suffering from type 2 diabete [118]. *C.spinosa* which was commonly used as a medicinal plant contained many biologically active chemical groups including, alkaloidsglycosides, tannins, phenolic, flavonoids, triterpenoids steroids, carbohydrates, saponins and a wide range of minerals and trace elements. It exerted many pharmacological effects including antimicrobial, cytotoxic, anti-diabetic, anti-inflammatory, antioxidant effect and many others[119]. Flavonoids, the most abundant secondary metabolites in fruit and vegetables, have been shown to interact with cellular signal pathways controlling the proliferation, differentiation and apoptosis of various tumour cells, including those derived from the alimentary tract[120].

Flavonoids are one of the most diverse polyphenolic natural products[121]. Many research have been done to identify and quantify the flavonoids that may be found in *C. spinosa* due to the fact that it has a high concentration of flavonoids. Compounds belonging to the flavonoid family, such a quercertin and rutin, have been identified in *C.spinosa* [122]. Alkaloids are a diverse group of secondary natural metabolites containing one or more nitrogen atoms in their structure[123].

Fruits of *C.spinosa* contain a significant amount of compounds with many health benefits. Three new alkaloids, capparisine A (1), capparisine B (2), capparisine C (3), and two known alkaloids, 2-(5- hydroxymethyl-2-

formylpyrrol-1-yl) propionic acid lactone (4), and N-(30 -maleimidy1)-5-hydroxymethyl-2-pyrrole formaldehyde (5) were isolated from the fruits of C. spinosa L[124]. The main components of root are capparispine, cadabicine 26-O-β-D-glucoside, capparispine 26-O-β-Dglucoside, and stachydrine, seeds contain glucocapparin [125].

The literature indicates that most of the studies focus on the leaves and roots. In present study, we are highlighting on the fruits, so their phytochemical content and therapeutic potential were examined. In our study Fruit aqueous extract of the *C. Spinosa* L. contains some compounds (2,2-'Di hydroxyl-4',6'-di methoxy chalcone), Glafenin , Acetildenafil and 6,7-Di Methyl tetra hydropterin as shown in Figure(1-9).

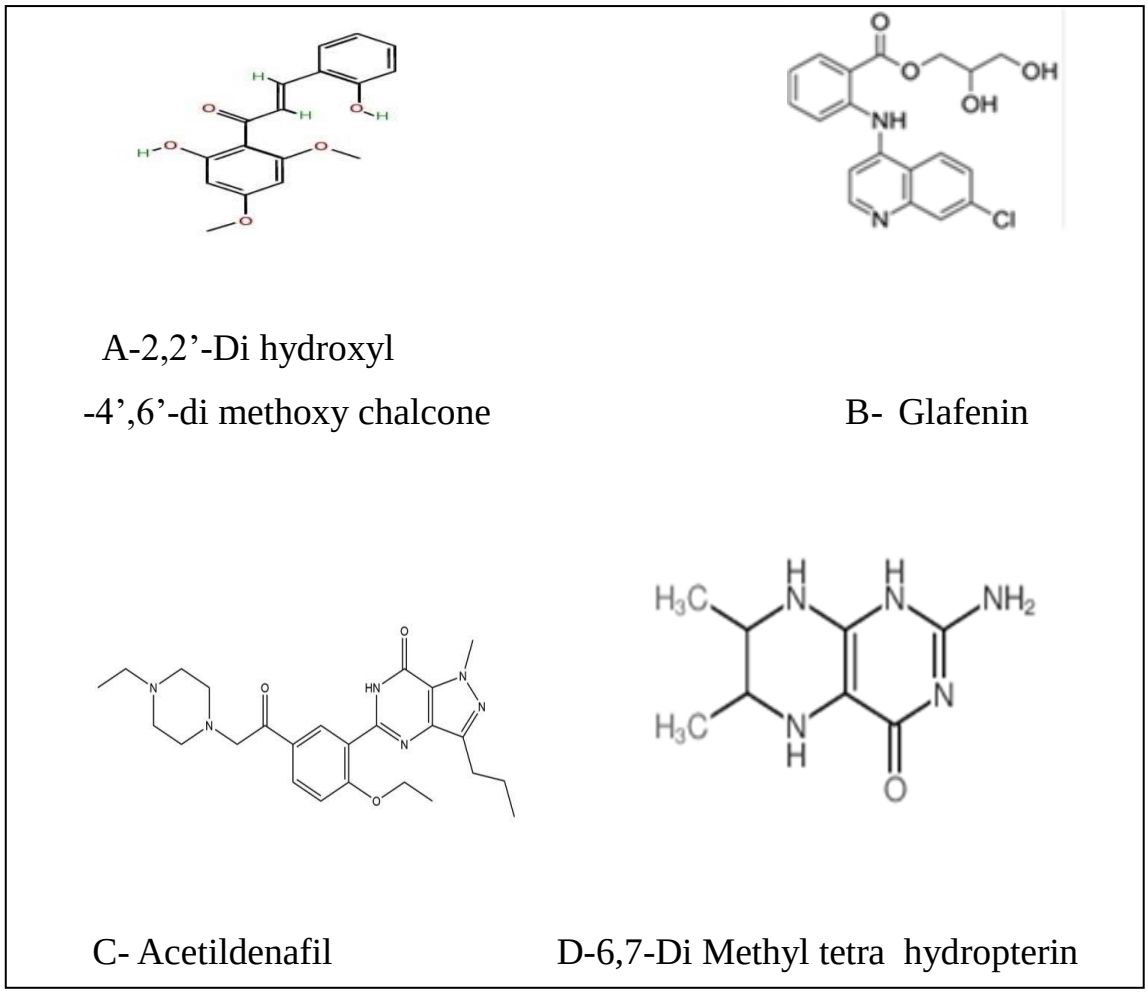


Figure (1-9) A- Flavonoid and B,C,D – alkaloids[125]

The Aims and Objectives of Study:

1. To Synthesise high-quality zinc oxide (ZnO) nanoparticles using only environmentally friendly methods, employing an extract from *C.spinosa* L. as a powerful chemical reduction agent .

2. Using different techniques such as Ultraviolet spectroscopy (UV-Vis), Fourier Transform Infrared Spectroscopy (FTIR), and Gas Chromatography-Mass Spectrometry Analysis (GC-MS). The ZnO-NPs were identified through X-Ray Diffraction (XRD), Dynamic Light Scattering (DLS), zeta potential (ζ), and Field Emission Scanning Electron Microscope (FESEM).

3. Study of Biological activities, cytotoxicity (MTT), hemolysis, antioxidant (DPPH), antibacterial and Lactate Dehydrogenase (LDH) inhibition effect .

CHAPTER TWO

Materials and Methods

2- Materials and Methods

2.1 Equipment:

The devices listed in Table (1-2) were used in conducting the experiments of current study:

Table (2-1): Devices

Ser	Devices	Company	Origin
1	Bath sonicator, WHC- A10H	Daihan Scientific	China
2	Centrifuge	Hettich-D-78532	Germany
3	Dynamic light scattering (DLS) and zeta potential	Malvern	UK
4	Field emission scanning electronic microscope (FESEM), 5 KV	TESCAN	USA
5	FT-IR spectrophotometer, FTIR-8400S	Shimadzu	Japan
6	Furnace	Carbolite	UK
7	Gas chromatography Mass analysis spectroscopy GC-MS	YL	Korea
8	Hotplate Magnetic Stirrer	medilab	Korea
9	Incubator	Binder	USA
10	Incubator/CO_2	BioBase	China
11	pH meter, pH 7110	InoLab	Germany
12	UV-Vis spectrophotometer, UV-1800	Shimadzu	Japan
13	Oven	Memmert	Germany
14	X-ray diffraction (XRD), LabX-XRD6000	Billerica, Ma,	USA
15	The enzyme-linked immunosorbent assay (ELISA)	Thermo Scientific	USA

2.2 Chemicals

All materials and chemicals, with their company and origins , used in this study are listed in table (2-2):

Table(2-2) Chemicals

Ser	Chemicals	Molecular formula	Company\ origin
1	Ammonia solution	NH_4OH	Fisher Chemical.U.K
2	Ascorbic acid	$C_6H_8O_6$	Sigma-Aldrich.U.K
3	Deionized water	H_2O	Sigma-Aldrich.U.K
4	Dimethyl Sulfoxide (DMSO)	$(CH_3)_2SO$	Sigma-Aldrich.U.K
5	2,2-diphenyl-1-picrylhydrazyl (DPPH)	$C_{18}H_{12}N_5O_6$	Sigma-Aldrich.U.K
6	Iodine	I	Sigma-Aldrich.U.K
7	Ferric Chloride	$FeCl_3$	Sigma-Aldrich.U.K
8	Sodium Hydroxide	NaOH	BDH. England
9	Sodium Decyl Sulfate (SDS)	$NaC_{12}H_{25}SO_4$	Sigma- Aldrich.U.K
10	MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide	Sigma-Aldrich.U.K
11	Methanol	CH_3OH	Sigma-Aldrich.U.K
12	Potassium Iodide	KI	Sigma-Aldrich.U.K
13	Phosphate Buffered Saline(PBS)	$C_{l2}H_3K_2Na_3O_8P_2$	Sigma- Aldrich.U.K
14	Lactate Dehydrogenase(LDH) Assay kit		BIOLABO.France
15	Zinc acetate dihydrate	$Zn(CH_3COO)_2.2H_2O$	Sigma-Aldrich.U.K

2.3.Plant collection

C.spinosa L. was collected from Ali Algharbi town of Maysan Governorate Iraq, during the period from April and June 2022 The plant was classified in the Biology department of Science college at the university of Misan. The extract of *C.spinosa L.* fruits was performed according to the previous study [126] with a few modifications. The *C. spinosa* plant's fresh fruits were collected, washed with tap water, and then washed again with deionized water. After that, the fruit samples were left to dry. In a pristine electric blender as shown in the figure(2-1), the dried fruits were ground into a fine powder. After that, 100 mL of deionized water was added to 40 g of the powdered dried fruits, which was then boiled for two hours at 90 °C while being magnetically agitated. The solution of extract was filtered using sterile Whatman No. 1 filter paper after cooling to room temperature and stored at 4 °C for further analysis.

Figure (2-1) Dry powder of extract fruit *C. spinosa*

2.4. Qualitative Detection of Bioactive Compounds:

The following tests have been used to detect Bioactive compounds .

2.4.1. Phenols test:

Add (1-2) a drops of 1% $FeCl_3$ solution after dissolving (0.1 g) of the sample in (1) ml of purified water. The presence of phenols is indicated when the colour becomes blue or green, which is a good sign[127].

2.4.2. Detection of Flavonoids test:

When a yellow deposit forms after adding 1 ml of the Alcoholic potassium hydroxide reagent (5) N to 1 ml of the sample, the result is positive and shows the presence of flavonoids[127].

2.4.3. Alkaloid test:

Wagner's reagent was ($KI+I_2$) used to detect alkaloids; few drops of the reagent were added to one milliliter of the sample; and when an precipitate emerges, the result is positive and indicates the presence of alkaloids[127].

2.4.4.Detection of Tannins Test:

When a white deposit is discovered, add 1 ml of water lead acetate)1%(to 1 ml of extract since this indicates the presence of tannins[127].

2.4.5. Detection of Saponin test:

Add 1 ml of the model and 1 ml of the Mercury Water Chloride Reagent (5%) together. Positive results and the presence of soap are indicated white precipitate appeared [127].

2.4.6. Detection of lipid:

When a fat is heated strongly in the presence of a dehydrating agent such as KHSO4, the glycerol portion of the molecule is dehydrated to form the

unsaturated aldehyde, acrolein, which can be distinguished by its irritating acrid smell and as burnt grease[128].

