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Green Synthesis of Zinc and Nickel Oxides Nanoparticles and Study of their Biological Applications

A thesis submitted to

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By

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B.Sc. in chemistry 2016

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بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

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We, the members of the examining committee, certify that after reading this thesis entitled **"Green Synthesis of Zinc and Nickel Oxides Nanoparticles and Study of their Biological Applications"** and have examined the student **(Baraa Yousif Hussein)** in its contents, that in our opinion, it is accepted as a thesis for the degree of master of science in chemistry.

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Dedication

All praise to Almighty Allah who gives me the ability to write this thesis. It gives me pleasure to dedicate this thesis to my beloved parents, my wonderful brothers and my lovely sister. They have been supporting me since the beginning of my study. Also, I would like to dedicate this thesis to my supervisor **(Prof. Dr. Ahmed Mishaal Mohammed)**, this work would have not been possible without his great support and supervision.

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Baraa Yousif

Abstract

Nanotechnology has recently emerged in many applications, including medical use. It provides alternatives to the development of controlled release systems for the treatment of different diseases and reduces drug side effects. Nano-biotechnology Focuses on nanoparticle synthesis and their application. It plays an important role in carcinogenic treatment. In addition, many species of pathogenic bacteria have developed high resistance to the commercial anti-biotics, thus, nanomaterials can be used to prevent their growth. The grape extract is rich in phytochemicals such as polyphenols, flavonoids, and antioxidants, which are capable for reducing of metal salts. The phytochemicals found in grape extract have high therapeutic benefits, making them an attractive alternative to traditional toxic reducing agents.

In this study, zinc oxide (ZnO) and nickel oxide (NiO) nanoparticles were successfully prepared by the green method from aqueous grape extract (*Vitis vinifera*) to generate multi-functional metal oxide nanoparticles. Therefore, this study aims to evaluate the efficiency of ZnONPs and NiONPs in vitro for destroying of human breast cancer cell line (MCF7) and human brain cancer cell line (AMGM5), as well as the effectiveness of these nanoparticles in suppressing the growth of *Staphylococcus aureus* and *Klebsiella pneumonia*.

To characterize and examine the zinc and nickel oxides nanoparticles, physical diagnostic techniques were used such as UV-Visible spectroscopy, Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), atomic force microscope (AFM), scanning electron microscope (SEM), and transmission electron

microscope (TEM) which demonstrated that these nanoparticles have unique chemical and physical properties. The anti-cancer properties of ZnONPs and NiONPs were determined against human MCF7 and AMGM5 cell lines by using the cytotoxic MTT assay. The anti-bacterial properties were also identified against *S. aureus* (G^+) and *K. pneumonia* (G^-) by using an agar well diffusion assay and MIC assay., biosynthesized nanoparticles have shown high efficiency as anti-cancer and anti-bacterial agents in a dose-dependent manner.

In conclusion, our findings revealed that the green biosynthesis of ZnONPs and NiONPs from aqueous grape extract (*Vitis vinifera*) gave in sight and encouraging preliminary results for future work to be used in the therapeutic approach as an anti-cancer and anti-microbial medications.

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List of Symbols and Abbreviations

Symbols and abbreviation	Full name
Abc	Absorbance
AFM	Atomic force microscope
AMGM5	Ahmed Majeed Glioblastoma Multiform-2005
Cm	Centimeter
COX-2	Ciclo-oxygenase-2
CVD	Cardiovascular Diseases
D	Dimension
DLA	Daltons lymphoma ascites
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
e.g	Example
EDTA	Ethylene diamine tetraacetic acid
ER	Estrogen receptor
FT-IR	Fourier transform infrared
G	Gram
G^-	Gram negative
G^+	Gram positive
GBM	Glioblastoma Multiform
H	Hour
HAI	Hospital acquired infections
HIV	Human immunodeficiency virus
HPV	Human Papillomavirus Vaccines
ICAM-1	Intercellular adhesion molecule-1
IFN- c	Interferon-c
IL	Interleukin
JCPDS	Joint Committee on Powder Diffraction Standards
K.	*Klebsiella*
LDL-c	Low-density lipoprotein
LiBs	Lithium ion batteries
µg	Microgram
µL	Microliter
m	Meter
M	Molar
MBC	Minimum bactericidal concentration
MCF	Michigan cancer foundation

Symbols and Abbreviations	Full name
MCP	Chemotactic protein-1
MIC	Minimum inhibitory concentration
Min	Minute
mL	Milliliter
mm	Millimeter
M.W	Molecular weight
MRI	Magnetic resonance imaging
MTT	Thiazolyl Blue Tetrazolium Bromide
NF-jB	Nuclear Factor-jB
NiONPs	Nickel oxide nanoparticles
Nm	Nanometer
Nrf2	Nuclear factor (erythroid-derived 2)-like
^{o}C	Centigrade/Celsius
pH	Power of hydrogen
ROS	Reactive oxygen species
rpm	Revolutions per minute
RPMI	Roswell park memorial institute
S.	*Staphylococcus*
SDM	Mean of standard deviation
SEM	Scanning electron microscope
SIRT-1	Silent information regulator 2/sirtuin-1
SMC	Smooth muscle cells
TEM	Transmission electron microscope
UV	Ultraviolet
VCAM-1	Vascular cell adhesion-1
XRD	X-ray diffraction
Zn^{+2}	Zinc (II) cation
Zn^{0}	Zinc atom
ZnO	Zinc oxide
ZnONPs	Nanoparticles
ZnONPs	Zinc oxide nanoparticles
θ	Theta
λ	Wavelength
<	Less than
>	More than
%	Percent

CHAPTER ONE

1. Introduction

1.1 Grape

Grape is a smooth, fleshy, juicy fruit that grows on vine trees. Grapes appear in clusters that usually contain between six and three hundred fruits. Fruits vary in color and are black, red, or green-yellowish [(1)].

The studies of grape seeds and leaves showed that humans had been eating and feeding grapes since prehistoric times, grape is one of the most popular fruits produced in the world, with approximate 75 million tons per a year [(2)].

Also an grapes, which are sometimes referred to as *Vitis vinifera* L. (Vitaceae family), is one of the most global valuable fruits, used in nutrition (as fresh and/or dried fruits) [(3)], it is also for manufacturing medical purposes, the perfume and other commercial concern of the industries over time [(4,5)].

In addition, grape extract has a great potential as a reducing agent. The phytochemicals found in grape extract have a high therapeutic benefit, which are also environmentally benign, rendering them an enticing alternative to traditional toxic reducing agents [(6)]. Figure (1-1) display an image of the grape plant.

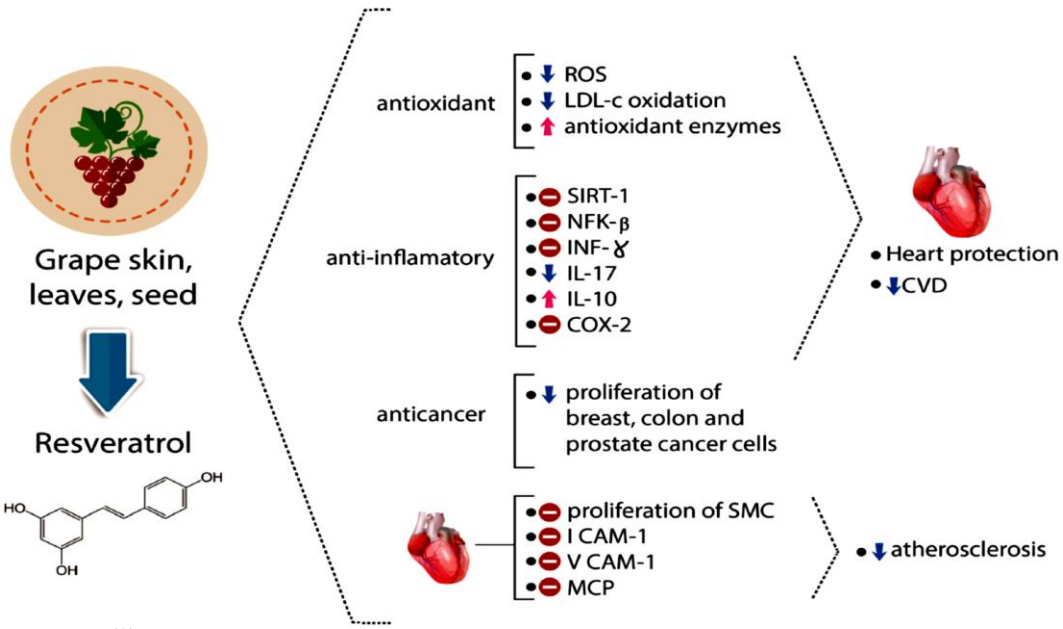
Figure (1-1): Shows image of the grape (*Vitis vinifera*) L. (Vitaceae family)

1.2 The benefits of grapes

Grape is considered fruits with good nutritional and therapeutic value [7]. The nutritional value of grapes of all kinds are characterized by the presence of a good percentage of sugars that are quickly absorbed and digested, as glucose sugar and fructose sugar are highly concentrated in grapes [8].

Grapes are rich in vitamins such as vitamin C and they also contain vitamin B. It contains a good proportion of mineral elements and mineral salts such as potassium, calcium and sodium. Grapes also contains substances with a therapeutic effect, as it contains a compound known as resveratol, which is characterized by its positive effect in reducing of arteriosclerosis, as it has a direct and noticeable effect in reducing of cholesterol, and then reduces of heart disease [9].

It also reduces bad cholesterol low density lipoprotein (LDL-c) in the blood. There are some acids in grapes that have a role in preventing the accumulation of free radicals, thus it is a good anti-



cancer [9]. As shown in Figure (1-2).

Figure (1-2): Shows the origin of resveratrol in the grape and its potential effects on health [10]

Green grape (also known as white grapes) is a rich source of carbohydrates and many of vitamins, such as vitamin C, K and A. It contains an anti-oxidant known as flavan-3. It also contains of potassium and iron, which are important for the health of blood circulation and arteries. Experts may recommend for focusing on green grapes because they contain an enough amount of resveratrol, which is an important compound as an anti-inflammatory agent. Experts believe that green grapes, in particular, contribute to enhance the digestion process, clean toxins from the body, and prevent the formation of stones in the gallbladder and kidneys [11]. Figure (1-3) shows structures of some phenolic compounds in grapes.

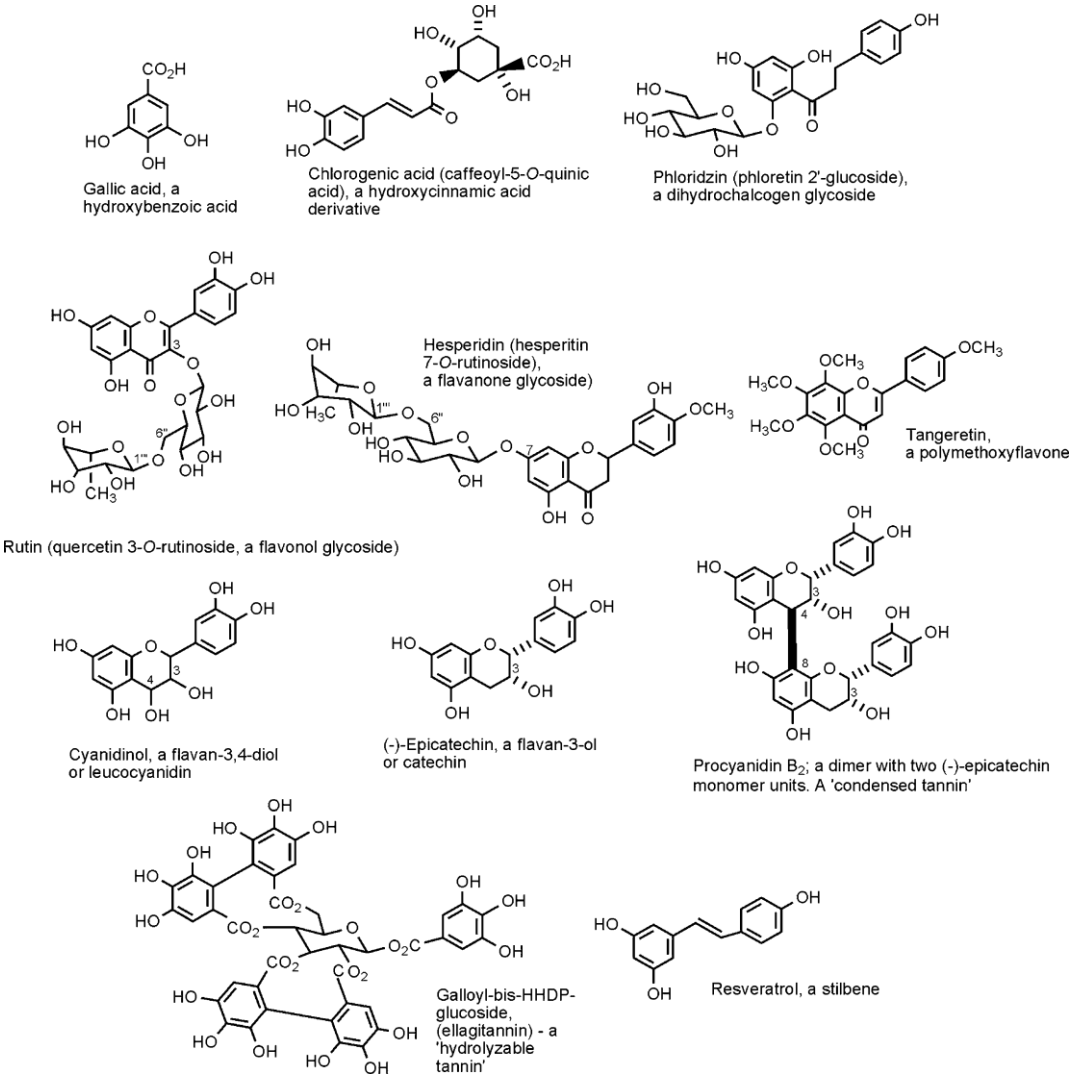


Figure (1-3): Structures of some phenolic compounds in grapes [11]

1.3 Nanotechnology

The word "nano" meaning one billionth of a unit (10^{-9}) which is derived from the Greek word "nanos" meaning very small or dwarf [12,13]. Nanotechnology is a multidisciplinary science. The new nano wide substances (1-100) nm can be associated with a variety of applications [14]. The new characteristics of nanoparticles include large areas, thermal conductivity, size, load capacity, shape, crystal structure, morphological surfaces and allow materials to be incorporated into biomedical and biotech [15,16].