2.5. Biosynthesis of ZnO NPs Method

ZnO NPs were greenly synthesized following the previous study [129]. with some modifications. A hot plate with a magnetic stirrer was used to heat 30 mL of *C. Spinosa L.* fruit aqueous extract to 85 °C. when the mixture reached 60 °C three grams of zinc acetate dihydrate was added, and the mixture was then allowed to boil until the extract converted into a paste. After that, it was put in a ceramic crucible and heated for three hours at 350 °C in a furnace. After gathering the residue powder, it was centrifuged after being re-suspended in deionized distilled water. The powdered residue collected for the subsequent analysis.as shown in figure(2-2)

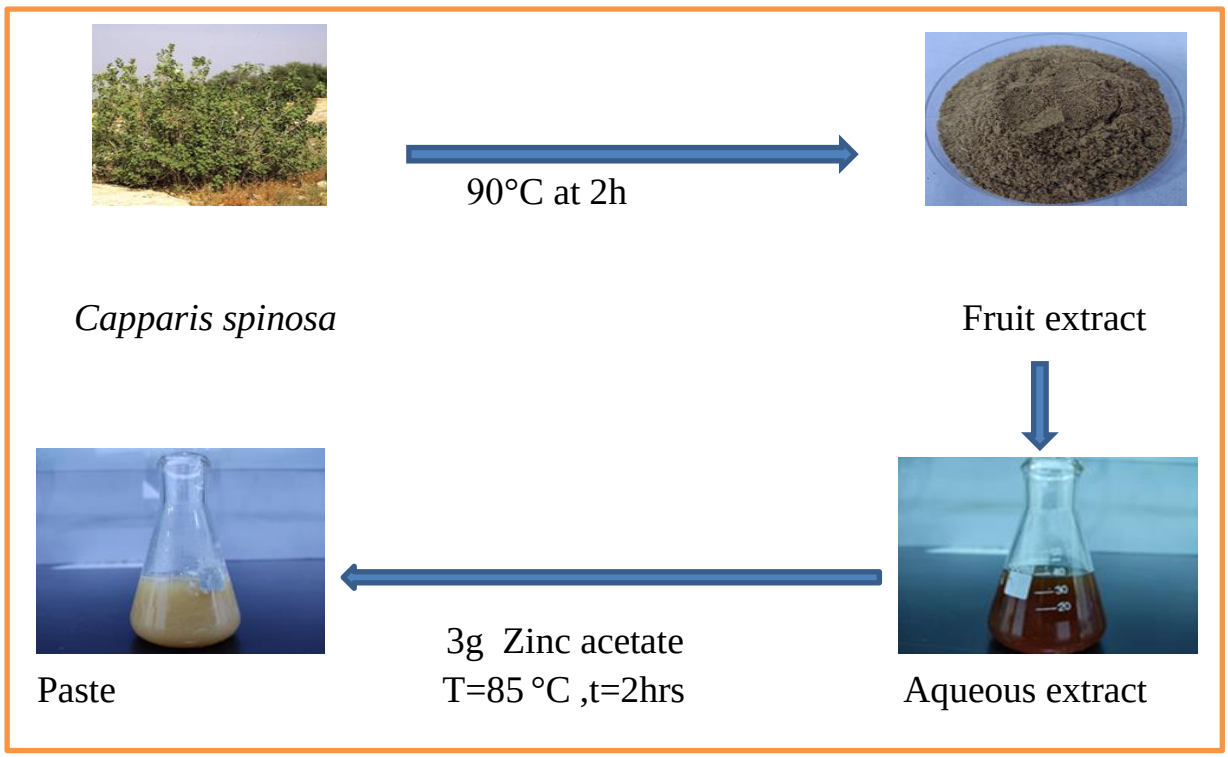


Figure (2-2) Schematic Biosynthesis of ZnO NPs using *C.spinosa* (fruit)

2.6. Characterization of ZnO NPs

2.6.1. UV–Vis Analysis

Sample Preparation After our centrifugation operation was finished, the pellets was collected, the superannuated was ejected, distilled water was added to the pellets. forming a stock solution, for further analysis [130].

Experiment Method Analysis of the UV-Vis spectrophotometer was done using stock solution. These analyses allowed for the observation and identification of the optical characteristics of zinc oxide nanoparticles. Water was first used as a blank reference. Nearly 4 ml of the extract and 4 ml of biologically produced ZnO NP solution were combined and placed inside the spectrophotometer. To prove that the synthesised green ZnO NPs were created, light in the 200-800 nm range was generated in a UV-Vis spectrophotometer [131].

2.6.2. Fourier Transform Infrared Spectroscopy (FT-IR).

FTIR instrument was utilized to examine the samples over the wavelength range of 400–4000 cm^{-1} in order to identify their functional groups s presented in the chemical compounds of the aqueous extract of c.spinosa fruit and stock solution which were responsible for the reduction of Zinc ions and the stabilization of the synthesized nanoparticles.

2.6.3. Gas Chromatography-Mass Spectrometry (GC-MS)

The GC- Mass analyses were performed using a CDS analytical ion source of 200 ºC and a detector of 250 ºC . The temperature of the samples had been elevated to 600 ºC for 15 seconds at a rate of 20 C/ms. A carrier gas (helium) drives the effluents out of the interface to GC; the solvent employed is deionized water.

2.6.4 X-Ray Diffraction Spectroscopy

The crystalline structure of the powdered sample was examined using a Bruker D8 Advance diffractometer with CuKα radiation (λ = 1.5418 Å).

Procedure. X-ray diffraction spectroscopy was utilised to investigate the structure of zinc oxide nanoparticles. X-ray radiations were utilised in this procedure. X-ray radiations were produced by the cathode ray tube in the spectroscope. When these radiations were blasted on the sample, the structure of zinc oxide nanoparticles was assessed. The spectrum was created using a diffractometer [132].

The Debye-Scherrer formula may be used to compute crystallite size.

$D = k\lambda / \beta \cos \Theta$

D= Average crystallite size (Diameter of the crystal)

β = Line broadening in radians (Full width at half maximum)

Θ= Bragg angle is the angle obtained from 2Θ value corresponding to maximum intensity peak in XRD pattern.

λ= X-ray wave length of the Cu-kα radiation.

K= 0.9 Scherrer constant

2.6.5. FESEM and EDX

FESEM was used to determine the shape and size of the obtained ZnO NPs. FESEM slides were created by putting gold on sample to increase conductivity. under acceleration voltages of 30 kV. using the EDS equipment, the EDX analysis was done to identify elemental sition of the sample in the synthesised ZnO NPs.

2.6.6. DLS and zeta potential

ZnO NPs were examined for size and surface charge using a particle size analyzer through DLS and Zeta potential.

2.7.Biological Evaluation of Synthesized Nanoparticles

2.7.1 MTT Assay

To evaluate the effect of ZnO NPs on cell proliferation in vitro, a Healthy L929 fibroblast cell line (ATCC, USA) was used. The cells were essentially grown in DMEM medium containing 10% fetal bovine serum. [133]. Cells were plated into 96-well dishes with 100 µL of DMEM and kept at 37 °C in a humidified, 5% CO2 incubator. To investigate the effect of ZnO NPs concentration and exposure period on cell viability, different concentrations of green-produced ZnO NPs (7.5,15,30,60, and 120 µg/mL) were added to each well and mixed with distilled water before being incubated for 24, 48, and 72 h. The diluent was used in the same quantity in the control cells (distilled water). Following the exposure period, the media in each well was changed to a new medium (100 µL) containing 5 mg/mL of MTT solution at (24, 48, and 72 h). After being incubated for four hours in the dark, the formazan crystal of the MTT reduction was dissolved in DMSO, and the absorbance was then determined using a microtiter plate ELISA reader ZnO NPs effect was assessed using the proportion of reduced MTT dye control absorbance at 570 nm. Three times each experiment was performed. The viability of the cells was determined by their capacity to transform the yellow dye MTT into a blue formazan crystal .The below formula was used to determine the percentage of cell viability

$$\%\textbf{Cellular viability} = (\textbf{OD specimen}/\textbf{OD control}) \times \mathbf{100}$$

2.7.2 Hemolytic Activity assay

The hemolysis test was utilized to assess how green ZnO NPs and the fruit extract from *C. Spinosa* affected RBCs. Fresh human red blood cells were obtained by healthy 20–30-year-old volunteers . Centrifugation was used to prepare the blood sample, which required 8 minutes at 1500 rpm. A clean pellet was then obtained by washing three times with PBS. The collected RBCs were suspended in PBS (10% v/v). All samples were combined with various concentrations of *C. Spinosa L.* and ZnO NPs (7.5, 15, 30, 60, and 120 μg mL−1) and incubated at 37 °C for 60 minutes. Following this, all samples were centrifuged at 5000 rpm for 4 minutes. The sample supernatant was then transferred to a 96-well plate. Using a plate reader, the absorbance values of the supernatants were calculated at 570 nm. The positive control was SDS in PBS (0.1%) and the negative control was PBS solution. The following formula was used to calculate the percentage of hemolysis in RBCs [134].

$$H\% = \frac{S.A - N.C.A}{P.C.A - N.C.A} \times 100\%$$

Where H indicates hemolysis, S for sample, A for absorbance, C for control, N for negative, and P for positive

2.7.3. Antioxidants Evaluation

Using the DPPH free radical scavenging method, the antioxidant potency of the ZnO NPs and *C.spinosa L*. extract was assessed, with ascorbic acid acting as the positive control. The standard solution of ascorbic acid was employed as a positive reference, and 1 mL of the biosynthesized ZnO NPs solution was diluted with methanol to obtain various concentrations (37.5–

300 µg/mL). The mixture was then stirred and left in the dark at room temperature. After an hour of incubation, the absorbance (A) of each sample was measured using a UV-visible spectro-photometer at 520 nm to determine the measures of radical scavenging activity the absorption bands change from violet to yellow, as shown in Figure (2-3) . Using the following equation to calculate the percentage inhibition (%) [135].

$$\text{Inhibition } \% = \frac{(A\ \ C - A\ \ S)}{(A\ \ C)} \times 100\%$$

Where A refers to Absorbance, C= Control, and S=Sample.

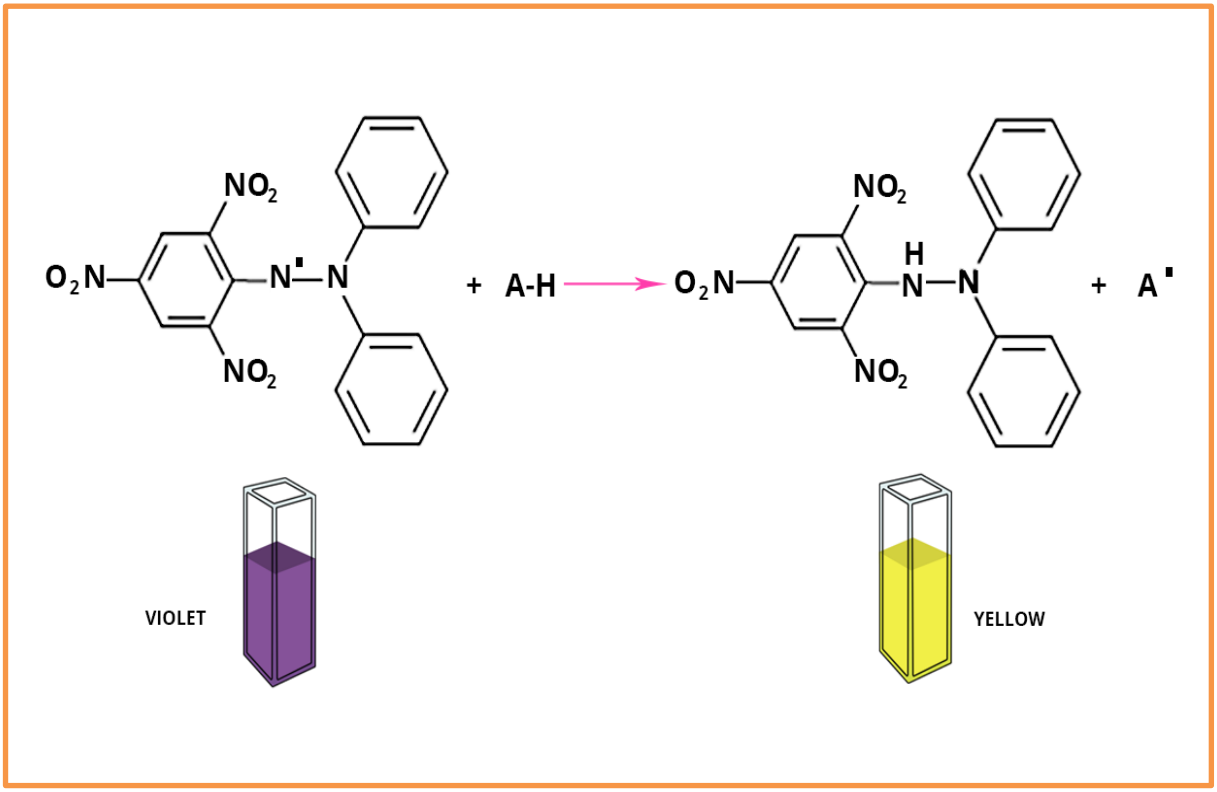
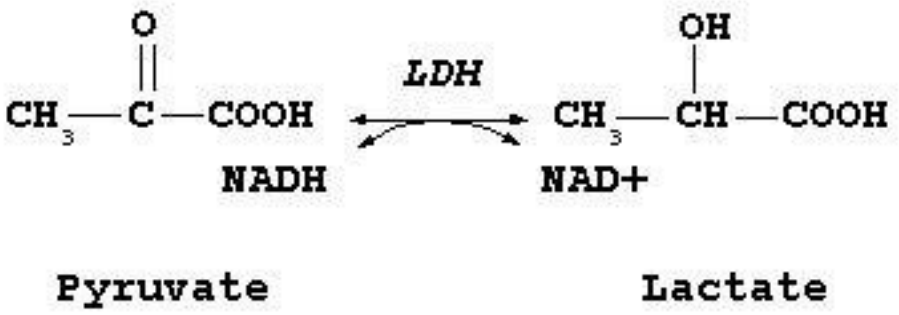


Figure (2-3) Reaction DPPH with AH [135]

2.7.4. An Inhibitor effect on Lactate dehydrogenase Enzyme

Estimation of LDH enzyme activity was evaluated following the method described by the instructions from manufacturers (BIOLABO SAS, France) . using tris pH 7.2 (80mmol/L) as buffer, pyruvate (1.6 mmol\L) as a substrate NADH (0.2 mmol\L) as coenzyme [136].



Enzyme activity is specificated as the LDH enzyme that produces 1 µM of lactate per minute at 37 °C. Diluted solutions (75, 100, 125, 160, 210) ppm of *Capparis Spinosa* L. fruit extract, were add as inhibitors of LDH enzyme according to [137]. 1 ml of the working reagent put in a water bath for 5 min at 37 C, then 20 µL was add from both the inhibitor and pool of serum(from patients with hereditary haemolytic diseases). The activity was measure after 30 seconds, 1 and 2min at 340 nm, Residual activity was estimate by the equation (1).

UI\L=(ΔAbs/min) x 8095……..(1)

While the % inhibition was measured by equation (2)

% inhibition = 100 - (Activity UI\L with inhibitors x 100…….. (2)
Activity UI\L without inhibitors

2.7.5 Anti-Bacterial Activity Testing

In this study, two types of standard bacterial strains were used, *Staphylococcus aureus* (ATCC25923) and *Escherichia coli* (ATCC25923) as positive and negative gram stains respectively which were provided by the American Type Culture Collection (ATCC) . The culture media, it was be prepared with nutrient agar (NA), nutrient broth (NB), and Müller-Hinton agar (MHA) using the wells diffusion method. Preparation of bacterial colonization one colony whose age is not above 24 hours, grown on brain heart infusion agar , transported by a loop from nutrient agar to the plate, the plates were placed in the Incubator at (37℃) for 24 hours. The sterile tips approximately (6 mm) in diameter were used to make 4 wells placed on Muller Hinton Agar (MHA). Plates were spread of one colony carry by swab from "fresh overnight cultures" to the Muller Hinton Agar (MHA) plate by a loop. 4 holes with a diameter of 6 mm were filled with 100 ,200 μg mL-1 of *C. spinosa* fruit extract and others filled with ZnO NPs, a blank well was carried by adding solvent alone (distilled water) to act as a negative control. After an incubation period under 37 °C for 24 hr. zones of growth inhibition were measured. All of the experiments were conducted in triplicate [138] .

CHAPTER THREE

Results And Discussion

3.1 Characterization of the *C.Spinosa* L. fruit Extract and the Synthesized ZnO NPs

3.1.1 Qualitative analysis:

Table (3-1): Qualitative phytochemical analysis of *C.spinosa* (fruit parts) extract.

Chemical Tests	Aqueous Extract
Saponins	+
Phenols	+
Flavonoids	+
Tannins	+
Alkaloids	+
Lipid	+

(+) : shows the presence of the given chemical constituent.

(-) : shows the absence of the given chemical constituent

3.1.2. Visual Inspection

The formation of ZnO NPs was confirmed by a visual color change and UV-Vis spectral analysis. As shown in Figure (3-1), the colors of the zinc acetate solutions changed to Creamy after irradiation. This indicates that the metabolites in the aqueous extract of *C. spinosa* L. could be responsible for the reduction of zinc ions for synthesizing ZnO NPs. Comparing the experimental studies in the literature, similar color changes were observed in the green synthesis of ZnO NPs [139]. Several parameters influence the biological basis of green synthesis procedures, including solvent, temperature, pressure, and pH levels (acidic, basic, or neutral). Plant metabolite functional

groups such as amine, hydroxyl, and carbonyl may interact with metal ions and decrease molecules to nanoscale size [140]. This is related to the presence of powerful phytochemicals such as flavonoids, polyphenols, ketones, aldehydes, amides, alkaloids, carboxylic acids, saponins, and vitamins in diverse plant extracts, notably fruits.

Figure(3-1). The visual appearance of the synthesized ZnO NPs using *C. spinosa* L. fruit extract: (a) fruit extract solution; (b) zinc acetate; and (c) mixture of zinc acetate and aqueous fruit extract

3.1.3. UV-Vis Spectral Analysis

To further verify the results, the formation and stability of the synthesized ZnO NPs were determined by measuring the absorption spectrum in the 200–800 nm wavelength range against the aqueous extract of *C. spinosa* L. as the reference. As shown in Figure (3-2), the maximum absorption peaks at 374 nm confirmed the green synthesized ZnO NPs. Furthermore, a peak with 290 nm was observed in the absorption spectra of the aqueous extract of *C. spinosa* L. The obtained absorption peaks in the visible range are formed from the excitation of free electrons due to the color change [141]. The position and shape of the absorption peaks of metallic nanoparticles are strongly related to particle size and morphology. A sharp, single absorption peak corresponds to spherical nanoparticles [142]. Metal ions are transformed into metal nanoparticles by the phytochemicals that are found in the plant extract. As a result, the plant extract has stabilizing and reducing capabilities. UV-Vis spectroscopy was used to observe the progression of this reaction [143]. When electromagnetic waves interacted with electron conduction band oscillation in the spectrum of UV-visible spectroscopy, peak absorption was coupled by the surface plasmon resonance (SPR), accurately simulating metal ion reduction and nanoparticle creation. Because there are numerous OH groups available, it is possible to make nanoparticles. The fruits of *C. spinosa* L. contain large amounts of alkaloids such as capparisine A, capparisine B, and capparisine C, as well as other flavonoid compounds such as rutin, quercetin, and tetrahydroquinoline acid, which are recognized for their ability to reduce metal ions and act as stabilizing agents in the nanoscale dimension [144]. These compounds are also known to be antioxidants and free of harmful substances.

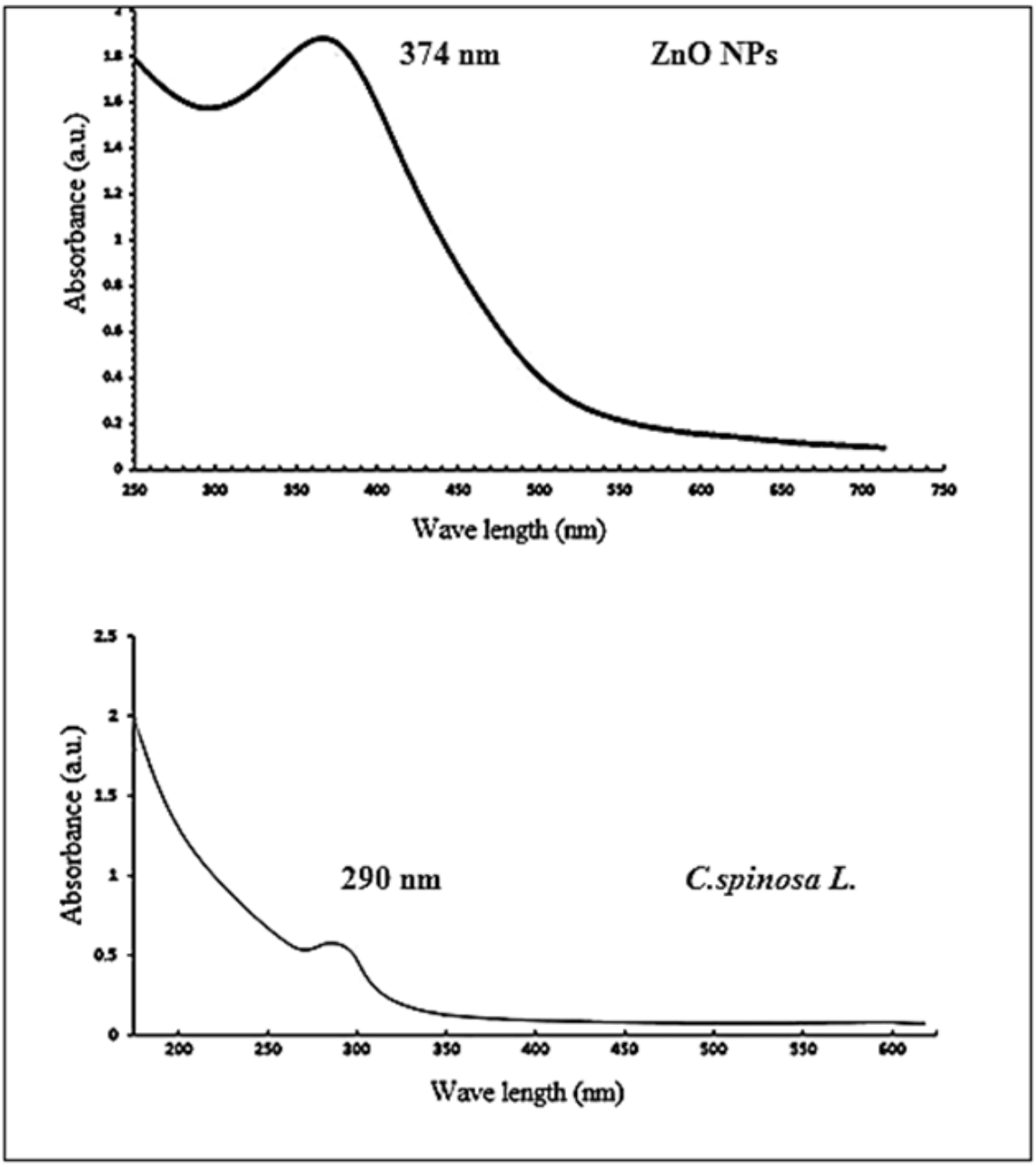


Figure. (3-2). UV–Vis spectroscopy analysis of the *C. spinosa* extract,

and ZnO NPs

3.1.4. FTIR Analysis

The FTIR analysis was performed to confirm the presence of the functional groups of the metabolites of *C. spinosa* Lfruit . over the surface of the synthesized nanoparticles. The FTIR spectrums of the green synthesized ZnO NPs are shown in Figure (3-3). The stretching vibration of the hydroxyl group(O-H) is related to the peak in the FTIR spectrum that appears between 3291 and 3464 cm^{-1}, which is attributed to the phenolic compound in the plant extract that is involved in the synthesis of NPs [145]. The peak at 1650 and 1716 cm^{-1} in the FTIR spectrum of *C. spinosa* L. extract represents the stretching bands of (C=C) and (C=O) functional groups; an identical peak can also be seen at this location in the FTIR spectra of ZnO NPs . The organic functional groups at 1635 cm^{-1} may not have entirely broken down because ZnO NPs' annealing duration was barely one hour. This results in the vibrational peak we observed in our sample. This finding is consistent with the information provided by Faisal, Shah et al. [146]. The peak at 2977 cm^{-1} is caused by the aliphatic (C-H) groups. The peaks at 1380 and 1382 cm^{-1} indicate the (C-O-H). Between 492 and 422 cm^{-1}, metal-oxygen (Zn-O) stretching can be seen [147]. Therefore, it can be said that the fruit extract of *C. spinosa* L. acts as a capping, reducing, and stabilizing agent for ZnO NPs synthesized synthetically. It also contains functional groups with (O-H) and (C=O). The biomolecules in the plant extract help create nanoparticles by acting as effective capping agents. The capping agents appear to stabilize NPs through a number of mechanisms, including electrostatic stability, steric stabilization, hydration force stabilization, and van der Waals forces. The stability of nanoparticles determines their use and applications.