Nanotechnology can be characterized by the regulation of shape and size on the nano-scale as the design, characterization, manufacture and application of materials, devices and systems [17]. Nanoparticles (NPs) synthesis and self-assembly are considered to be the foundation of nanotechnology [18].

The obvious example of nanoparticles is the atomic or molecular biosystems. For instance, the length of a typical bacterium is 200 nanometers and the diameter of a DNA strand is a mere two nanometers. Also, submicron (100-1000 nm), nano 2 (1-100 nm) and atomic (or angstrom, less than 1 nm) are however the three categories of microfabrication systems as shown in Figure (1-4) [19,20].

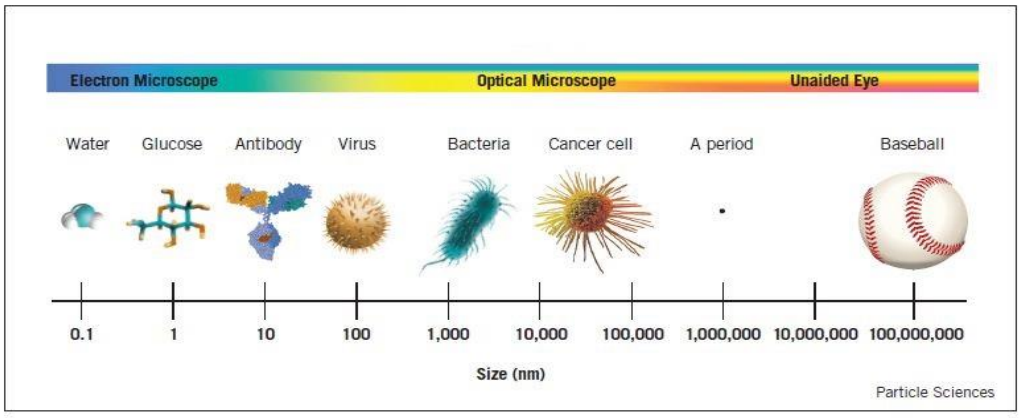


Figure (1-4): The sizes of different objects in nanometers [20]

Nanotechnology has gained broader prominence due to its applications like cosmetic products and skincare products that are use as abrasives, automotive polishes and drug delivery systems. Nanotechnologies are used for physics, chemistry and materials science. The large to volume proportion and the increased reactivity is due to their small size, nanoparticles produced with diameter < 100 nm that exhibit exclusive physicochemical characteristics [21,22].

As nano technologies not only resume miniaturisation unpretentiously from the micron meter level down to the nanometer

level due to their limited size, which gives wide areas, but it can also result in abrupt or radically different characteristics than the bulk. Nanotechnology are involved in various applications such as solar cells, batteries, electronic chips, cosmetics, delivery of pharmaceuticals, catalysis systems and sensors etc., enabling them to make significant progress in reaching the global market [23].

1.4 Applications of nanotechnology

Still today, a host of dangerous and diverse disorders remain a significant concern, including diabetes, cancer, Parkinsons and Alzheimer's disease, as well as different types of severe inflammatory and infectious diseases (e.g. HIV). Nanomedicine is an application of nanotechnology, have shown valuble result in health and medicine field [24].

Nanomaterials and nano electronic biosensors are used in nanomedicine. Nanomedicine will benefit molecular nanotechnology in the future. The medical field of nanoscience application has a lot of possible advantiges which will be beneficial wold wide. Early identification and prevention, as well as proper diagnosis, treatment, and follow-up of pathogens, are all possible with nanomedicine [25].

Biological examination has become more adaptive and versatile, and certain nanoscale particles are used as tags and stickers. With the advent of nanodevices such as gold nanoparticles, gene sequencing has become more effective. These gold nanoparticles can be used to identify genetic sequences in a sample when tagged with small fragments of DNA. Damaged tissues may be replicated or healed with the help of nanotechnology [26]. These artificially activated cells are used in tissue engineering, and have the potential to revolutionize organ transplantation and artificial implant placement. It is feasible to

develop advanced biosensors with new features. These biosensors can be used in astrobiology to shed light on the discovery of lifes origins. Sensors for cancer diagnostics are also being developed with this technology [27]. Figure (1-5) shows the applications of green nanotechnology.

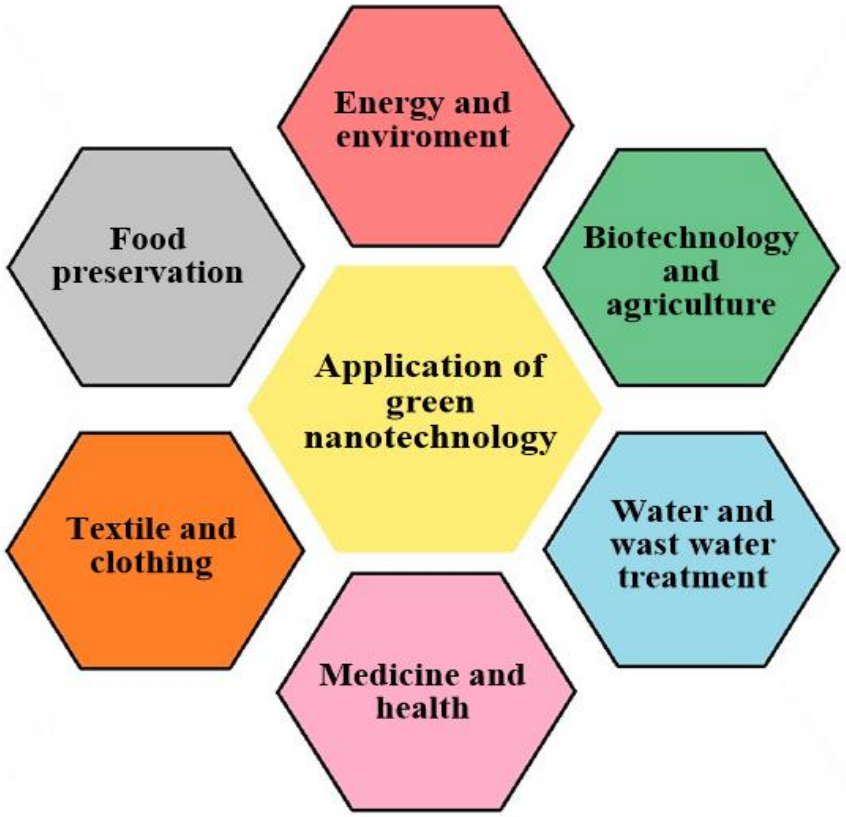


Figure (1-5): Applications of green nanotechnology [27]

1.5 Nanomaterials (NMs.)

Nanomaterials are chemical compounds or materials formed on a nano-scale with at least one external dimension [28]. Nanomaterials contain a melting point or a lower transitional step temperature compared with the bulk material. The increased energy from the surface makes nanoparticles reactive. Other characteristics, such as mechanical, electric, optical and magnetic properties differ from those of bulk substances nanostructures [29].

Nanomaterials are currently used for numerous strategies and priorities of human health protection, such as screening, diagnosis and treatment. The dimensions of the nanomaterials allow them to react with the biomolecules more efficiently [30].

1.6 Classification of nanomaterials (NMs)

As shown in Figure (1-6), NMs can be classified into four types : zero dimension (0D), one dimension (1D), two dimensions (2D) and three dimensions (3D) [31].

1- 0 D nanomaterials have both of their nano-scale lengths. Examples of 0D nanomaterials contain spherical materials nanoparticles and certain cubes and polygons.

2- 1 D nanomaterials at the nano-scale and one at the macroscale have two dimensions. Examples of 1D nanomaterials are nanowires and nanofibers.

3- 2 D nanomaterials, such as nano thin films and nanosheets, have just one nano-scale dimension on their three faces.

4- 3 D nanomaterials do not have nano-scale dimensions, but they contain nano-scale material building units that are limited to their 3D macrostructures [31,32].

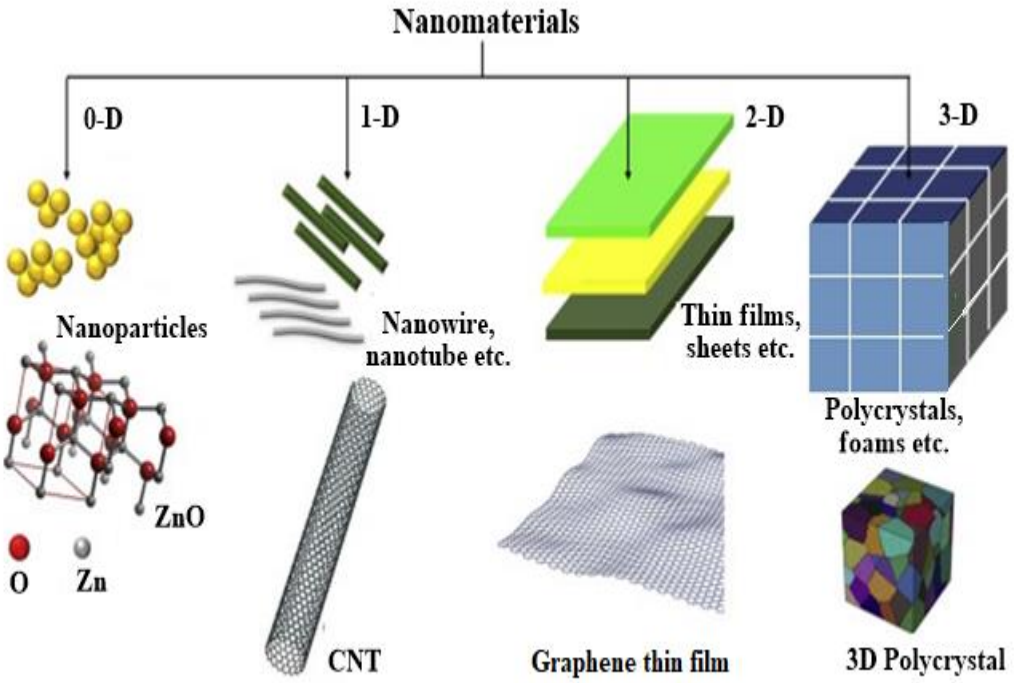


Figure (1-6): Classification of nanomaterials according to their dimensionally [32]

1.7 Nanoparticles

Nanoparticles (NPs) are small particles of at least one diameter of less than 100 nanometers. Recent advancements in nanotechnology have allowed researchers to synthesize NPs with dual modalities, combining diagnostic and therapeutic capabilities within a single NPs formulation. This gives NPs a distinct benefit over conventional therapies. As a result, NPs carry a lot of promise for theranostic applications and are widely used for personalizing nanomedicine-based therapies [33,34].

Organic and inorganic NPs are existed in nature and often used in nanotechnology, especially nanomedicine, due to their unique inherent properties. Inorganic NPs can be synthesized by bottom-up or top-down approaches. However, the bottom-up approaches allow for a wide range of flexible and controlled distributions of sizes (nm to μm scale), surface chemistry and physical properties where the materials

imply. For the prevention, diagnosis, and identification of diseases, inorganic NPs have been extensively studied in both preclinical and clinical research [35-37].

1.8 Applications of nanoparticles in the medical field

1- Pharmaceutical industries: Because nanoparticles are too small, the drug can be delivered not only to diseased tissues, but to infected cells with great accuracy, meaning that drug penetration improves greatly, which is also useful in reducing the side effects of the drug because it targets directly infected cells only, thus it reduces the symptoms of the side effects, which may occur on diseased cells, and also reduces the side effects that may occur from the drug targeting unplanned parts [38,39].

2- Diagnostic medical imaging: Nanoparticles are also used in medical radiation dyes. The dyes reach to the exact places to be diagnosed and are linked to them, this makes the diagnostic image matter more clear [40].

3- Treatment or repair of cellular damage: Nanoparticles can also be used for cancer treatment. Where nanoparticles reach to the clusters of cancer cells. They are heated by specific frequency waves (Radiofrequency), which leads to kill cancer cells without harming neighboring normal cells. If this technique is proven effective and safe, it may exchange with chemotherapy or radiotherapy, which have many side effects [41,42].