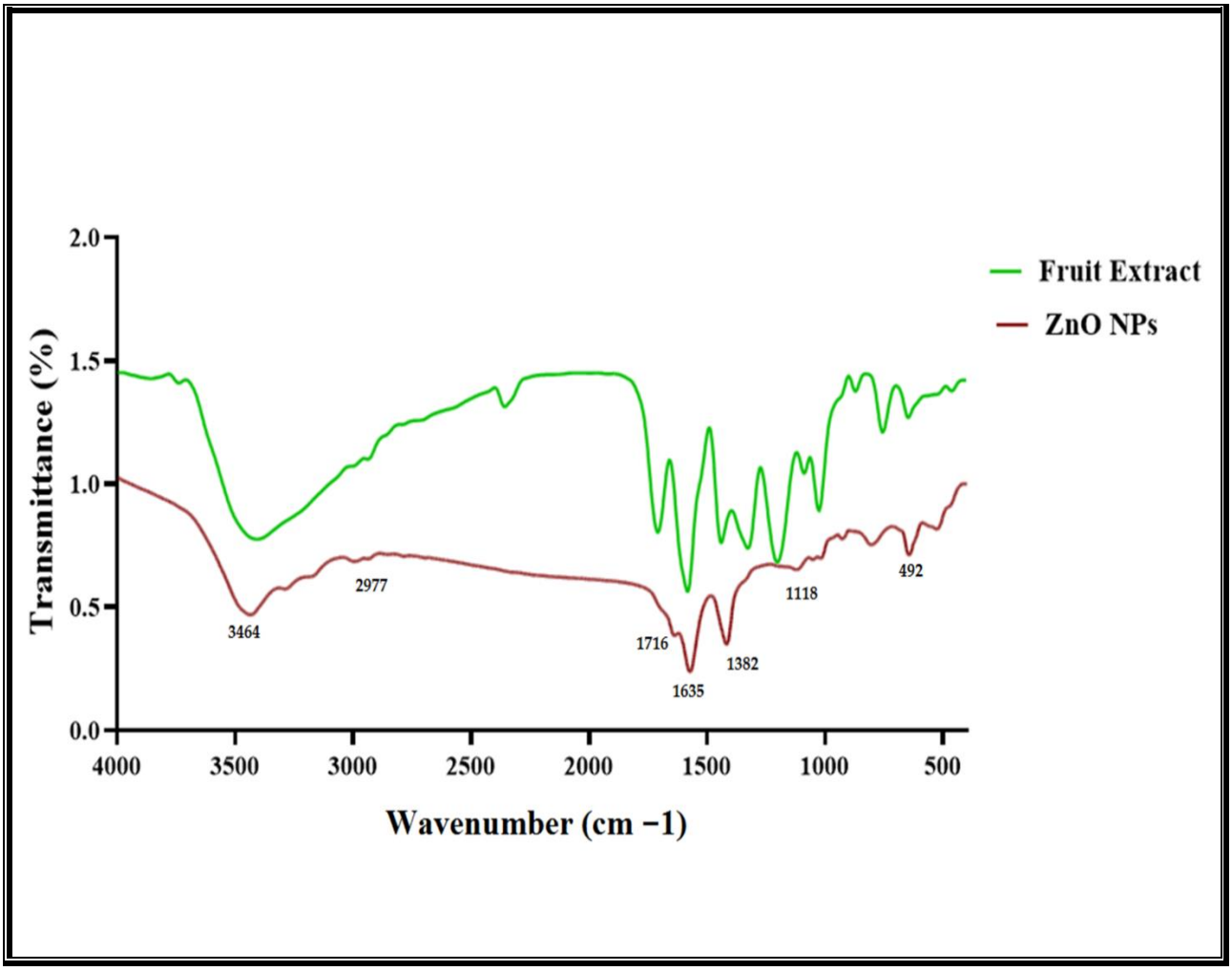


Figure (3-3). FTIR analysis of the *C. spinosa* L. extract and ZnO NPs.

3.1.5 GC- Ms

Table (3-2) shows the organic compounds in the aqueous extract of the fruit of *C.spinosa* , which were characterization using GC-MS technique. The composition and weight of each organic compound were obtained within a sample, and the Table also shows the most important molecular formulas and Retention time. Fifteen different chemical compounds were obtained with different retention times. Figure(3-4) depicts the highest peak of the chemicals in the aqueous extract of the C.spinosa plant's fruit.

Table (3-2) The main chemical composition in the aqueous fruit extract of *Capparis Spinosa* L. by GC-MS

NO	Name	RT	Area %	M.wt	Chemical formula	Classification
1	N-Nitroso pi peridine	3.548	34.24	114	$C_5H_{10}N_2O$	Alkaloid
2	3-Methyl-1-butanol	3.749	35.01	88	$C_5H_{12}O$	Alcohol
3	2,2’-Di hydroxyl-4’,6’-di methoxy chalcone	4.102	45.75	300	$C_{17}H_{16}O_5$	Flavonoids
4	Anti biotic k25 2b	4.952	7.88	453	$C_{26}H_{19}N_3O_5$	Alkaloid
5	2-Docosa hexaenoyl-1-stearoyl-sn-glycero-3-pho	5.091	7.55	791	$C_{45}H_{78}NO_8P$	Sterols
6	Glafenin	5.208	11.61	372	$C_{19}H_{17}ClN_2O_4$	An anthranilic acid derivative
7	Colchicine	5.658	6.25	399	$C_{22}H_{25}NO_5$	Alkaloid
8	Acetildenafil	6.038	5.18	466	$C_{25}H_{34}N_6O_3$	Alkaloid
9	7,8-Dihydro-1-Biopterin	6.848	4.34	239	$C_9H_{13}N_5O_3$	Alkaloid
10	Rhodamine 6G Cation	7.491	13.45	443	$C_{28}H_{31}N_2O_3$	Alkaloid
11	Buprenorphine glucuronide	7.761	5.35	643	$C_{35}H_{49}NO_{10}$	Alkaloid
12	Hepta Carboxy porphyrin 1	9.172	32.82	786	$C_{39}H_{38}N_4O_{14}$	Alkaloid
13	(6S)-5-Methyl tetra-hydro folic acid	10.078	3.92	459	$C_{20}H_{25}N_7O_6$	Alkaloid
14	Lutein	10.85	29.04	568	$C_{40}H_{56}O_2$	Flavonoids
15	6,7-Di Methyl tetra hydropterin	11.95	10.78	195	$C_8H_{13}N_2O$	Alkaloid

3.1.6. XRD Analysis of the Resulting ZnO Nanoparticles

Figure (3-4) shows the XRD pattern of ZnO NPs that were made using *C. spinosa* L . The XRD diffraction peaks at 2θ = 31.81°, 34.42°, 36.24°, 47.55°, 56.66°, 62.96°, 66.42°, 67.93°, 69.12°, 72.62°, and 77.02° correspond to (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202) planes, respectively. These peaks match those of (JCPDS card No. 36-1451), confirming the hexagonal wurtzite structure of the Biosynthesis of ZnO nanoparticles [148].

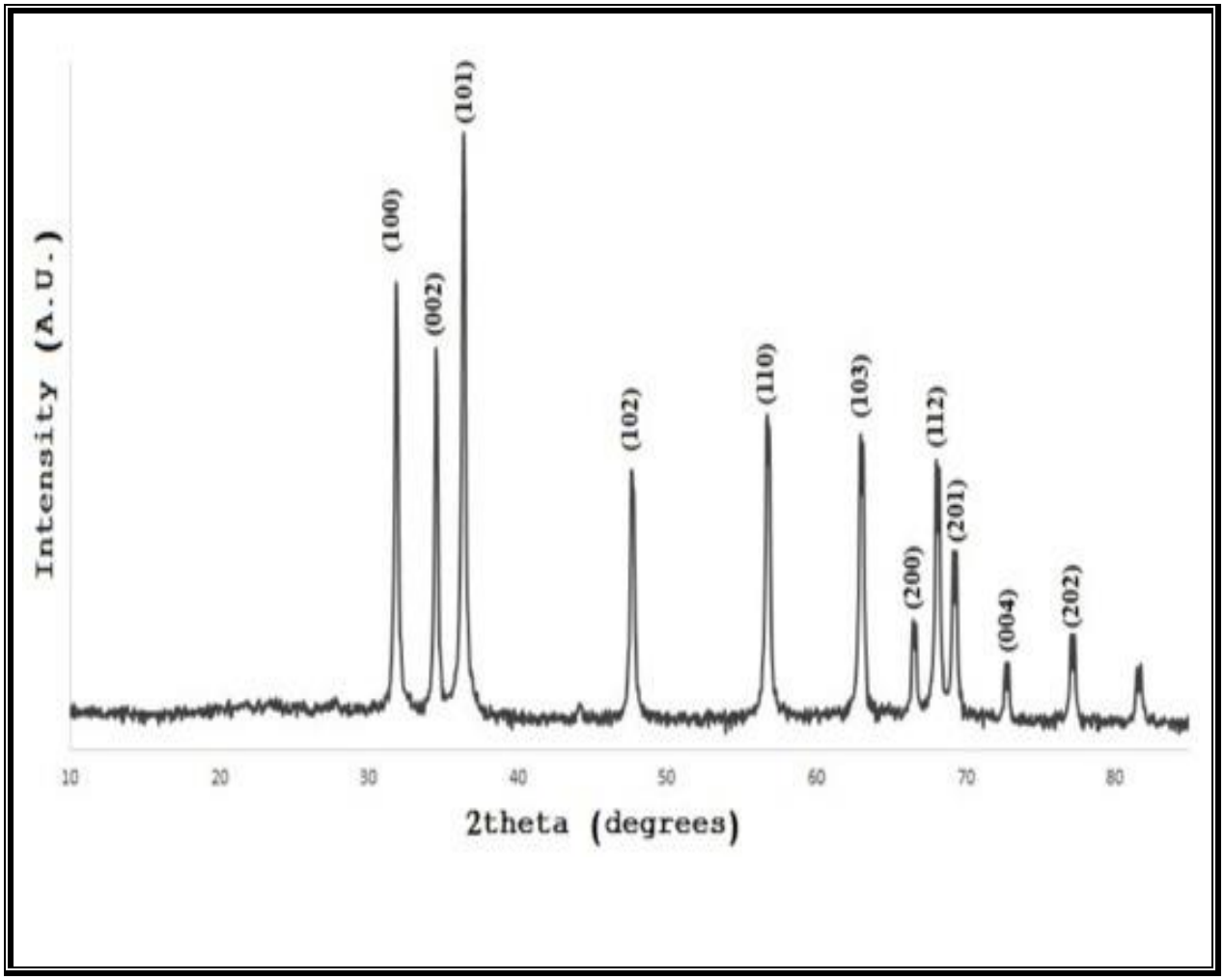


Figure (3-4). XRD Analysis of ZnO NPs created by *C. spinosa* L.

3.1.7. The FESEM and EDX Results

FESM analysis demonstrated that the synthesized ZnO NPs were almost spherical and monodispersed. The synthesized ZnO NPs had an average size of 37.49 nm (Figure 3-5). The FESEM picture of the obtained ZnO NPs shows their homogeneous distribution and nearly spherical shape. The large majority of the ZnO NPs that were produced appeared extremely spherical and in the nanometer range. The green synthesis nanoparticles, which exhibit considerable aggregation development, were infrequently present in the generated ZnO NPs. This is due to the increased surface area and strong affinity of biosynthetic NPs, which accumulated or aggregated together. The EDX analysis was performed to determine qualitative levels of elements (Figure. 3-6). that could have contributed to the creation the of ZnO NPs as shown in table (3-3). The EDX analysis showed that the substance under investigation contained green produced ZnO NPs.

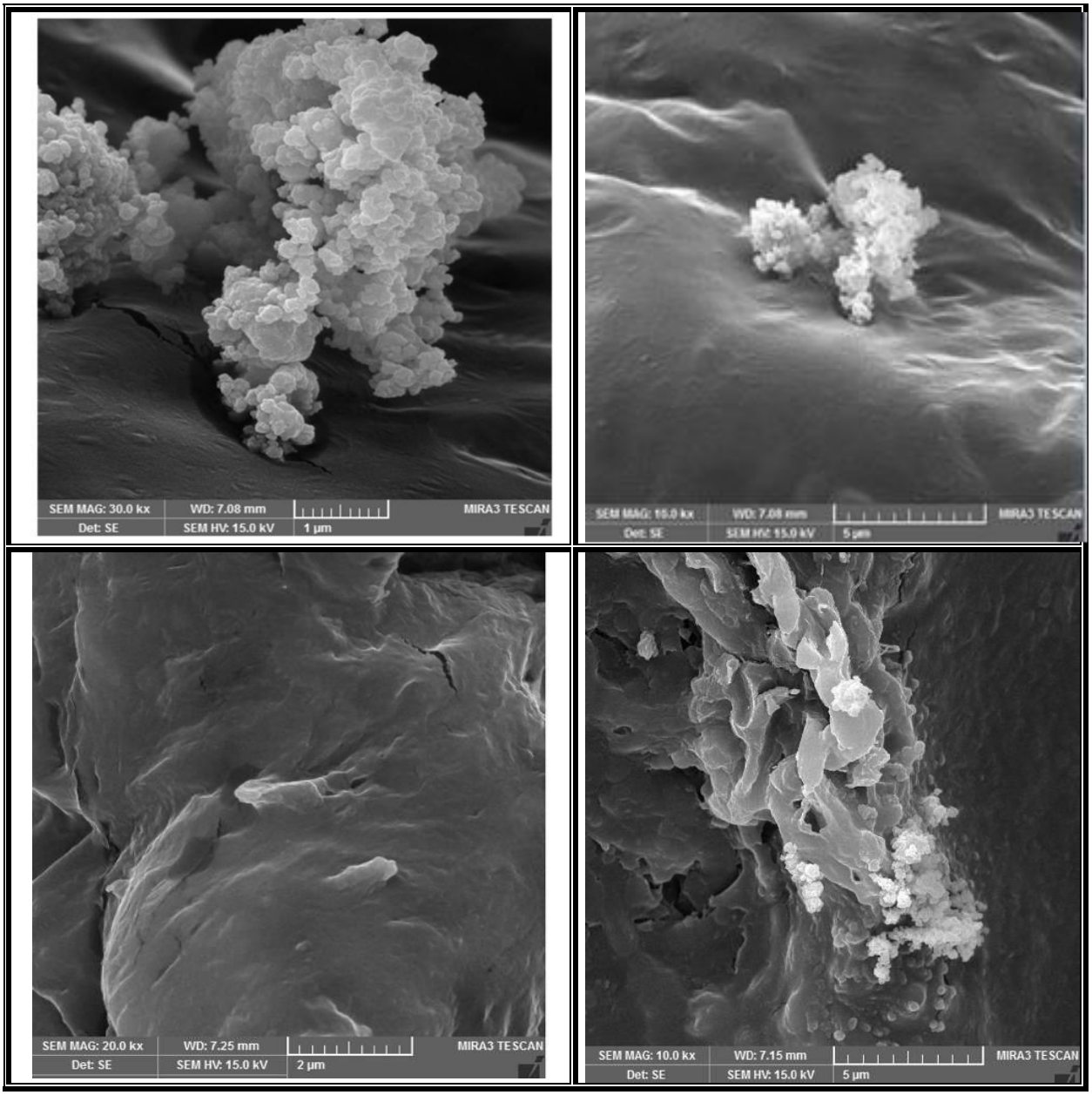


Figure (3-5). FESEM analysis of bio-synthesized ZnO NPs

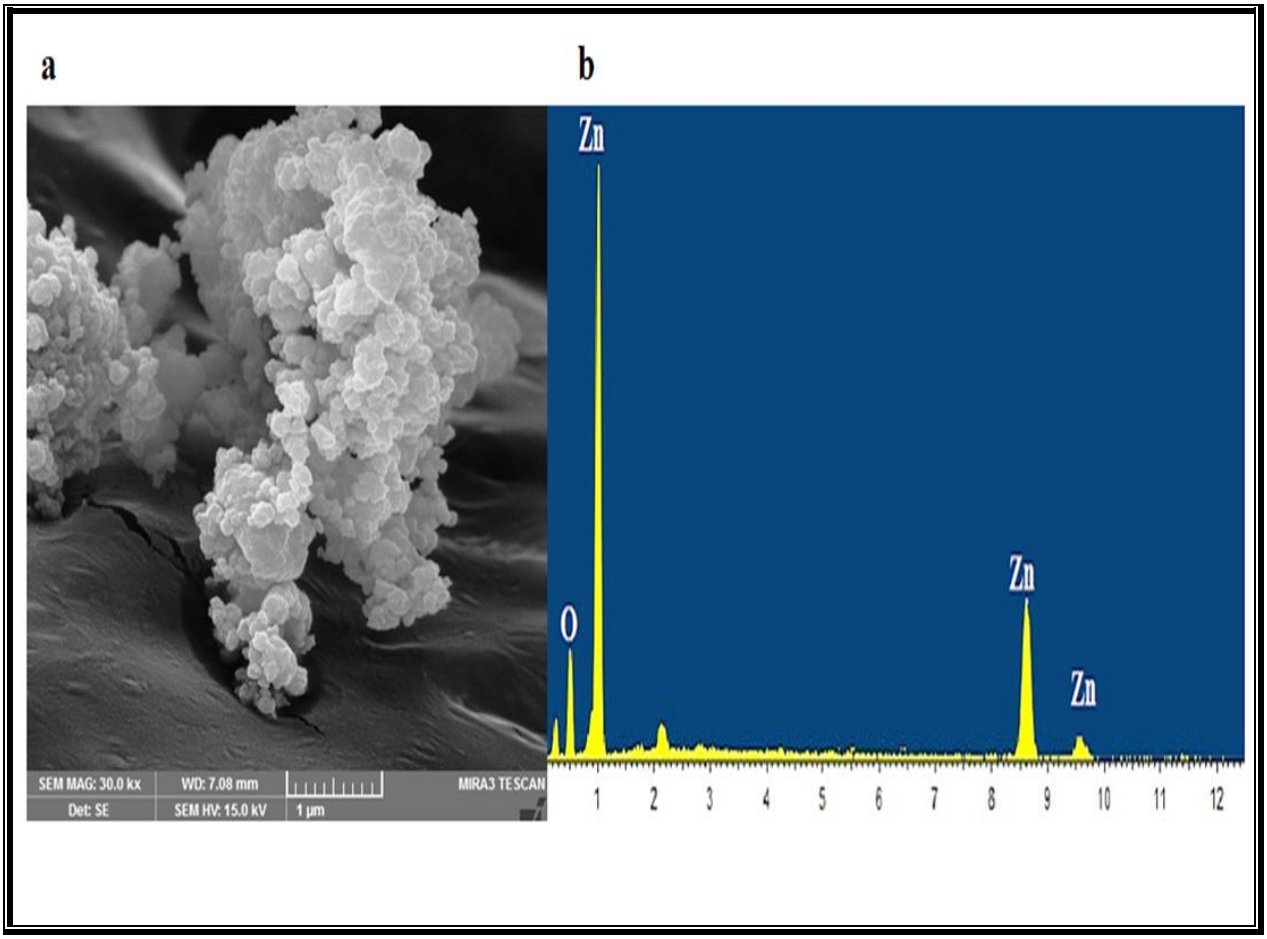


Figure (3-6) a- FESEM of ZnO NPs b-EDX Spectra of ZnO NPs

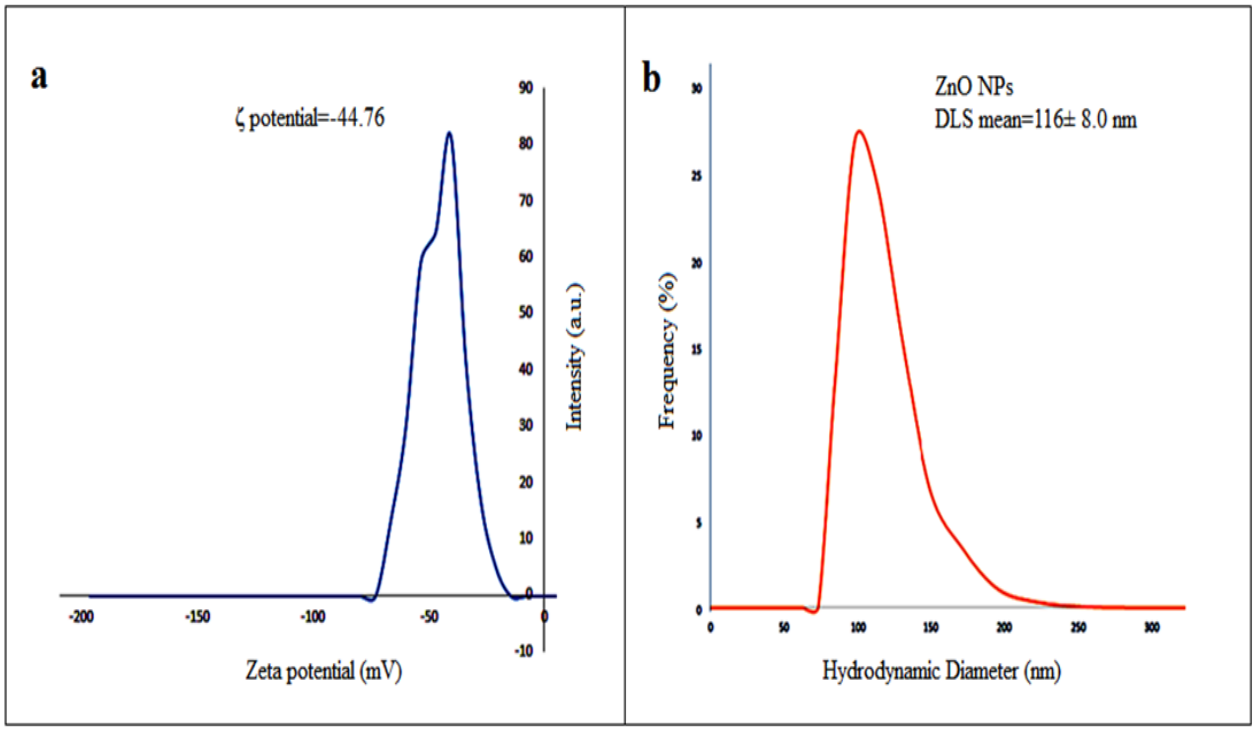
Table (3-3) describes ZnO NPs' elemental components

Components	wt.%	Atomic (%)
Zn	80.72	50.59
O	19.28	49.41
Total	100	100

3.1.8. DLS and Zeta Potential Analysis

The hydrodynamic diameters, zeta potentials, and polydispersity indexes (PDIs) of the synthesized ZnO NPs were determined by DLS analysis. The size distribution histogram of DLS showed that ZnO NPs' average hydrodynamic diameters were determined to be 116 ± 8.0 nm Figure (3-7)b. DLS measures the diameter of the particles in solution based on the Brownian motion. DLS analysis measures particle size including the hydration layer of water molecules; therefore, the average diameters of the synthesized ZnO NPs were greater than the diameters measured with FESEM and XRD analysis [149]. The ZnO NPs showed zeta potentials of −44 ± 76 mV Figure (3-8)a. It is known that a high negative charge prevents aggregation of nanoparticles via electrostatic repulsion and increases the stability of particles. PDI values of the synthesized ZnO NPs were calculated as 0.452 ± 0.092. A PDI value less than 0.7 indicates monodisperse particle size distribution [150]. It is well known that a nanomaterial's physicochemical properties, such as its size, shape, surface area, zeta potential, and composition, have a substantial impact on the results of its cytotoxicity. Considering that ZnO NPs exhibit size-dependent substantial cytotoxicity, more soluble and smaller NPs offer more useful toxicological information on nanomaterials in this context. The biological activity of ZnO-NP is significantly influenced by their shape, size, and concentration. DLS average sizes are larger than those determined from FESEM images; this is likely because ZnO NPs observed in FESEM are only viewed on the surface of the agglomerates. In contrast, DLS measures the particles' as three-dimensional, which might include a biomolecular coating. Such discrepancy between FESEM and DLS average size has been reported previously and is related to the specifics of the method. In order to examine the surface charge and stability of the synthesized NPs, a zeta-potential investigation was conducted.

The results revealed that the synthesized NPs have good stability, as evidenced by the emergence of a distinct peak at roughly −44 ± 76 mV. The effective stability of ZnO NPs and reduction of metal ions may be due to the extract's high protein and flavonoid content.