4- Nanoparticles are used in the diagnosis of some microbial diseases: The particles stick to anti-bodies which label the microbes inside the body. It is possible to capture signals from the nanoparticles to diagnose infection with this or that microbe [43,44].

5- Treating cancer diseases (a nano-submarine used to destroy cancer): Conventional chemotherapy works by killing both cancerous and non-cancerous cancer cells. Nanotechnology can produce micro-vectors that carry drug doses. These vectors are designed in such a way to prevent the immune cells in the body to recognize them [45]. These submarines perform two operations when they reach to the tumor area:

   a. First, they block the capillaries that feed the cancerous tumor.

   b. Their chemical or radioactive components are released to fisht the cancer cells only.

This technique was applied against group of mice at the American cancer center (Memorial Sloan Kettering). The cancerous mice were able to live more than 300 days after this treatment, while the mice that did not receive the treatment did not live more than 43 days [46,47].

1.9 Synthesis of nanoparticles

Physically, chemically and biosynthetically, NPs can be synthesized in two ways: Top-down and bottom-up approaches. According to Revaprasadu and Mlondo (2006), due to the regulation of the scale, form, and ease of management of these NPs, the chemical synthesis is the preferred method for the future applications [48].

1- Top-down approach

In accordance with this approach, it is aimed to reduce the material from macro dimension to nano size by giving energy to the raw material chemically or mechanically. Grinding, abrasion and chemical methods are frequently used in these processes. Metal nanoparticles and produced in this way generally have a high size. The

method of grinding with high energy balls can be given as an example of this approach [49].

2- Bottom-up approach

In this technique. Nanoparticles are obtained by giving energy to atomic or molecular sized materials through chemical reactions Figure (1-7).

This approach is preferred because it is cheaper and more effective in nanoparticle synthesis. Applications of this approach can be divided into two categories which include vapor (gas) phase (e.g., pyrolysis, inert gas condensation) and liquid phase (e.g., solvothermal reaction, sol-gel, biological method and microemulsion method) [49]. The bottom-up method on the other hand, depends on the self-assembly of atoms into nano-scale structures [15]. Examples of the bottom-up method are sedimentation and reduction methods, including sol-gel, chemical reduction, green synthesis and spinning [50].

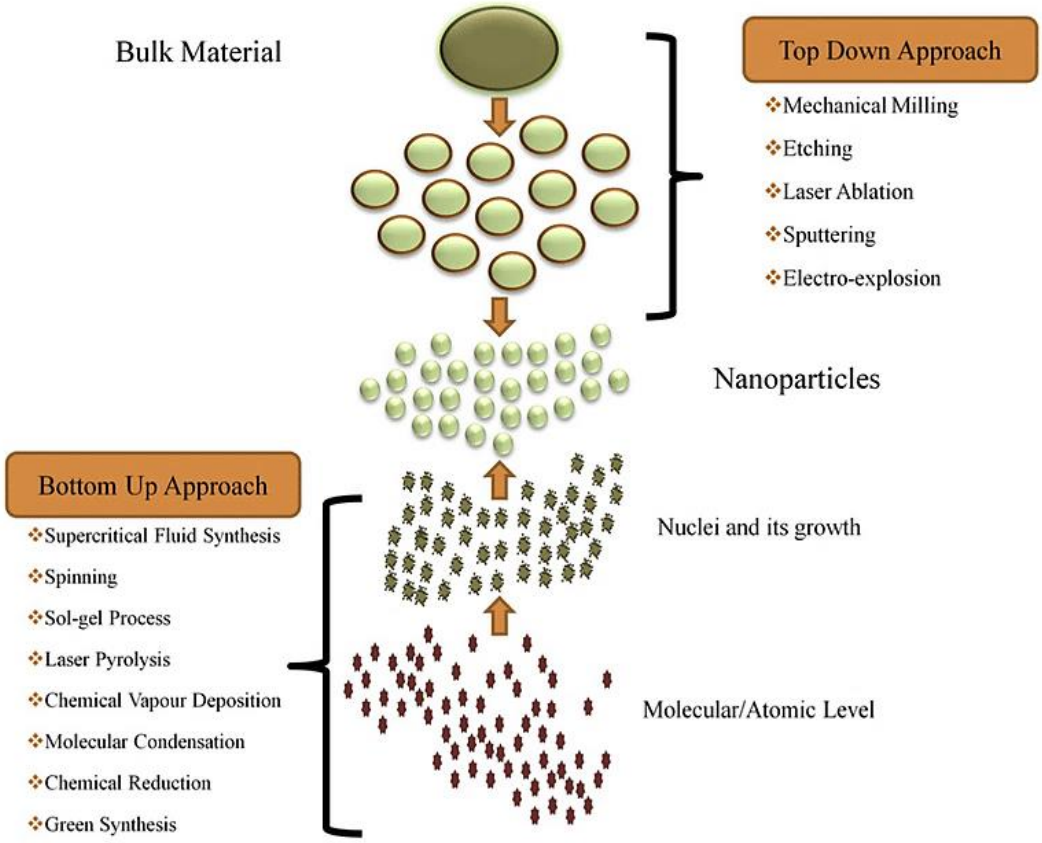


Figure (1-7): A schematic diagram shows different approaches for NPs synthesis

1.9.1 Physical synthesis

Physical synthesis uses the top-down approach that entails directly producing NPs or ordered assemblies from bulk materials by the generating atoms using different diffusion techniques. Dependent on the bulk substrate and the target effect, these methods of delivery or elimination could be mechanical, chemical, electrochemical, etc. Milling is one of the most commonly used methods for grinding big molecules. When the last size of molecules is obtained, milling become correctly controlled by computer assisted numerical systems [51].

1.9.2 Chemical synthesis

The bottom-up methodology is the cornerstone to approach the chemical synthesis. The basis of this method of NPs synthesis is done

when a metal salt is dissolved in a solvent and reduced to the state of zeroth valence. Starting materials are molecular elements, accompanied by a subscript indicating the number of atoms of that element in the molecule, in order to form complex clusters, chemical reactions, nucleation, and development [52,53]. Metal atoms formed by a solution reduction are primarily insoluble, which by a slow aggregation leads to clusters called embryos. The embryos that are moderately sized are either dissociated or mature to become stable. When the embryos have reached a certain scale of size, as stable particles, they separate from the solution and are referred as nuclei. The nuclei expand to nanosize particles and then metal atoms are diffused onto the particles and/or the nanosize particles are aggregated to form the final NPs. The density and shape of the NPs depends on the dominant phase [54].

NPs produced by aggregation are mainly have a spherical shape and a lower density [55]. If a capping agent does not shield the surface, there will be normal interactions between particles to decrease their high surface energy that induces the aggregation. Chemical or biological molecules as well as polymers may be capping agents [56]. They are responsible for processes of charge or steric stabilization that stop further agglomeration, making them integral components of NPs. As they synthesize particles that are bound to a substrate or set in a matrix, which limits their ability in applications, most physical methods have a drawback. The right choice of organic moieties as capping agents can decrease or increase toxicity in environmental systems [57,58].

1.9.3 Biological synthesis

The bottom-up technique is used for biosynthesis approaches such as chemical synthesis methods, whereby the main reaction is the reduction of the metal salts to form NPs. The use of toxic materials, poor content conversions, is complicated and inefficient purifications and high energy needs concern nearly all of the NPs synthesis methods. Biosynthetic methods based on biological microorganisms or plant extracts have emerged as a simple or feasible alternative to chemical and physical synthesis methods.

Green synthesis offers numerous developments because it is cheep, environmentally sustainable, conveniently scalable for large scale synthesis, and high pressure, electricity, temperature. In addition harmful chemicals do not need to be used [(59)]. There are typically three important conditions for green synthesis: Choosing a solvent medium; choosing an environmentally friendly reducing agent; and choosing non-toxic substances for NPs stability [(60)]. The natural material extract is thought to act as a reducing agent in the biosynthesis to processed of metal NPs [(61)]. Bacteria, fungi, algae and plants synthesize the NPs. Since noble metal NPs in their applications are in touch with humans, there is an increase need to develop eco-friendly processes to synthesize NPs for preventing exposure for toxic chemicals. Most of the techniques, however, are still in the developmental stage. Numerous issues with NPs stabilization, crystal growth regulation, and particle aggregation are frequently encountered [(62)]. Plant biosynthesis have an advantage over other systems in terms of morphological characterization, molecular distribution, primary and secondary metabolite processes, it allow an autotrophs to respond under a variety of conditions [(63)]. Other benefits plant biosynthesis are:

1- They are situated along a broad variety of ecological borders which make them readily accessible.

2- They are safe to deal with.

3- The valuable metabolites are stocked with them.

4- They make it easy to interpret every chemical protocol as green.

Their metabolites are seen as gems that, especially when related to metallic NPs synthesis, which have not been used to their maximum potential [64]. It can be preferable over other biological processes to use plants for NPs synthesis because:

1- It is readily available.

2- It reduces the need for costly equipments and the experience in the tissue culture.

3- It can be adapted in a non-aseptic setting for large scale synthesis of NPs [63]. As shown in Figure (1-8).

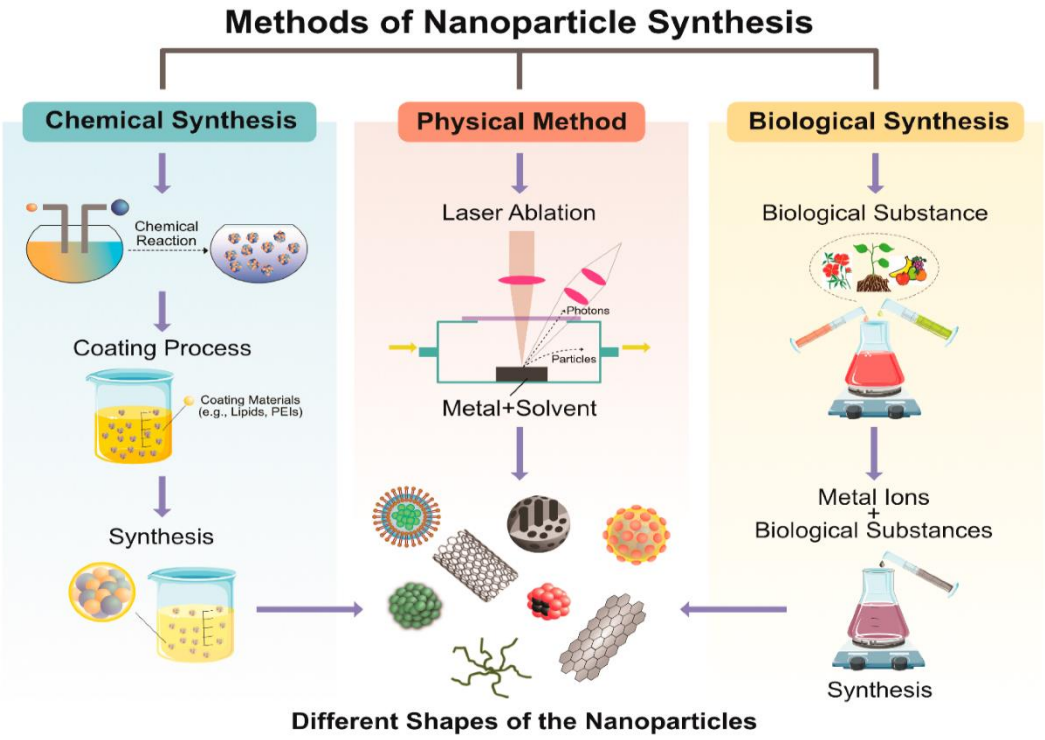


Figure (1-8): Methods of nanoparticle synthesis

1.10 Nanoparticles of metals and metal oxides

Metal oxide nanomaterials show a rich compositional and thus deliver tremendous potential in applications such as catalysis, optics, electronics, energy storage, biomedicine, etc. The combination of different characteristics such as morphology, form, scale, surface chemistry and crystal structure leads to the functional characteristics[65].

Metal oxide nanoparticles have a high surface area, mechanical stability, special composition, catalytic and redox properties that are beneficial and uncommon, and biocompatible [66].

The emergence of microbial anti-biotic resistance is on the increase at an unprecedented pace. Existing drugs are becoming ineffective which, requiring new solutions. Nano-scale metallic oxide NPs have emerged as a possible candidate due to lower toxicity, high resilience, greater stability and selectivity, among other for methods approaching the problem [67].

The value of using inorganic metal oxides for biomedical applications is that they contain essential human mineral elements and they one productive at very low concentrations [68].

Nanoparticles of metal oxides are also used of cancer theranostics, neurochemical testing and surgical implants. Some metal oxides have been used successfully as nanoprobes for cell isolation, labeling and gas sensing. They are often used as contrasting agents and for direct distribution of therputants in Magnetic Resonance Imaging (MRI) [69].

Metal oxides have the ability to satisfy our ever growing energy requirements by delivering revolutionary energy storage solutions in addition to numerous biomedical applications [70].

In recent decades, major research is the manufacture of energy-smart devices such as Lithium Ion Batteries (LiBs) and supercapacitors has been supported [71].

1.11 Zinc oxide

Zinc oxide (ZnO), an n-type metal oxide semiconductor, is being investigated for various applications due to its tunable and multifunctional spintronic, photonic, and morphological properties [72].

ZnO is an inorganic compound that crystallizes into two main structures: Wurtzite (hexagonal) and zinc blende (hexagonal) or (cubic). Figure (1-9) shows a hexagonal structure that is more stable in ambient conditions, ZnO is characterized by a 3.37 eV direct and broad band gap and has 60 mev of high excitation energy. ZnO has efficient pyroelectric and piezoelectric functions [73].

Table (1-1): Physical and chemical properties of zinc oxide [73]

Property	Value
Symbol	ZnO
Mass density	5.60 g/cm^3
Molar mass	81.39 g/mol
Melting temperature	1975°C
Density	19.3 g/cm^3
Direct band gap	3.37 eV
Crystal structure	Hexagonal wurtzite
Type of semiconductor	n-type
Refractive index	2.0041

Because of its intriguing properties, ZnO has been successfully used in a variety of applications, including metal insulator semiconductor diodes, miniaturized semiconductor lasers, optically transparent electrodes, nanogenerators and ultraviolet photo detectors, surface acoustic instruments, and gas sensors, among others [74].

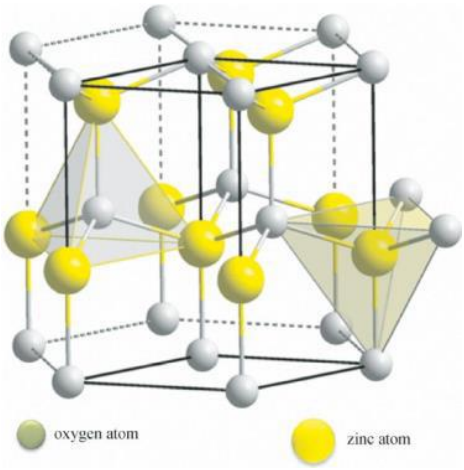


Figure (1-9): The crystal structure of ZnO

The diverse chemical and physical properties of zinc oxide are widely used in many fields in a host of applications. In several fields of chemistry, physics and materials science, metal oxide nanoparticles play an important role. Metal oxides nano parts can exhibit unusual physical and chemical properties due to their small size and high density of angles and edge surfaces [75].

1.12 Zinc oxide nanoparticles and properties

Recenly, zinc oxide nanomaterials have developed new properties as a result in the rapid advancement of nanotechnology. The large surface area of the nanoparticles has an advanced catalytic propensity with respect to the size and catalytic activity of ZnONPs with their diameter below 100 nm [76].

ZnO is among five zinc compounds identified as safe band [77]. ZnO has been studied in its possible medicinal uses, such as sunscreens, lotions and cosmetics, owing to its biocompatible nature. ZnO has also been used for drug delivery and bioimaging [78]. In order to develop them as an anti-microbial and anti-cancer agent, comprehensive research is being carried out on ZnO nanoparticles [79].

1.13 Applications of zinc oxide nanoparticles

1.13.1. Sunscreens

The major benefit credited to ZnONPs is that they can be used as sunscreens. Spreading and scattering light, zinc oxide particles between (200 – 400) nm distinctly white on the skin. Yet, nanoparticles absorb and dissipate UV light. They absorbing visible wavelengths, invisibly makes sunscreens on the skin, thus NPs gain the consumer wanted characteristics [80].

1.13.2 Textile coatings

ZnONPs textile coatings have more air permeability than their counterparts that produce larger particles [81]. Moreover, these coatings have an anti-bacterial feature and nanoparticles properly amended ensure the self cleaning of the substrate [82].

1.13.3 Anti-microbial effect

Anti-microbial activity is an advantage of ZnO at the nano-scale. Zinc oxide is used for accelerating wound healing in the form of ointments and creams. It is also used in dental fillings for temporary purposes [82]. It is highly involved in the fight against foodborne bacteria which has been shown to be used as a preservative and food packaging component [83].

1.13.4 Rubber production

It is common for ZnONPs to use good thermal conductivity in the manufacture of various linked rubber products [84]. Thermal conductivity of rubber is very low. The addition of a filler that, for example, can be modified to prevent aggregation, ensures high thermal rubber conductivity, while retaining the elasticity of zinc oxide nanoparticles. In addition, ZnONPs enhance flexibility and speed the process of vulcanization [85].

1.13.5 Sensors

Zinc oxide is used also as a semiconductor which has many applications in electronics and electrical engineering, due to its potential to emit light under the influence radiation. Their higher sensitivity is achieved by the use of nanometric particle sized sensors for carbon monoxide and other gas detection [82,86].

1.13.6 Cancer therapy

Although extracellular ZnO has been shown to be biocompatible, their higheist concentrations of intracellular ZnO are apparent to increase cytotoxicity and oxidative stress caused by zinc mediated protein imbalance [87]. One of ZnONPs cytotoxicity mechanisms for cancer is inducing oxidative stress on cancer cells, the ZnO nanoparticles have a unique capacity to do. This property is because that ZnO is a semiconductor. ZnO causes the development of reactive oxygen species (ROS), which causes oxidative stress and, lead to exceed, cell death due to the anti-oxidant capacity of the cell [88]. Several in vitro experiments have shown that ZnONPs have selective cytotoxicity against cancer cells [89,90]. Zinc oxide nanoparticles are multifunctional as shown in Figure (1-10).

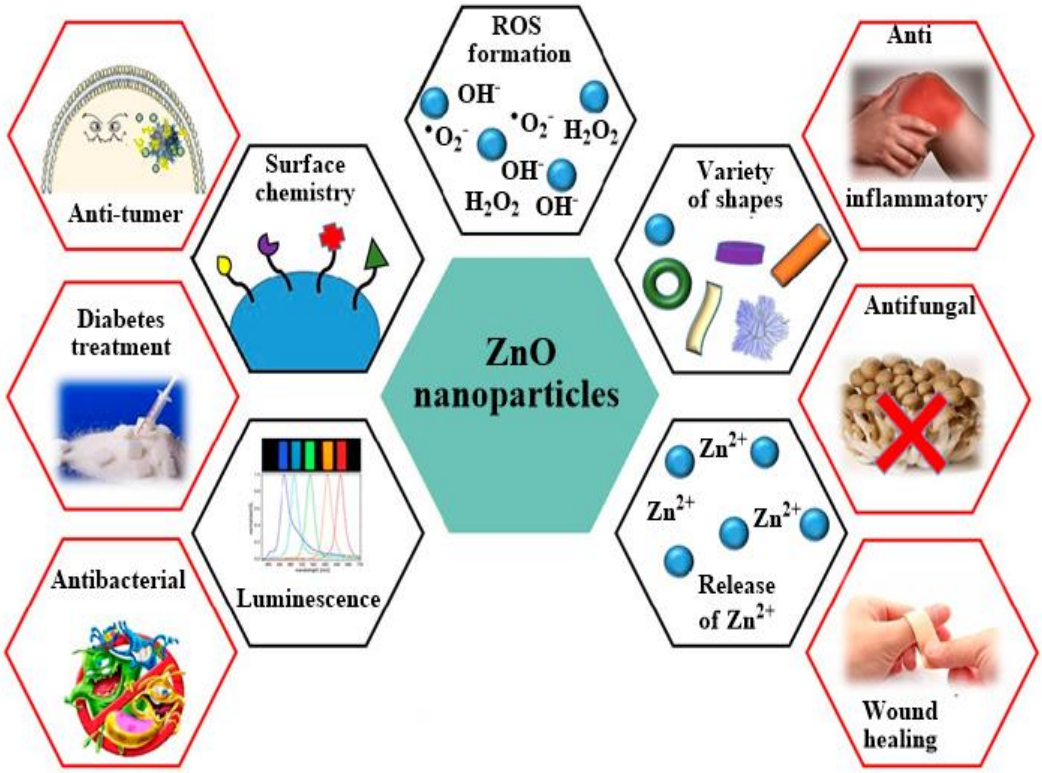


Figure (1-10): Zinc oxide nanoparticles are multifunctional

1.14 Nickel oxide

Nickel oxide (NiO) is a semiconductor with a p-type and well known for its excellent electrical and optical properties, and chemical stability with a broad band gap (3.6 - 4.0) eV. The NiO structure is identical to the sodium chloride (NaCl) structure, known as the rock salt structure Figure (1-11) [91].

Table (1-2): Physical and chemical properties of Nickel oxide [91]

Property	Value
Symbol	NiO
Color	Green powder
Molar mass	74.69 g/mol
Mass density	6.67 g/cm^3
Melting point	1957 °C
Band Gap	(3.6 - 4) eV
Crystal structure	Rock salt (Octahedral)
Type of semiconductor	p-type

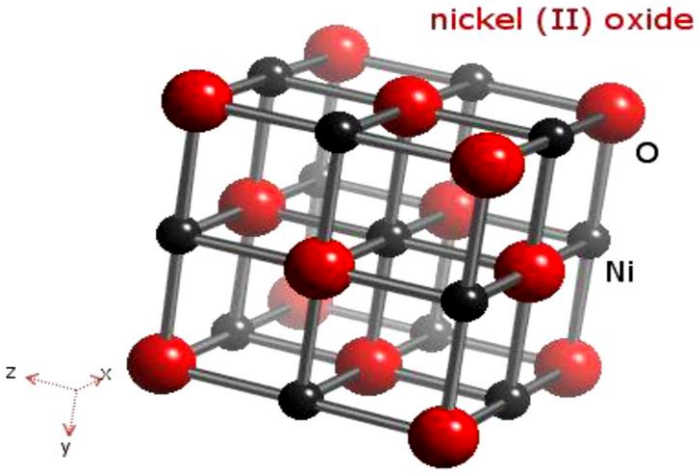


Figure (1-11): The crystal structure of NiO

Although NiO particles are extremely high ionization and high isoelectric point, they are anti-ferromagnetic. Due to specific characteristicof NiO particles, they are desirable material for study [92].

NiO particles are used for electrochemical storage, anodic electrochromic smart screens, gas meters, battery materials, etc. [93].

It sees use in the adsorption of inorganic contaminants and dangerous dye as an active ingredient. NiO nanoparticles has been studied for multiple medicinal applications as a result of its anti-inflammatory nature. A possible anti-oxidant, anti-cancer and anti-microbial nature has been demonstrated by recent research [94].

1.15 Nickel oxide nanoparticles and properties

Biologists and chemists are particularly interested in NiONPs because of their numerous uses in battery electrodes, magnetic materials, heterogeneous catalysts, gas sensors, electrochromic films, and solid oxide fuel cells, as well as their ability to aid in the adsorption of inorganic pollutants and dyes. By producing ROS, inducing oxidative stress, and releasing (Ni^{2+}) inside the cell, NiONPs

have shown toxicity against a variety of microbial agents and microalgae [95].

Nanoparticles like NiO are particularly promising in advanced studies due to their use in catalysis, gas sensors and optoelectronics. Metal oxides are low cost components used for a wide variety of optical applications. NiO was examined as chemical sensors, photocathodes in dye-sensitized solar cells, catalysts, magnetic materials, due to its chemical stability and magnetic or optical characteristics [96,97].

1.16 Applications of nickel oxide nanoparticles

1.16.1 Anti-cancer activity

Nickel oxide nanoparticles cause cytotoxic effects in cancerous cells, when compared to other treatments such as chemotherapy and surgery. The treatments goal is to destroy cancer cells without harming healthy cells [98].

1.16.2 Anti-bacterial activity

Nickel oxide nanoparticles are highly resistant to common anti-biotics and due to their excellent characteristics, such as large surface areas, exhibits altitude action against bacteria and viruses, These drug-destroy or suppress bacteria development [99].

1.16.3 Toxic chemicals

Metal oxide particles have ensured effective adsorption content. And a decomposition of a large amount of volatile and permanent

chemicals within a variety of solid substances for the treatment of toxic chemicals [100].

1.16.4 Electronics

Nickel nanoparticles take crucial components used for microchips into consideration and produce resistors which are also of huge effect, due to the excellent chemical and physical properties of NiONPs[101].

1.17 Cancer

Cancer Is a group of diseases involving abnormal cell growth with the potential to spread to other parts of the body, affecting populations in all countries and all regions. Usually, it is not possible to determine exactly why one person gets cancer and another person doesn't get it. However, some of factors may increase the risks of an individual developing cancer [102]. Risk factors for cancer include being vulnerable to toxins or other ingredients. Other factors involved, such as age and personal history. In addition, diet, physical activity, weight, alcohol intake, sun exposure, and infection exposure such as hepatitis, human Papillomavirus Vaccines (HPV), and human immunodeficiency virus (HIV) are risk factors of cancer [103].

The medication to kill cancer cells is chemotherapy, which is operated by preventing the division and growth of cancer cells. It has a more important effect on tumoral cells than normal cells. However, it is widely understood that the medications used for chemotherapy may cause harm to healthy tissue. Therefore, several of side effects associated with the use of chemotherapy, such as nerve injury, problems with pregnancy, or heart damage [104].

1.17.1 Classification of cancer

Cancers can be classified based on the similarities between the cancerous cell and a healthy cell. Examples of types Cancers [105]:

1- Carcinomas: These cancers originate from the epithelium and constitute the largest group of general cancers, especially in breast, prostate, lung and pancreatic cancer.

2- Sarcoma: These cancers stem from connective tissue (ie, bone, cartilage, fat, and nerves).

3- Lymphoma and leukemia: These two cancers stem from the blood forming cells.