Figure(3-7). Zeta potential (a) and DLS (b) spectra of the obtained biosynthesized ZnO NPs

3.2 Application for ZnO NPs

3.2.1. Cytotoxicity Results

To assess the cytotoxic effect of greenly obtained ZnO NPs utilizing *C. spinosa*. The MTT assay was used for various concentrations of ZnO NPs, including 7.5, 15, 30, 60, and 120 µg mL−1 on L929 normal fibroblast cells and cultured for different periods: 24, 48, and 72 h (Figure 3-8).

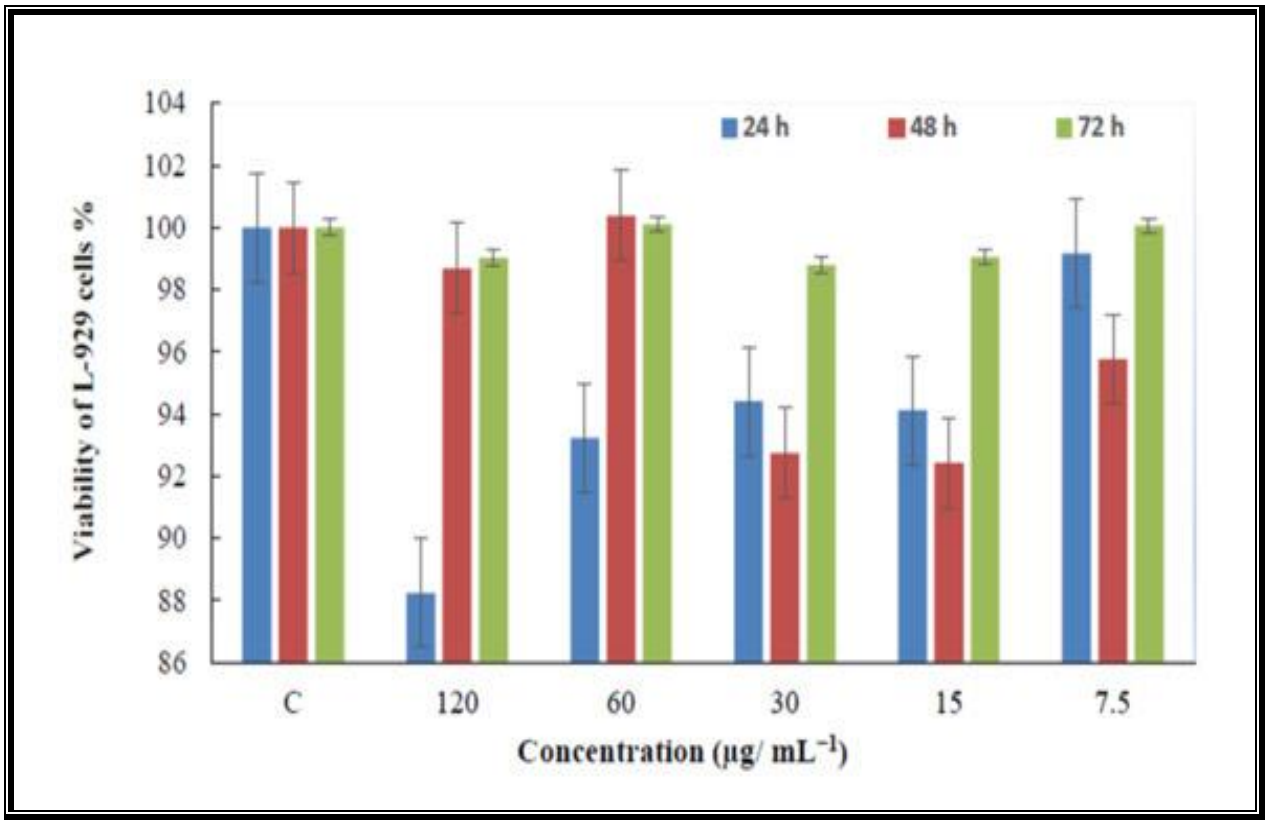
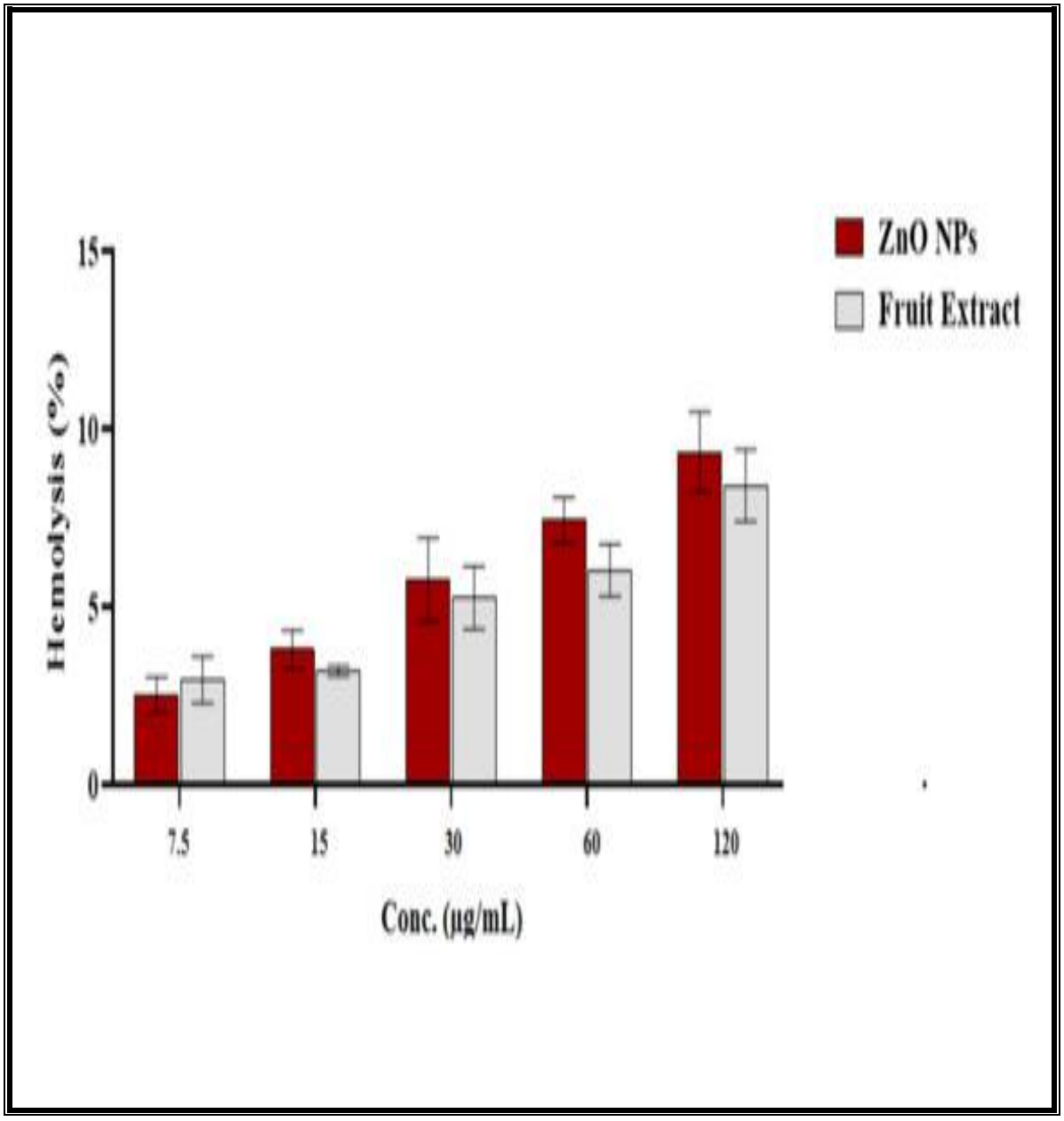


Figure (3-8). Cell viability using green ZnO NPs

3.2.2. Hemolysis Test

To test the biocompatibility of ZnO nanoparticles in blood, hemolytic activity testing was performed (Figure 3-10). Hemolysis results from direct or indirect harmful effects against the RBC membrane and is a reliable indicator of a substance's biological incompatibility. One of the tests for determining a biomaterial's hemolytic potential is the determination of whether it is safe when in contact with blood [151] The red blood cells undergo hemolysis when, as a result of the broken cells, they free hemoglobin. We primarily used the ZnO nanoparticles made from the fruit extract of *C. spinosa* L. for the hemolysis experiment. In the other cytotoxicity tests, this ZnO NPs sample displayed the least harmful effects of the other samples. ZnO nanopowders as well as extracts of *C. spinosa* L. showed low hemolytic activity compared to the control, at dosages between 7.5 and 120 μg/mL, as shown in Figure (3-9). These results support the possibility that future in vivo investigations using green synthesized ZnO NPs generated using Capricorn *spinosa* L. fruit extract could yield promising results for medication administration.



Figure(3-9). Hemolytic activity of fruit extract and bio-synthesized ZnO nanoparticles**.**

3.2.3. Antioxidant Activity Analysis of ZnO-NPs

The capacity of ZnO-NPs to scavenge free radicals was assessed using the DPPH test. Free radicals such as DPPH have been widely employed to examine the antioxidant strength of inorganic substances and nanomaterials [152]. The DPPH solution was made with a deep violet hue that, after the addition of ZnO-NPs, turned pale yellow, which often denotes the antioxidant capacity of ZnO-NPs [153]. ZnO NPs and *C. spinosa* L. extract were tested using the DPPH method for their antioxidant activities, with ascorbic acid acting as the standard reference. According to Table (3-4) and Figure (3-10), the biosynthesis of ZnO NPs using *C. spinosa* L. extract displayed good antioxidant activity at dosages of 37.5–300 μg/mL, which varied from 23% to 96%. IC50 was about 43.68 ± 0.04 μg/mL in comparison to 81 ± 0.054 μg/mL ,for plant extract. However, there was a difference in the antioxidant potential between *C. spinosa* L. extract and the ZnO NPs. While both samples promptly responded to and decreased the diverse range of DPPH free radicals , there was a difference in antioxidant capability between the extract and ZnO NPs, which may be caused by the chemical composition of each sample analyzed [154]. Our results are consistent with those of a recent work by Thavamurugan, S et al. [155] that investigated the antioxidant activity of Ag NPs using *C. spinosa* fruit extract. Previous studies have demonstrated the significant antioxidant activity of *C. spinosa* L. extract [156].

Table (3-4). Ascorbic acid, ZnO NPs, and plant extract IC50 values.

Sample	**IC_{50} (μg/mL)**
C. spinosa fruit extract	81 ± 0.054
ZnO-NPs	43.68 ± 0.04
Ascorbic acid	26.4 ± 0.05