4- Sex cell tumors: These tumors usually occur within the gonads (ovaries and testes).

5- Glioblastoma: It is a tumor that resembles immature or fetal tissue and is more common in children.

1.17.2 Symptoms and causes

Cancer can effect a wide range of cells, the profile of its effects very complex. Invasion and compression of adjacent tissues by solid tumors can contribute to disturbance of the functioning of body tissues and organs. Swelling, discomfort, and jaundice can be common symptoms. Systemic symptoms are more invasive; most common symptoms of cancer are weight loss, fatigue, waste, and anemia, swollen liver, bone pain, and neurological pains [106,107].

Sarcomas and hematopoietic malignancies are a result of oncogene activation, while carcinomas are a result of tumor suppressor deactivation [108]. The DNA mutation that occurs to trigger or inactivate all these gene types is a result from a number of assaults. Ionizing radiation contributing to chromosome fusions, are due to its

ability to induce chromosomal translocations. This may contributes to the development of gene product with a new features, or upregulation of the gene if the proto oncogenes are merged with a powerful promoter [109]. Cells can become cancerous by carcinogenic chemicals such as tobacco smoke, either by a direct DNA mutation or through encouraging mutated cell proliferation [110]. Some forms of viruses can add exogenous oncogenes, facilitate replication or inhibit apoptosis, resulting to malignancies [111].

1.17.3 Breast cancer

Breast cancer is the most often diagnosed cancer in women, and it's the most common cause of cancer mortality, 23% of all cases and 14% of cancer deaths. Thus, the economic and psychological impact of this cancer must be tackled by research [112]. Breast cancer is a set of molecular cancers that arise from the epithelial cells of the breast, rather than a single disorder, which is evident in recent years [113]. Cell lines play an important role in the molecular diagnosis of breast cancer because they can be used in a variety of lab research settings, including in vitro cancer models [114].

Michigan Cancer Foundation (MCF-7) cells are a very important candidate in breast cancer, because they are found in abundance in estrogen receptor (ER)-positive breast cancer cells and numerous sub-clones [115]. MCF-7 is a type of cancer cell. Dr. Soule and his collaborators at the Michigan Cancer Foundation observed a pleural effusion in a 69-year old woman with metastatic disease in 1973 [116].

1.17.4 Brain cancer

Brain cancer is an irregular brain division that exponentially increases and invades the healthy tissues around it. The brain is one of the most essential organs in the body, if not the most crucial. It is

responsible for the signal transfer to the rest of the body. The brain must be protected and any damage are disastrous which cause some bodily functions prevented. The importance and fragility of the brain, survival rates for brain cancers are reasonably low [117].

Ahmed Majeed Glioblastoma Multiform-2005 (AMGM5) has developed a cell line. This cell line was taken from a multiform human brain glioblastoma (GBM). The most common malignant brain tumor is glioblastoma multiforme, representing about 50 percent of all malignancies in the central nervous system [118]. This tumor is characterized by a rapid division of the cells, normal brain invasion, and high vascularization [119].

1.17.5 Nanotechnology for cancer treatment

Has started recently, because of its enormous ability to lead to a revolutionary paradigm to overcome the problems of current chemotherapeutic devices. Nanotechnology has drawn considerable interest in cancer therapeutics [120].

A range of nano vehicle systems are with nano-scale sizes, for example, favour intracellular endocytic absorption, high drug packaging, and accurate tumor network targeting. The therapeutic efficacy of chemotherapy loaded medications would be greatly increased by these artificially manufactured nanomaterials. While they also reduce non-specific toxicity, make it easier to manufacture safe and useful cancer therapies. In addition, enormous attempts have been made to produce multi-functional therapy nanosystems for both cancer detection and treatment that can distribute drugs specifically to tumors and simultaneously track their therapeutic response by visualizing legions of tumors in the body [121,122]. Many current anti-cancer regimes do not adequately distinguish between healthy and cancerous

cells with respect to cancer treatment. for normal body tissues, this random action also results in chronic toxicity and crippling adverse effects, including bone marrow suppression, neurotoxicity, and cardiomyopathy. Nanomedicine and nanotechnology have provided a more focused strategy which promised major advances in cancer treatment [123-125].

1.18 Bacteria

Bacteria are prokaryotic cells with range less than (1-10) μm in size. Their semi-rigid walls consist of phospholipids that are responsible for preserving the bacterias three-dimensional structure. Gram-positive bacteria have dense cell walls composed of several peptidoglycan and teichoic acid layers, while Gram-negative bacteria have comparatively thinner cell walls but more complex structures comprising many layers of peptidoglycan, polysaccharides, proteins and lipids [126]. Bacterial survival relies on homeostasis of the membrane lipid and bacteria which have the ability to change compositions of the lipid to acclimatize various conditions [127].

1.18.1 *Klebsiella pneumoniae*

Klebsiella pneumoniae (*K. pneumonia*) is a Gram-negative, rod shaped bacterium belongs to the Enterobacteriaceae family that is usually active in bacterial pneumonia, urinary tract, and wound infections acquired in the hospital and population [128]. *K. pneumoniae* is a significant cause of hospital acquired infections (HAI), it is the third major HAI pathogen that was identified as the onset of pneumonia at approximately 48 h after hospital admission. Level of mortality in *K. pneumoniae* has a high degree exceeding 50 percent. *K. pneumoniae*, affect approximately 13 percent of all induced infections, which can also colonize wound/surgical sites [129].

1.18.2 *Staphylococcus aureus*

Staphylococcus aureus (*S. aureus*) is a Gram-positive bacterium in cocci shape with a diameter of (0.5-1.0) μm. It can be seen in grape-like clusters or in single and pairs non-motile cells [(130)]. The cell membrane of *S. aureus* is composed of an amorphous protective coat. Peptidoglycan is the main ingredient of the cell membrane, and is 1/500 of the mass of the membrane [(131)]. *S. aureus* induces later diseases and bloodstream infections of both adults and children, such as bacteremia and sepsis [(132)].

1.18.3 Nanotechnology for anti-microbial treatment

Certain inorganic and organic nanomaterials have been shown to have powerful anti-microbial properties that are rarely expressed in their bulk forms. Some of these nanomaterials, in particular, can cause anti-biotic resistance by compromising current resistance pathways. Anti-microbial drug delivery nanoparticles, also have distinct advantages over traditional anti-biotics in terms of overcoming resistance and having fewer side effects. Anti-microbial nanomaterials may be used in medical devices to resist microbial adhesion and inflammation. The use of nanomaterials as vaccine adjuvants and delivery vehicles elicit more successful microbial infection immune responses [(133)]. These nanoparticles make it impossible for microbes to produce resistance. It is not shocking nowadays, with nanotechnology that becomes more common and implemented in medicine. It is not surprising seeing nanoparticle technologies being applied to combat anti-biotic resistance [(134)]. For anti-microbial resistance therapies, there are many approaches for using NPs. Nanoparticles may also be added by a combination with current clinically associated anti-biotics in order to modify and improve their physiochemical properties and to

defeat mechanisms of anti-microbial resistance; or as anti-microbial agents themselves, as colloidal formulations. Silver (Ag), zinc (Zn), copper (Cu), titanium (Ti), and gold (Au) are a case of report [135].

Synthesis and inhibition or interruption of translation and transcription during protein synthesis and synthesis of nucleic acids as well as cell wall structure are the three most critical targets for anti-biotic action. Nanoparticle technologies have also been used to inhibit the bacterial anti-oxidant system and cause a formation of reactive oxygen species (ROS) in bacteria to affect the bacterial respiration process [133].

In order to overcome bacteria, nanotechnology should now deliver a new therapeutic approach. Microbe drug resistance mechanisms can be overcome by nanoparticles, including reduction absorption and increase the efflux of microbial cell medicines. Finally, nanoparticles may be applied the infection site to target anti-microbial agents. Higher drug doses can be administered at the infected site, thus overcome resistance with less patient side effects [136].

1.19 Literature review

The literature review includes a number of papers that reference zinc oxide and nickel oxide nanoparticles using biological strategies and biological applications.

Vijayakumar, S., *et.al* [137] used the co-precipitation approach to study green synthesis of zinc oxide nanoparticles using the aqueous leaf extract of Laurus nobilis (Ln-ZnONPs). Ln-ZnONPs had a higher

anti-bacterial efficacy against (*S. aureus*) (G^+) bacteria compared to (*Pseudomonas aeruginosa*) (G^-) bacteria. Moreover, Ln-ZnONPs were an effective inhibitor for the viability of human A549 lung cancer cells. Under a phase contrast microscope, the morphological improvements in Ln-ZnONPs treated A549 lung cancer cells were detected.

Zare, E., *et.al* [138] noticed green synthesis of zinc oxide ZnO nanoparticles by using Cuminum cyminum (cumin) as a novel natural source and zinc nitrate [$Zn(NO_3)_2$] as a Zn^{2+} source. The effect of anti-microbial nanoparticles in various bacteria strains was measured using the disk diffusion technique and broth MIC and MBC, which revealed that both Gram-positive and negative bacteria were susceptible to zinc oxide nanoparticles.

Ngoepe, N., *et.al* [139] studied synthesis of ZnO nanoparticles from the water extract of the Monsonia burkeana plant. The anti-bacterial behavior of Monsonia burkeana-derived ZnO nanoparticles was observed against both Gram-negative and positive bacterial strains, which may be due to their small particle sizes. Farthermore, cytotoxicity experiments revealed that ZnO nanoparticles inhibited the growth of A549 lung cancer cell lines.

Suresh, J., *et.al* [140] studied synthesize of biocompatible ZnO nanoparticles from zinc nitrate using a green technique from leaf extracts of the Costus pictus D. Don medicinal plant. By using the agar diffusion process, the biosynthesized zinc oxide nanoparticles display significant anti-microbial activity against bacterial and fungal organisms. The anti-cancer activity of synthesized ZnO nanoparticles have been shown against Daltons lymphoma ascites (DLA) cells, as well as they used anti-bacterial and anti-fungal.

Sharmila, G., *et.al* [141] studied green synthesis of zinc oxide nanoparticles using Tecoma castanifolia leaf extract was reported. Both Gram-positive and Gram-negative bacteria had outstanding anti-bacterial activity. The anti-oxidant activity of ZnO nanoparticles was found to increase as the concentration of the nanoparticles increased. ZnONPs had greater anti-cancer activity with an IC_{50} value of 65 g/mL, indicating that they had better cytotoxic effects on the proliferation of the A549 cell line. The results of this analysis show that the pharmacologically active compounds contained in green synthesized ZnO nanoparticles pave the way for their use in biomedicine and nano-drug delivery systems.

Selim, Y., *et.al* [142] studied the synthesis of ZnONPs from Deverra tortuosa extract. ZnONPs were synthesized in this analysis using an environmentally friendly extract of D. tortuosa aerial parts as a reducing and capping agent. The MTT assay was used to compare the possible anti-cancer efficacy of two cancer cell lines (human colon adenocarcinoma “Caco-2” and human lung adenocarcinoma “A549”) to their activities on the human lung fibroblast cell line (WI38). Against the two cancer cell lines tested, both the aqueous extract and ZnONPs demonstrated extraordinary differential cytotoxicity.

Mohana, S., and S. Sumathi. [143] studied the synthesis of ZnONPs from Agaricus bisporus aqueous extract and assess their in vitro biological activity. Spectroscopic techniques were used to characterize the biosynthesized ZnONPs. The nanoparticles' stability was confirmed by a zeta potential of 20.5 mV. ZnONPs which have spheroid-shaped structures, according to SEM and TEM analyses. Anti-bacterial activity was measured using the agar well diffusion method, which revealed that gram-positive bacteria had a larger zone

of inhibition. The ZnONPs is shown to be cytotoxic against SW-620 cell lines in an MTT assay (human colon cancer).

Dulta, K., *et.al* [144] studied the biosynthesis of ZnONPs in plant B. ciliata rhizome extract. The synthesized ZnONPs demonstrated the anti-microbial activity against Gram-negative bacteria, with the lowest minimum inhibitory concentration value of 6.25 g/mL. The ABTS and DPPH assays were used to determine the free radical scavenging ability of zinc oxide nanoparticles, which revealed scavenging behavior with IC_{50} = 63.8 and 118.7 g/ mL respectively. Human cervical cancer (HeLa) and human colon cancer (HT-29) cell lines displayed perfect selective cytotoxicity when exposed to ZnONPs.

Chen, H., *et.al* [145] investigated the cytotoxicity in human cervical epithelial cancer HeLa by using ZnONPs. ZnONPs was synthesized from Aspergillus terreus culture filtrate. The cytotoxicity of synthetic ZnONPs was studied via the MTT test and Western blot analysis investigated the expression of apoptotic proteins. In ZnONPs treatment, HeLa cells were shown to be concentrates-dependent cytotoxic and apoptotic because they decreased dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx), and increased reactive oxygen species (ROS), and decreased mitochondrial membrane potential (MMP). The mycosynthesized ZnONPs induce apoptosis in HeLa cells by the persuasion of oxidative damage and apoptotic protein modulation. A. terrus synthesized ZnONPs may also be used to treat cervical cancer as an important chemotherapeutic agent.

Ezhilarasi, A., *et.al* [146] studied Moringa oleifera plant extract for green synthesis of nickel oxide nanoparticles. Using different concentrations of nickel oxide nanoparticles, in vitro cytotoxicity and cell viability of human cancer cell HT- 29 (Colon Carcinoma cell

lines) as well as anti-bacterial tests against various bacterial strains were examined. MTT assays on cell viability and morphological studies revealed that the synthesized NiO nanoparticles have cytotoxic activity against human cancer cells. Various zones of inhibition (mm) obtained revealed that NiO nanoparticles have effective anti-bacterial activity against various Gram-positive and Gram-negative bacterial pathogens.

Kganyago, P., *et.al* [147] noticed used a local medicinal plant, Monsonia burkenea, to study the synthesis of NiO nanoparticles. Gram-negative bacteria, such as *E. coli* and *P. aeruginosa*, were selectively killed by Monsonia burkeana NiO particles. Furthermore, cytotoxicity tests revealed that the materials had no anti-proliferative effect on A549 lung cancer cells, but they could be used as drug delivery vectors for human cancers.

Khalil, A., *et.al* [148] noticed Sageretia thea (Osbeck.) aqueous leaf extracts can be used to biosynthesize of NiO nanoparticles, as well as their biological activities. Six pathogenic bacterial strains were tested for anti-bacterial activity (Gram-positive and Gram-negative). The lethality of brine shrimps was used to prove cytotoxicity. The application of MTT cytotoxicity to Leishmania tropica-KWH23 promastigotes and amastigotes revealed high percentages of inhibition at all concentrations. Assays including DPPH, tandem affinity purification, and total anti-oxidant capacity were used to determine a moderate anti-oxidant capacity. Protein kinase inhibition and alpha amylase inhibition have also been documented.

Abbasi, B., *et.al* [149] noticed the use of Geranium wallichianum for the green synthesis of NiO nanoparticles. NiONPs have been shown to have potent anti-fungal and anti-bacterial properties.

NiONPs have been found to be cytotoxic to HepG2 cancer cells (IC_{50}: 37.84 μg mL^{-1}). NiONPs were found to be consistent with human RBCs and macrophages (IC_{50}: >200 μg mL^{-1}) through toxicological testing, indicating that they are acceptable for variety of biomedical applications. Anti-oxidant activities, protein kinase inhibition, and alpha-amylase inhibition assays were also carried out.

Karthik, K., *et.al* [150] studied the green synthesis of NiO nanoparticles using Andrographis paniculata (leaf extract) as a starting material. The photodegradation of prepared nanoparticles (Evans blue) was also evaluated. The prepared nanoparticles were tested against the MCF-7 cell line for anti-breast cancer purposes.

Ibrahim, F., *et.al* [151] prepared green synthesized nickel oxide nanoparticles (NPs) using Terminalia chebula extract. Green NiONPs exhibited toxicity against breast cancerous cells in a dose dependent manner from 0 to 100 g/mL, exhibiting cell viability, reactive oxygen species (ROS) activity, and mitochondrial membrane potential liberation (MMP), with huge possibilities for various biological and biomedical applications.

Iqbal, J., *et.al* [152] used Rhamnus virgata (Roxb.) (Family: Rhamnaceae) as a possible stabilizing, reducing, and chelating agents to synthesize nickel oxide nanoparticles (NiONPs). Biogenic NiONPs were tested against five different Gram-positive and Gram-negative bacterial strains to see if they have any anti-microbial activity. Biogenic NiONPs have been shown to be highly effective ness against HepG2 cells, and NiONPs have been shown consistency with human RBCs (IC_{50}: > 200 g/mL) and macrophages (IC_{50}: > 200 g/mL), indicating that they are suitable for nanomedicine. Additionally, inhibition assays for protein kinase and alpha amylase were carried

out. NiONPs synthesized by Rhamnus virgata could have significant biomedical applications while causing minimal cytotoxicity in normal cells.

Ezhilarasi, A., *et.al* [153] studied biological synthesis of nickel oxide nanoparticles (NiONPs) from Solanum trilobatum leaf extract. The synthesized NiO nanoparticles outperformed A549 cells and were more selective against Gram-positive pathogens than Gram-negative pathogens in terms of anti-bacterial action. It also proved to be a photo catalytically active substrate by degrading 4-Chlorophenol effectively under UV light, as shown by TOC experimental findings.

Iqbal, J., *et.al* [154] used the green fabrication of NiONPs through fresh Rhamnus triquetra leaf broth (RT). Furthermore, using erythrocytes and macrophages, RT-NiONPs were exposed to various in vitro biological activities and they revealed distinct biosafe and biocompatibility potentials. NiONPs have shown to have anti-cancer properties in liver cancer cell lines. NiONPs have been shown to have important anti-microbial activity against a variety of bacterial and fungal strains. The current research revealed promising in vitro biological activities, implying that further in vivo trials in multiple animal models are needed to establish new drugs to treat a variety of chronic diseases.

Lingaraju, K., *et.al* [155] studied prepare biosynthesis of nickel oxide nanoparticles (NiONPs) from Euphorbia heterophylla (L.) leaves extract. For human erythrocytes, NiONPs demonstrate essential non-toxic effects, NiONPs showed significant bactericidal activity against pathogenic bacteria. NiONPs exhibit substantial cytotoxicity against human lung cancer (A549) and human hepatocarcinoma (HepG2) cell lines.

1.20 Aim of the present study

The main objectives of this research are:

1- Synthesis of ZnO and NiO nanoparticles from grape (*Vitis vinifera*) L. (Vitaceae family) extract using biological pathways.

2- Study the characteristics of zinc oxide nanoparticles and nickel oxide nanoparticles by using UV-Visible, FTIR, XRD, TEM, AFM and SEM.

3- Investigate the cytotoxicity effects of ZnONPs and NiONPs against the MCF-7 cell line and the AMGM5 cell line by using MTT assay.

4- Study the anti-bacterial activity of ZnONPs and NiONPs against *S. aureus* (G^+) and *K. Pneumonia* (G^-) using the agar well diffusion method.

CHAPTER TWO

2. Experimental part

2.1 Chemical materials

The following chemicals were used in this study as in Table (2-1).

Table (2-1): chemicals used in this study

No.	Materials	M.WT	Company	Manuf. Country
1	Nickel(II) Chloride Hexahydrate	237.71 g/mol	Anala R Normapur	England
2	Zinc(II) Chloride Dihydrate	148.3 g/mol	Riedel-de Haën	Germany
3	Deionized Water	18 g/mol	Chem-Lab	Belgium
4	Trypsin/EDTA	———	Capricorn	Germany
5	DMSO	———	Santacruz Biotech	USA
6	RPMI 1640	———	Capricorn	Germany
7	MTT Stain	———	Bio-World	USA
8	Fetal Bovine Serum	———	Capricorn	Germany
9	Muller Hinton Agar	———	Hi-Media	India

2.2 Instruments and apparatus

The following instruments and apparatus are used in this study Table (2-2).

Table (2-2): Instruments and apparatus are used in research

No.	Instruments	Manufacturing Company	Source
1	Analytical Balance	Mettler Toledo	Switzerland
2	Hot Plate Stirrer	Alfa (HS-860)	Iran
3	Centrifuge	Mindray	China
4	UV-Visible Spectrophotometer	Shimadzu-1800	Japan
5	Fourier-Transform Infrared Spectrophotometer (FT-IR)	Shimadzu-8400	Japan
6	X-ray Powder Diffraction (XRD)	Philips. PW1730	Holand
7	Atomic Force Microscope (AFM)	SPM-AA3000	USA
8	Scanning Electron Microscope (SEM)	Zeiss	Germany
9	Transmission Electron Microscope (TEM)	Zeiss	Germany
10	CO_2 Incubator	Cypress Diagnostics	Belgium
11	Microplate Reader	Gennex Lab	USA
12	Laminar Flow Hood	K&K Scientific Supplier	Korea
13	Micropipette	Cypress Diagnostics	Belgium
14	Cell Culture Plate	Santa Cruz Biotechnology	USA

2.3 Preparation of grapes extract

Fresh grapes were purchased in the month of september from the local market in Iraq. To remove the dust, the grapes were separated from the branches, seeds and washed several times with deionized

water. The grapes 250 g were smashed well with 250 mL deionized water to form a homogeneous mixture in the mixer. The product was filtered through Whatman No. 1 paper. A light yellow of clear solution was obtained as shown in Figure (2-1).

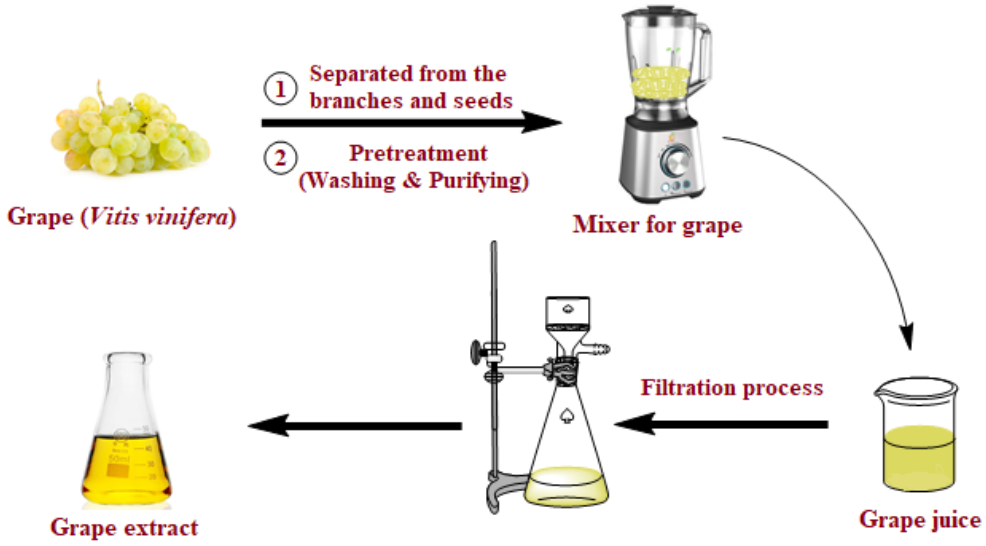


Figure (2-1): Preparation of grape extract

2.4 Green synthesis of ZnONPs

Zinc oxide nanoparticles were synthesized by mixing equal volumes of 0.1 M zinc chloride dihydrate solution ($ZnCl_2.2H_2O$) (M.W 148.3 g/mol) and 40 mL of the grape extracts and while stirring the mixture, 1M NaOH solution was added dropwise to adjust the pH of the mixture (monitored using a pH meter) to pH 8 as required. This mixture was stirred continuously for 2 hours and keep the heat at 60°C. The color was changed from yellowish white to white which confirm the formation of ZnONPs, after cooling down to 25°C, the solution was separated by centrifuging at 5000 rpm. for 10 min. The steps are as shown in Figure (2-2).

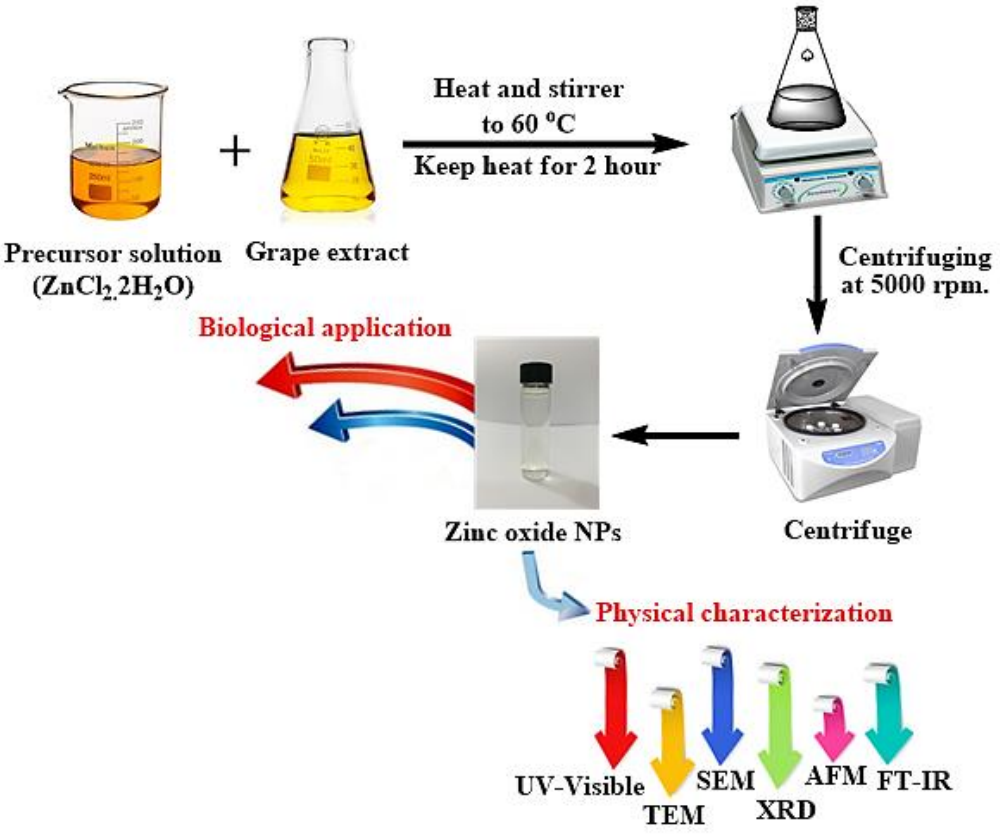


Figure (2-2): The steps of the green synthesis of ZnONPs

2.5 Prepare the precursor solution of ($NiCl_2.6H_2O$)

2.377g of nickel (II) chloride hexahydrate ($NiCl_2.6H_2O$) (M.W 237.71 g/mol) were mixed with 100 mL deionized water and then heated for ten minutes with amagnetic stirrer.