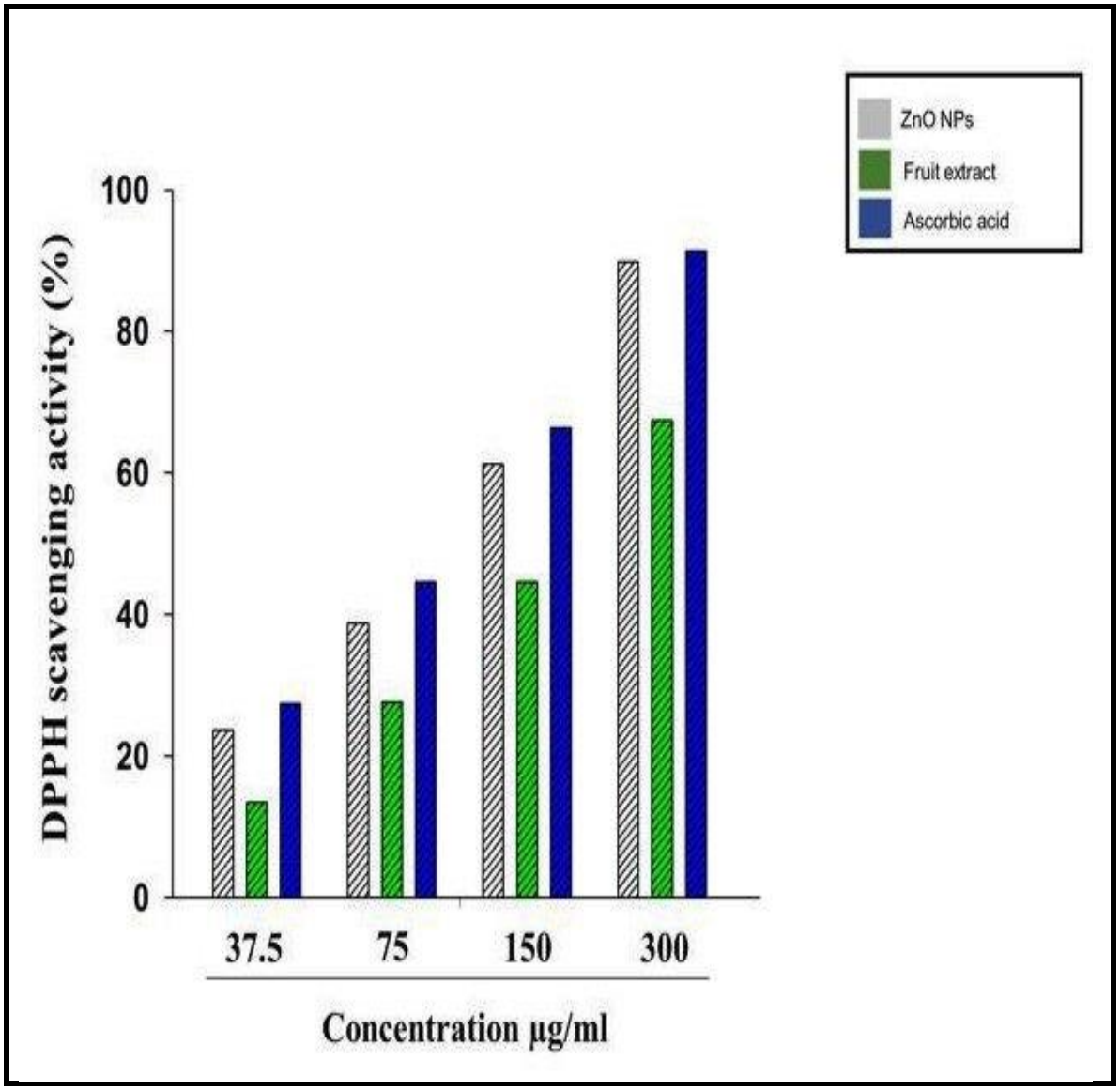


Figure (3-10). Antioxidant activities of *C. spinosa* L. Fruit extract and bio-synthesized ZnO NPs

The antioxidant potential of biosynthesized ZnO NPs may be connected to the complex of various antioxidant metabolites that are found within cells in plants and protect biological substances such as flavonoids and phenolic chemicals from oxidation and damage. After the metal ions' physical-chemical interaction with the functional groups of the plant extract, these compounds created a coating covering for the ZnO NPs, giving them a spherical morphology and a greater surface area [157].

3.2.4 An inhibitor effect on Lactate dehydrogenase enzyme

The results in the table (3-5) and figure (3-11) - shows Curve of the Michaelis– Menten equation between substrate concentration [S] and the reaction rate is half of V. While table (3-6,7) show the % inhibition activity of (LDH) enzyme estimated with 371UI\L using an effect of *Capparis Spinosa* L. Fruit extract and ZnO NPs at five diluted concentrations (75,100,125,160,210) and (7.5, 15, 30, 60,120,240) ppm respectively. In addition, figure (3-12) - had shown a drawing of (Line weaver Burk plot) equation to LDH enzyme in the serum of hereditary hemolytic patients. Data obtain from table (3-10) and figures (3-12, 13) the values of both Vmax and km without inhibitor effect were (333.3, 0.16) respectively, also the values of Vmax and km with inhibitor effect of *Capparis Spinosa L*. Fruit extract were (333.3, 0.13). Therefore, these data leading us to the type of inhibitor, which is competitive inhibition because of hydrogen bonding, which consist between the active site and a functional group in aqueous fruit, extract compounds.

Table (3-5) :The substrate values and LDH enzyme reaction rate before adding the extract as an inhibitor

V max	153	196	289	320	365	371
[S] m Mole/L	0.06	0.08	0.12	0.13	0.16	0.2

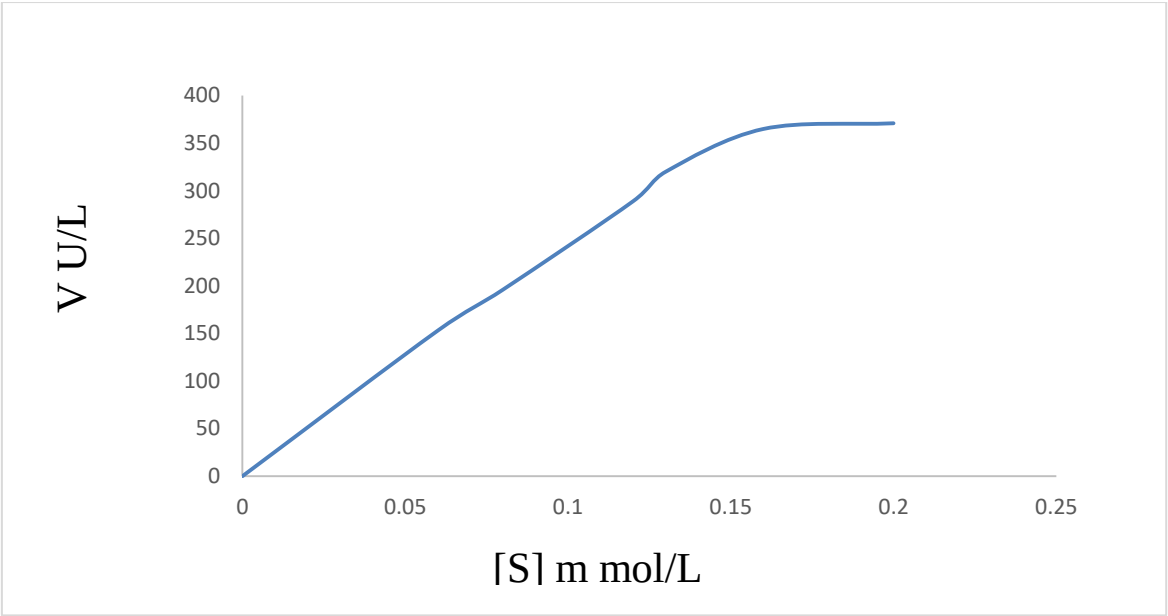


Figure (3-11) : Curve of the Michaelis–Menten Equation between Substrate concentration [S] and the Reaction rate is half of V.

Table (3-6): An inhibition Effective of *Capparis Spinosa L.* Fruit extract on LDH enzyme estimated with 371UI\L

Inhibitor conc. ppm	Enzyme Activity U/L	%Inhibition
75	303	18
100	247	33.4
125	202	45.5
160	192	48.2
210	153	59.5

Table (3-7) :The substrate values and LDH Enzyme Reaction rate after adding the *Capparis Spinosa* L. Fruit extract as an Inhibitor

V max	120	158	210	247	303	324
[S] m Mole/L	0.06	0.08	0.12	0.13	0.16	0.2

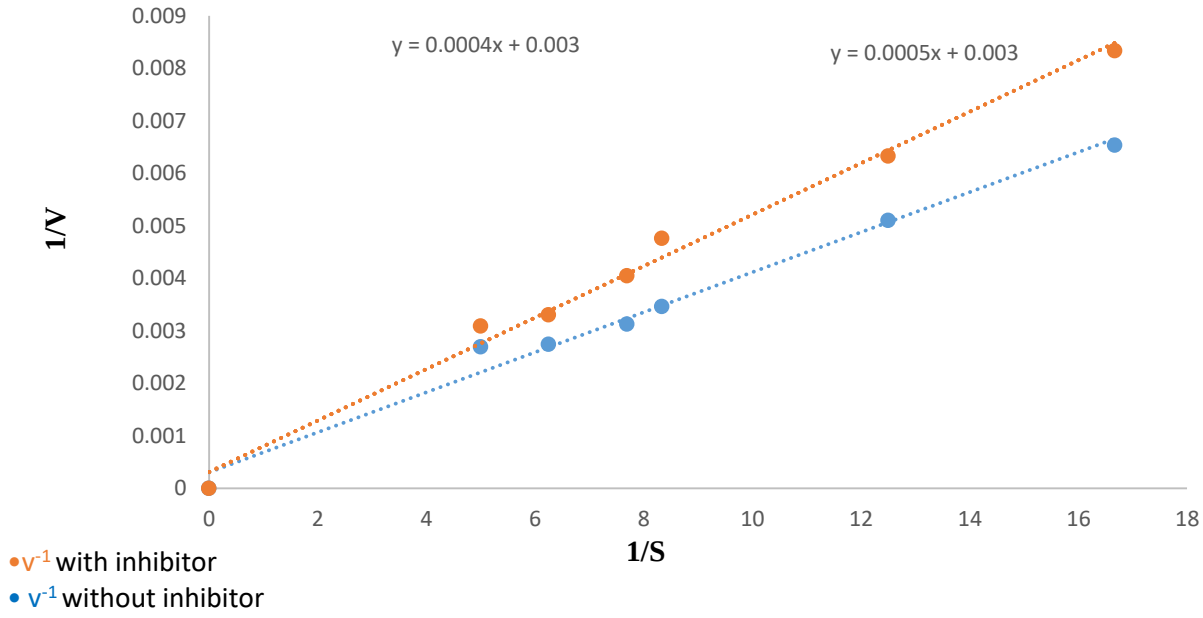


Figure (3-12):(Line weaver Burk plot) Equation of LDH enzyme with and without the inhibitory effect of *Capparis Spinosa* L. Fruit extract

Table (3-8): An inhibition effective of ZnO NPs on LDH Enzyme estimated with 371UI\L

Inhibitor conc. ppm	Enzyme Activity U/L	%Inhibition
240	113.3	69.4
120	150	59.5
60	194.3	47.6
30	170	54.17
15	234.77	36.7
7.5	160	43.12

Table (3-9): The substrate values and LDH Enzyme Reaction rate after adding ZnO NPs as an inhibitor

V max	120	150	189	292	267	201
[S] m Mole/L	0.06	0.08	0.12	0.13	0.16	0.2

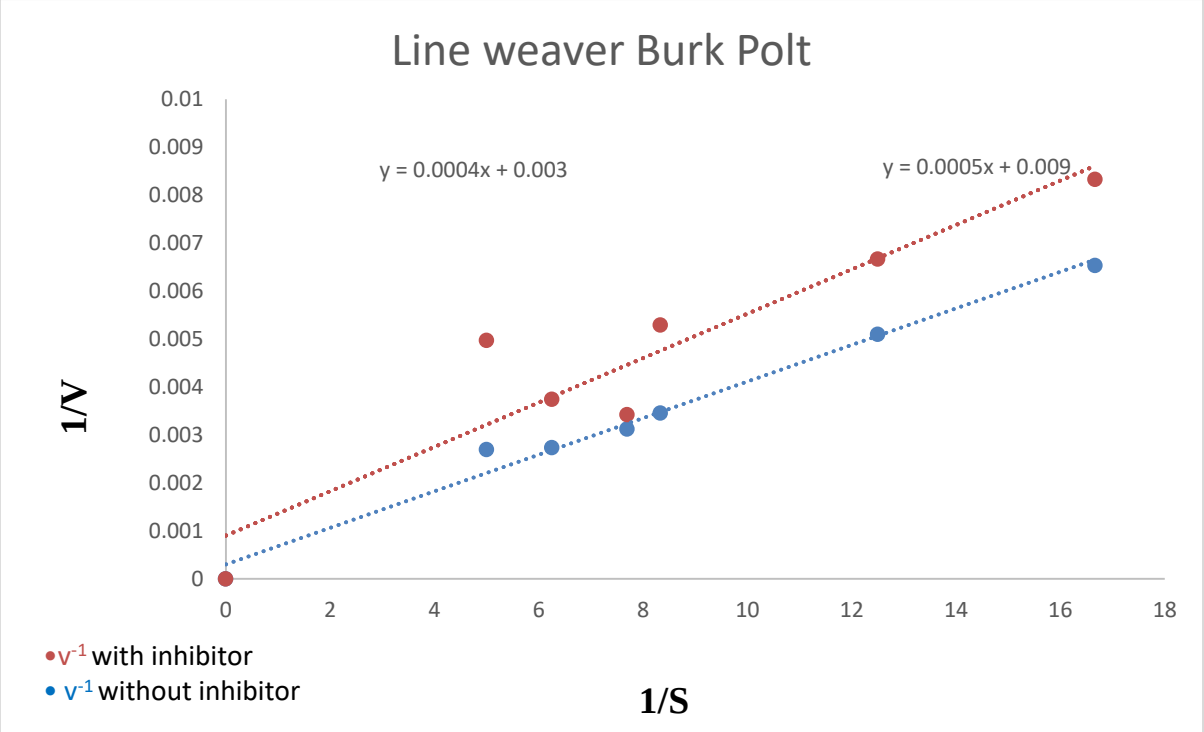


Figure (3-13): (Line weaver Burk plot) Equation of LDH Enzyme with and without the inhibitory effect of ZnO NPs

Table (3-10): The type of inhibition for each extract affecting on LDH enzyme

	Name of compounds	Type of Inhibition	K_m	V_{max} Absent of inhibitor	V_{max} With inhibitor
1	*Capparis Spinosa* L. Fruit aqueous extract	Competitive Inhibition	Without inhibitor 0. 16 With inhibitor 0.13	333.3	333.3
2	ZnO NPs using *C. spinosa* L. fruit extract	Un Competitive Inhibition	Without inhibitor 0.16 With inhibitor 0.04	333.3	111.11

3.2.5 Anti-Bacterial Activity of *C. Spinosa* L. Fruit extract and ZnO NPs.) .

The antibacterial activity of ZnO NPs and *C.spinosa* L. fruit extract was tested using Agar wells diffusion method, after 24 h of incubation at 37 ºC (table 3-11). ZnO NPs and *C.spinosa* fruit extract caused different inhibition zones ranging for the ZnO NPs from the highest (19 mm) against *S. aureus* at 200 mg/ml concentration.while the lowest inhibition zone was recorded (18mm) .on *E.Coli* at 200 μg /ml concentration the highest (17mm) while the lowest(15mm) at concentration 100 μg/ml. On the other hand, Other inhibition zones caused by *C.spinosa* L. ranged from the highest (17 mm) against *S. aureus* at 200 μg /ml concentration while the lowest inhibition zone was recorded (13mm) on E.Coli at 100 μg /ml concentration.

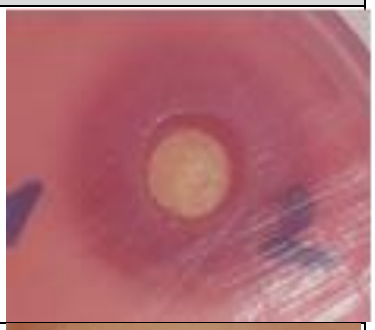
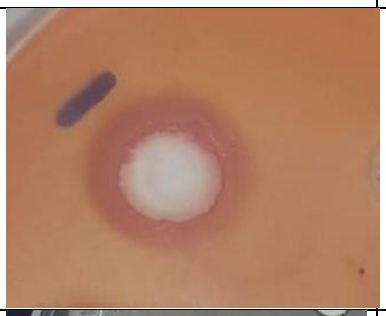
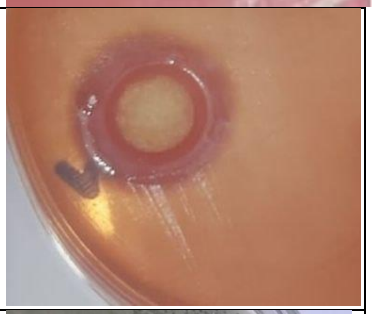
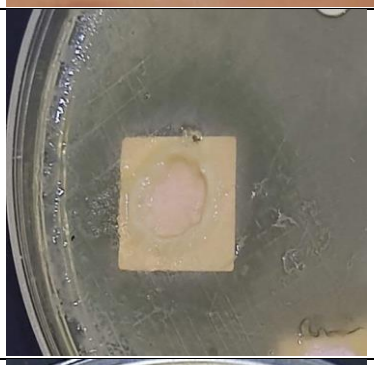
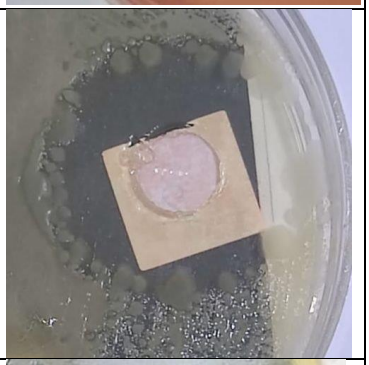
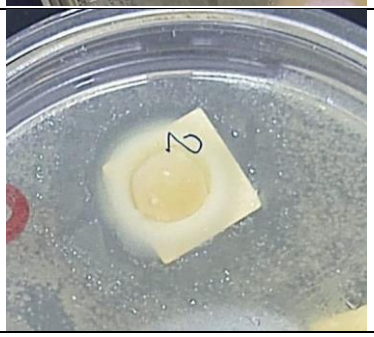
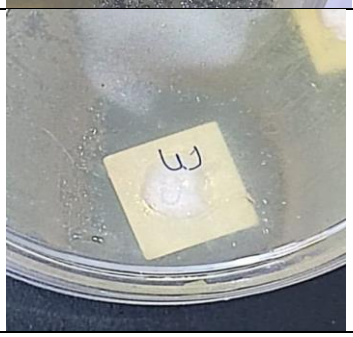
The possession of the *Capparis spinosa* L. Fruit extract an effect for killing germs, due to it possesses phenolic compounds and tannins ,the ability of tannins to form strong complexes with proteins is the most important aspect of their nutritional and toxicological effects that precipitate the proteins of the microorganism due to the formation of hydrogen bonds between the aromatic hydroxyl groups and proteins, which will lead to the inhibition of enzymes necessary for the metabolism of microorganisms[158].

The main mechanism proposed that ZnO NPs are exposed to bacterial cells and frequently followed by penetration into the cell, which might result in membrane damage [159].According to this theory, ZnO nanoparticles are gathered close to the bacterial cell membrane, and the size and shape of the particles affects how quickly they dissolve [160].Most importantly, it has been discovered that NPs with a positive charge are more hazardous because the positively charged ZnO NPs are electrostatically drawn to the negatively charged bacterial cell wall, increasing their potency [161].The function of the

bacterial electron transport chain is altered as a result of ZnO NPs' positive charge [162]. According to some theories, NP adsorption harms the cell by first interacting with it, creating a barrier between the cell wall and the cytoplasm and abnormal pit formations on the cell wall. This interaction depolarizes the cell and changes its negative charge, making the cell wall more permeable and allowing ions to enter the cell more easily, disrupting transport control [163].

Table: (3-11) antimicrobial activity of crude extract and ZnO NPs

Bacteria	Zone of Inhibition (diameter in mm)	
	C.spinosa L. fruit Extract (µg /mL)	
	100	200
S. aureus	16	17
E. coli	13	15
Bacteria	Zone of Inhibition (diameter in mm)	
	ZnO NPs(µg /mL)	
	100	200
S. aureus	18	19
E. coli	15	17

Concentration µg/ml		100	200
ZnO NPs	*S. aureus*		
	E. coli		
C.spinosa fruit extract	*S. aureus*		
	E. coli		

Figure(3-14) Antbacterial activity of ZnO NPs Synthesized using *C.spinosa* fruit Extract against Human Bacterial Pathogens.

CHAPTER FOUR

Conclusions and Recommendations

Conclusion

The present study demonstrated the cost-effective and eco-friendly synthesis of ZnO NPs was performed using *C.spinosa* L.extract as an effective reducing as capping agent. Through UV–Vis spectroscopy, the synthesis of the NPs has been confirmed by the peak at 374 nm whereas DLS, FESEM, and EDX indicated the size, shape, stability, the FESEM measurements showed that the ZnO nanoparticles were quasi spherical with an average size of 37.49 nm and DLS showed that ZnO NPs' average hydrodynamic diameters were determined to be 116 ± 8.0 nm. EDX and FTIR confirmed that the powder samples were pure ZnO nanoparticles without any defects or impurities . The capping of the synthesized ZnO NPs with bioactive compounds of the *C.spinosa* L. was confirmed with FTIR analysis. Also, the crystal structure of the NPs was confirmed by the XRD analysis. The ZnO NPs showed no cytotoxic activity against normal cells in a dose-dependent manner. Our data indicated that the synthesized ZnO NPs have an antioxidant effect using DPPH assay. The ZnO NPs has low hemolysis activity. The green synthesized ZnO NPs with *C.spinosa* L. extract has the ability to inhibit the Lactate dehydrogenase enzyme and anti bacterial activity against *S. aureus* and *E. coli*.

Recommendations

The following items may be suggested based on the findings of this study:

1. The qualities demonstrated by ZnO nanoparticles make them a potential material for pharmacological and medical applications. As a result, future study will include testing.

2. The antibacterial activity of ZnO nanoparticles using *C.spinosa* plant extracts, which may merit further investigation for therapeutic applications in the area of wound healing and tissue regeneration.

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الخلاصة

في السنوات الأخيرة، اكتسبت الكيمياء الخضراء الاعتراف باعتبارها نهجا قابلا للتطبيق لإنتاج الجسيمات النانوية. هدفت هذه الدراسة إلى استكشاف خصائص جسيمات أوكسيد الزنك النانوية (ZnO NPs) المنتجة باستخدام مستخلص ثمرة نبات الشفلح كعامل اختزال فعال. ركز التحقيق على جوانب مختلفة، بما في ذلك السمية الخلوية، وتأثير مضادات الأكسدة، والتوافق الدموي والأنشطة المضادة للبكتيريا، بالإضافة إلى العمل المثبط المحتمل على إنزيم لاكتيت ديهيدروجينيز (LDH). تم تأكيد توصيف المستخلص المائي لثمرة الشفلح وZnO-NPs باستخدام تقنيات مثل التحليل الطيفي للأشعة فوق البنفسجية (UV-Vis)، التحليل الطيفي للأشعة تحت الحمراء (FTIR)، كروماتوغرافي الغاز-مطيافية الكتلة (GC-MS). أثبتت هذه التحاليل وجود مركبات حيوية نشطة، مثل القلويدات والفلافونويدات والبوليفينول والستاتينات والفيتامينات، في المستخلص المائي. تم التعرف على ZnO-NPs من خلال حيود الأشعة السينية (XRD)، وتشتت الضوء الديناميكي (DLS)، وإمكانات زيتا (ζ)، والمجهر الإلكتروني لمسح الانبعاثات الميدانية (FESEM). أشارت النتائج إلى أن ZnO-NPs نقي للغاية وبلوري، ويظهر جزيئات متكتلة وكروية بمتوسط حجم حوالي 37.49 نانومتر. بالإضافة إلى ذلك، يبلغ الحد الأقصى لطول موجة الامتصاص للأشعة فوق البنفسجية 374 نانومتر، في حين كانت قياسات ζ المحتملة وDLS لـ 44.76- ZnO-NPs مللي فولت و116 ± 8.0 نانومتر على التوالي. قامت الدراسة أيضًا بتقييم نشاط مضادات الأكسدة لجسيمات اوكسيد الزنك النانوية باستخدام اختبار DPPH، بالإضافة إلى التوافق الدموي على كريات الدم الحمراء والخصائص السامة للخلايا من خلال اختبار MTT. أظهرت ZnO NPs توافقًا حيويًا جيدًا مع خلايا الدم الحمراء (RBCs) في تجربة النشاط الانحلالي، مع عدم وجود تفاعل انحلالي ملحوظ عند الجرعات من 7.5 إلى 120 جم / مل. علاوة على ذلك، أظهر المستخلص وأوكسيد الزنك نشاطا مضادا للجراثيم ضد بكتريا S.aureus وE.coli. ومع ذلك، أظهرت تراكيز المستخلص المائي القدرة على تثبيط إنزيم لاكتيت ديهيدروجينيز عند تركيز 0.13 ملي مول/لتر. في الختام، يحتوي المستخلص المائي لثمرة نبات الشفلح على مركبات نشطة بيولوجيا يمكن استخدامها كمثبطات لكل من الخواص المضادة للبكتيريا وإنزيم لاكتيت ديهيدروجينيز (LDH). أظهرت ZnO NPs المولدة حيويا عمليات تثبيط جيدة من حيث النشاط المضاد للبكتيريا، ونشاط مضادات الأكسدة، وأظهرت الحد الأدنى من السمية الخلوية.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة ميسان

كلية العلوم

قسم الكيمياء

التخليق الحيوي لجسيمات اوكسيد الزنك النانوية باستخدام مستخلص نبات الشفلح ودراسة الفعالية البايلوجية

رسالة مقدمة الى

كلية العلوم / جامعة ميسان جزء من متطلبات نيل

شهادة الماجستير في علوم الكيمياء

من الطالبة

سلمى عزيز نعمة الموسوي

بكالوريوس علوم كيمياء / جامعة ميسان (2014)

بإشراف

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2023 م

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