2.6 Green synthesis of NiO nanoparticles

In this experiment, 50 mL of 0.1 M nickel (II) chloride hexahydrate ($NiCl_2.6H_2O$) precursor solution were blended (1:1) with 50 mL of a grape aqueous extract. This was accomplished by steadily pouring 10 mL of aqueous extract into the precursor solution over a 5 minutes interval until a significant color change was observed. The final product was mixed for 2.5 hours with a magnetic stirrer at 65°C. The color was shifted from a light green to a dark green, indicating that NiONPs was formed. After cooling to 25^{o}C, the resulting solution

was then centrifuged twice at 3500 rpm to isolate it. The steps are as shown in Figure (2-3).

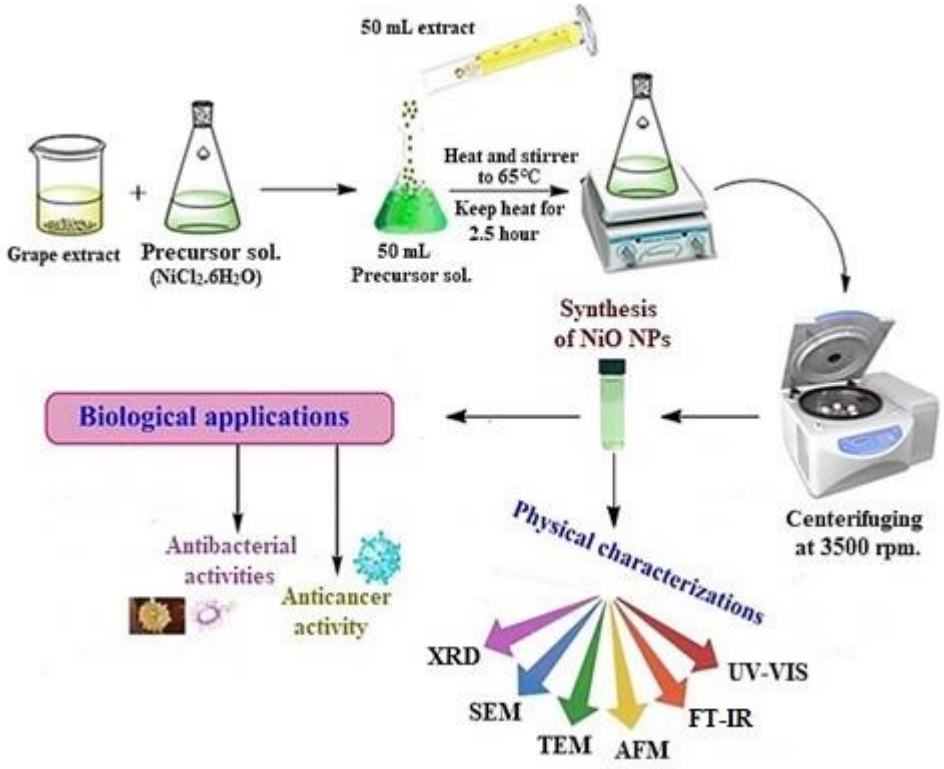


Figure (2-3): The steps of the green synthesis of NiONPs

2.7 Characterization of NiONPs and ZnONPs

The main purpose of these measurements is to investigate the type of morphology and the structure of the prepared nanomaterials.

2.7.1 UV-Visible spectroscopy

In this research, the nanoparticles solution were measured by UV-Visible, Shimadzu; 1800 (Japan) in the University of Baghdad/ College of science. All measurements were accomplished at the surrounding temperature in the quartz cell and the spectra of UV-Visible were recorded in the range of 200 - 1100 nm to charactrise the nanoparticles.

2.7.2 Fourier transform infrared spectroscopy (FT-IR)

Fourier transform infrared instrument was used for identifying the functional groups in the analysed nanoparticles. At the laboratory of University of Baghdad/ College of Science, the prepared nanoparticles were examined by FT-IR, Shimadzu; 8400 (Japan).

2.7.3 X-ray diffraction (XRD)

XRD is an analytical technique used for phase identification of a crystalline material. An X-ray diffractometer consists of an X-ray source, the sample holder and an X-ray detector. The X-ray diffraction scans were carried out on the samples in turn to ascertain their crystal structure as shown in Figure (2-4) [156]. The measurement via this technique was carried out in the laboratories of Tehran - Iran.

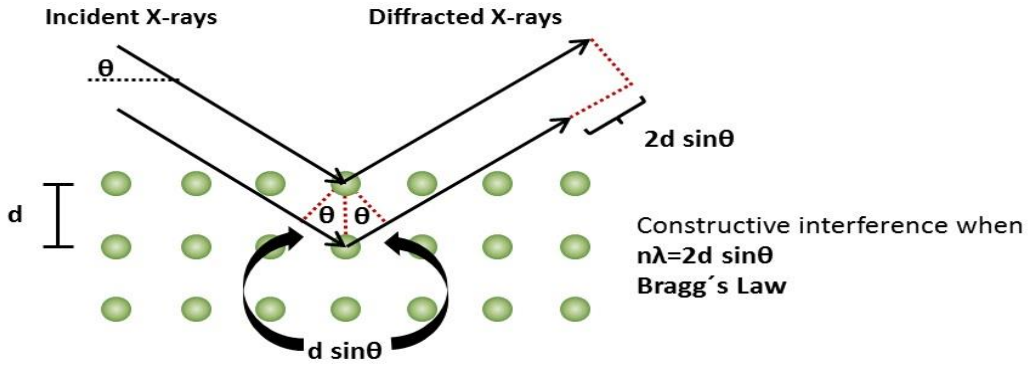


Figure (2-4): X-ray diffraction

2.7.4 Atomic force microscope (AFM)

AFM is commonly used to detect surface shapes of thin films deposited on substrates. It provides information about the nature of deposited materials and gives an estimate of surface roughness, surface shape, topography, thickness and hardness as shown in Figure (2-5). Atomic force microscopy can be performed in different imaging modes: Static force mode, lateral force mode, dynamic force mode,

and phase imaging mode. In addition, the AFM process includes a sharp-tip cantilever which is sensitive to the smallest force that comes into contact with the sample surface [157]. Measurement with this technique was carried out in the laboratory of Prof. Dr. Abdulkareem M. A. Alsammarraie Department of Chemistry at the College of Science, University of Baghdad, using a device of type (AA 3000 scanning probe microscope) of Taiwanese origin.

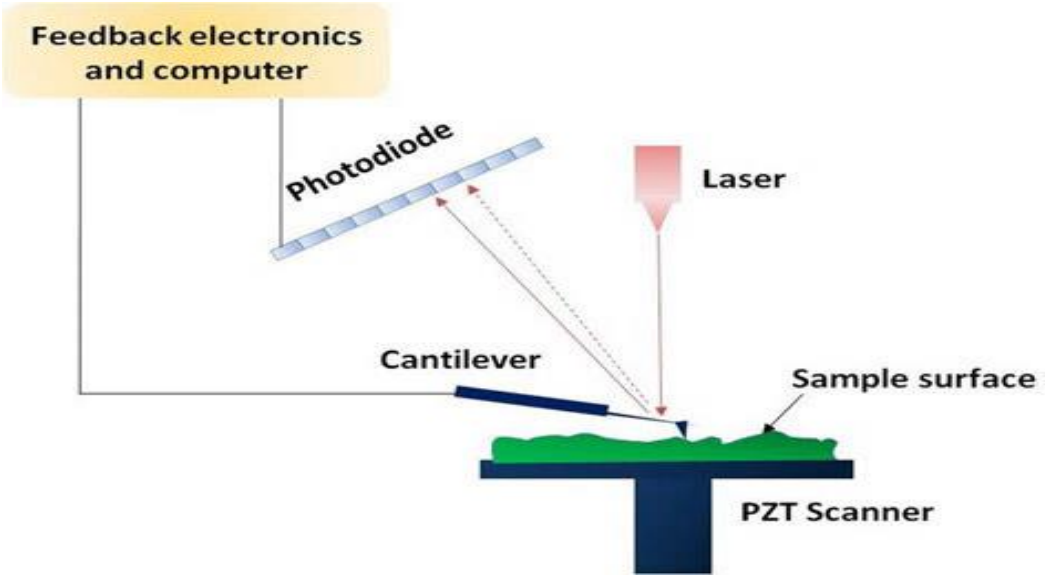


Figure (2-5): Schematic diagram of the operation of an AFM

2.7.5 Scanning electron microscope (SEM)

An SEM is a type of electron microscope that uses a fine beam of guided electrons to scan the surface of a sample. The microscope records details of the interaction between the electrons and the sample, result in an enlarge picture being created. The picture can be enlarged up to 2 millions times by SEM as shown in Figure (2-6).

The working principle of SEM, is based on an electron source emits range of high energy electrons toward a sample to produce a high-resolution image, using electromagnetic lenses, the electron beam is centered. The centered current scans the surface in a dot-rectangular shape as it approaches the specimen [158]. The device was

made in Germany type ZEISS and the measurments was carried out in the laboratories of Tehran - Iran.

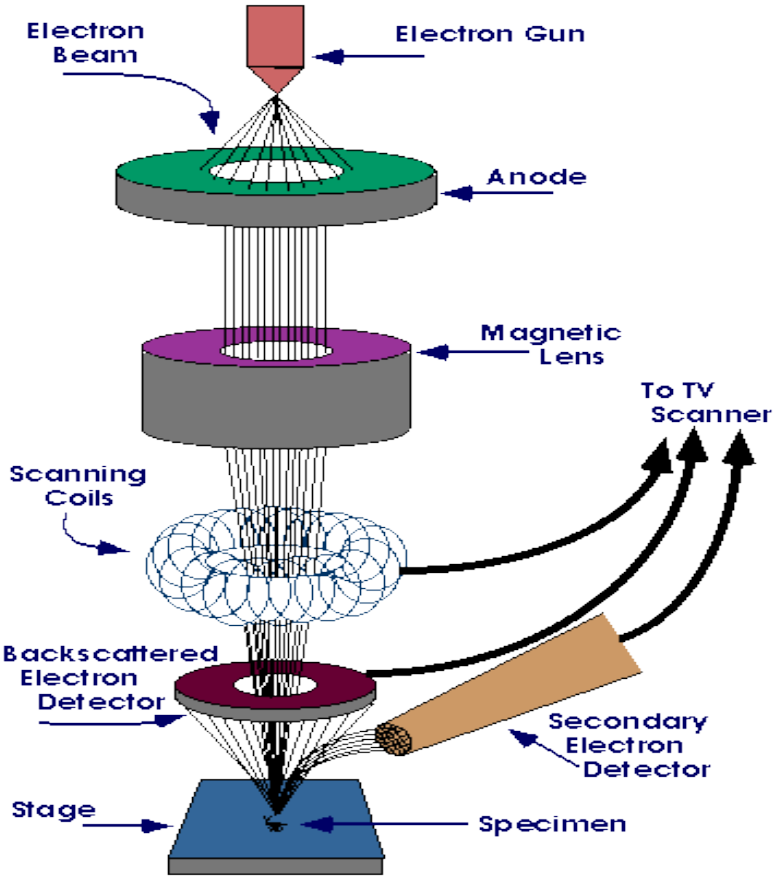


Figure (2-6): Schematic diagram of SEM system [158]

2.7.6 Transmission electron microscope (TEM)

A TEM is an electron microscope that creates a picture of samples internal structure using a large beam of electrons. A picture of the morphology, composition and crystal structure of a sample was created by passing an electron beam through it. The magnification capacity of TEMs ranges from 10 to 50 million times. The information was given at the atomic level, which is the best resolution available from any electron microscope.

The principle of TEM is based on, a pulse of electrons is sent into an ultrathin sample from an electron source. The electrons travel through the lenses below as they penetrate the sample. This

information is used to produce pictures that can be seen directly on a fluorescent screen or on a computer screen [159]. The device was made in Germany type ZEISS and the measurments was carried out in the laboratories of Tehran - Iran. as shown in Figure (2-7).

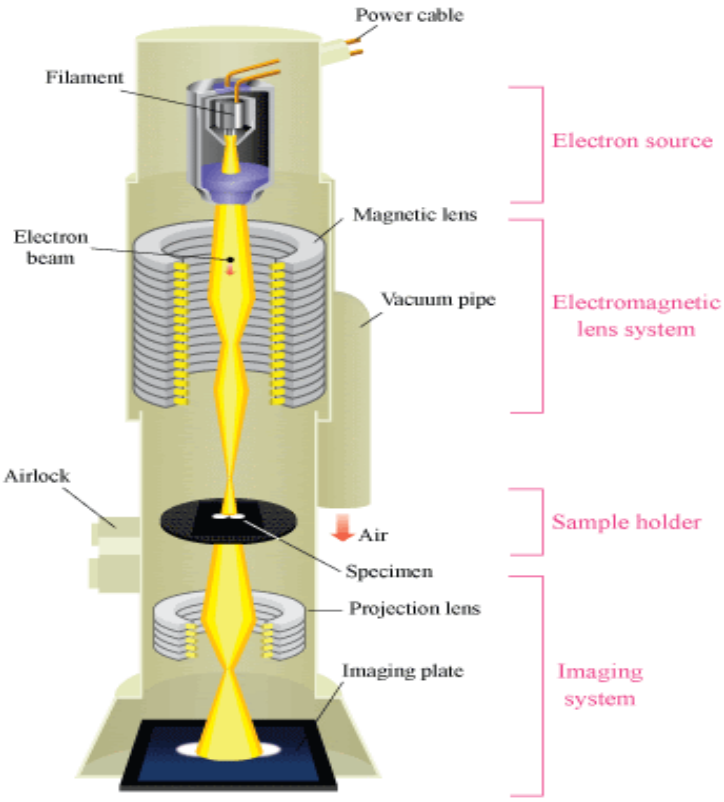


Figure (2-7): A schematic diagram of the TEM system

2.8 Anti-cancer activity

Anti-cancer and anti-bacterial were conducted in (Iraqi Biotechnology Company- Baghdad).

2.8.1 Cell culture

MCF-7 and AMGM5 cell lines were maintained in RPMI-1640 medium containing 10% fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged twice a week with Trypsin-EDTA, then grown at 80 percent confluence and incubated at 37 °C [160,161].

2.8.2 Cytotoxicity determination using MTT assay

To assess the cytotoxic impact of ZnONPs and NiONPs, an MTT assay was conducted using 96-well plates [162,163]. At 1x 10^4 cells per well, cell lines were planted. Cells were treated with the tested compounds at variety concentrations after 24 hours or when a confluent monolayer was achieved. After 72 hours of therapy, the viability of the cells was determined by extracting the medium, applying 28 litres of 2 mg/mL MTT solution, and incubating the cells at 37 °C for 2.5 hours. Following the removal of the MTT solution, the remaining crystals in the wells were solubilized by applying 130 µL of DMSO and incubating at 37 °C for 15 minutes with shaking [164]. The absorbance was measured at 492 nm using a microplate reader, and the assay was made in triplicate. The following equation was used to measure the rate of cell growth inhibition (the percentage of cytotoxicity) [165,166].

Inhibition rate = A − B/A x 100 …… (1)

Where A is the control (untreated cells) optical density and B is the samples optical density [167].

The cells were grown at a density of 1x10^5 cells mL^{-1} into 24-well microtitration plates and incubated at 37 °C for 24 hours to visualize the outline of the cells under an inverted microscope. The cells were then exposed for 24 hours to ZnONPs and NiONPs at the IC_{50} concentration. The plates were stained with crystal violet after exposure and incubated at 37 °C for (10 -15) minutes [165]. The stain was slowly rubbed away with tap water until all of the pigment has gone. The cells were observed at 100x magnification under an inverted microscope, and images were taken with a digital camera connected to the microscope [168 - 171].

2.9 Anti-bacterial activity

2.9.1 Determination of minimum inhibitory concentration (MIC)

Using an agar well diffusion method, the anti-bacterial activity of ZnONPs and NiONPs was examined against bacterial strain *K. pneumoniae* (G^-) and bacterial strain *S. aureus* (G^+). Prior culturing [172]. 20 mL of Muller Hinton agar were aseptically poured into sterile Petri dishes [173]. A sterile wire loop was used to capture the bacteria from their stock cultures. After culturing the organisms, a sterile tip was used to drill 6 mm-diameter wells in the agar plates [174]. Different amounts of ZnONPs and NiONPs (0.0025, 0.005, 0.01, and 0.02) M were pumped into the bored wells. The average diameter of the developed zones of bacterial inhibited by the respective ZnONPs and NiONPs concentrations was measured and reported after the cultivated plates containing ZnONPs and NiONPs. Test organisms were incubated overnight at 37 °C for 24 hours. The tests were carried out three times [175].

2.9.2 Statistical analysis

The obtained data were statistically analyzed using an unpaired *t-test* using Graph Pad Prism 6 [176]. The values were presented as the mean ± SD for triplicate measurements and P value (< 0.05) [177].

CHAPTER THREE

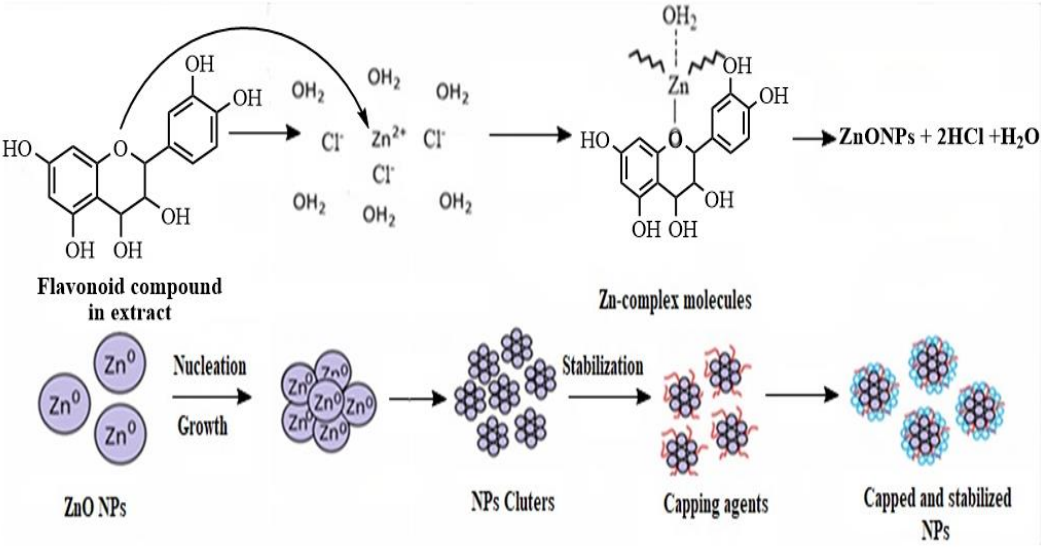
3 Results and discussion

3.1 Synthesis of zinc oxide nanoparticles

In this study, ZnONPs were synthesised by reducing of Zn^{+2} to Zn^{0}, which were generated by adding of zinc chloride dihydrate solution ($ZnCl_2.2H_2O$) to the aqueous extract of grapes (*Vitis vinifera*). This extract contains some of phytochemicals compounds like amino acids. Flavonoids and carbohydrates could act as reducing and capping agents. Ghanged in the color of the solution to white indicates the formation of ZnONPs.

3.2 Mechanism of ZnONPs formation

The key theory behind the biosynthesis of nanoparticles of ZnO may be that the natural materials produce the phytochemical substances such as saponins, phenols, terpenoids, which serve as both reducting and stabilizing (capping) agents. They reduce metal zinc to a zero valence state, and then oxide can be applied to the metal through calcinations. Another quite persuasive mechanism is that zinc ions in the natural extract solution can form a complexa with Zn^{+2} polyphenols or other phytochemicals. The synthesis of zinc hydroxide ($Zn(OH)_2$) is then followed by hydrolysis and, ultimately, after heating, it is decomposed, favouring the formation of ZnO nanoparticles [178-180]. Figure seen in (1-3).



Figure(3-1): Mechanism of ZnONPs formation [178]

3.3 Characterization of ZnONPs

3.3.1 UV-Visible spectroscopy measurement

The UV-Visible spectrum ZnONPs is shown in Figure (3-2). Nano-scale validation of the synthesized ZnO substance was demonstrated by a strongly blue-shifted maximal absorption of approximately 381 nm. For bulk ZnO, the maximal absorption was approximately 385 nm [181].

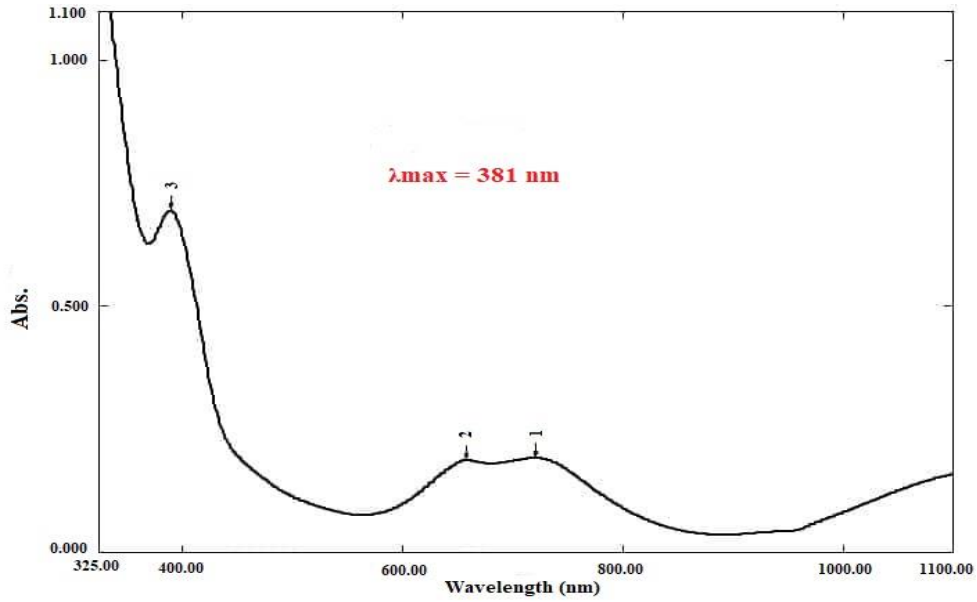


Figure (3-2): UV-Visible spectrum of ZnONPs

3.3.2 Fourier transforms infrared spectroscopy analysis (FT-IR)

FT-IR is technique used to idintify characteristics functional groups of surfaces. FT-IR tests have been carried out in order to identify potential biomolecules which have the effect of reducing and capping the metal ions of these ZnO nanoparticles. As seen in Figure (3-3), the FT-IR spectrum showed different characteristics between (400-4000) cm^{-1} peaks. In the FT-IR range of ZnONPs, the band around (3614.60, and 3739.97) cm^{-1} due to the vibrations extending of O–H group in water, alcohol and phenols while N–H extending in amines. The C–N stretch is shown around 2349.30 cm^{-1}. The band at 1535.34 cm^{-1} was ascribed to the C–C stretch in fragrant ring and C=O stretch in phenols and N–H bend in amine.

The band at 918.12 cm^{-1} was ascribed to the C–H bend in alkene and O–H bend in phenols. The absorption region at 763.81 cm^{-1} may have been associated with =C–H bending or C–C bending vibrations. The sharp peak observed in the range of (550 - 600) cm^{-1} was attributed to the vibrational photons of ZnONPs. In comparison with other studies, there was sharp peak observed in the range of (408 - 510) cm^{-1} which was attributed to the vibration of ZnONPs. This result indicated the successful production of ZnO nanoparticles [182].

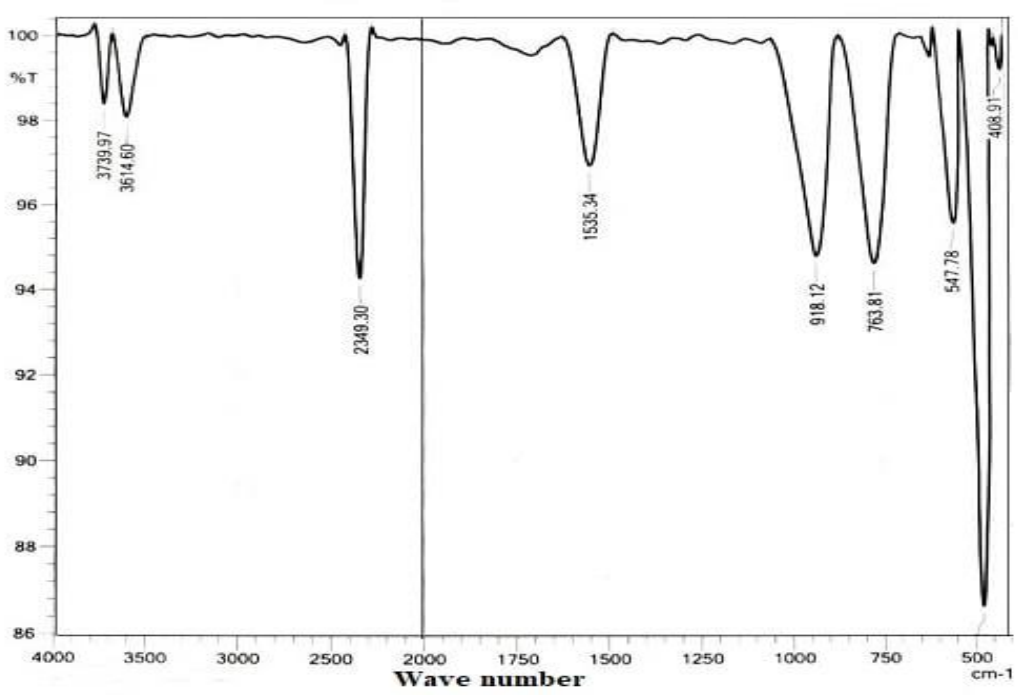


Figure (3-3): FT-IR spectrum of ZnONPs

3. 3.3 X-ray diffraction analysis (XRD)

Figure (3-4) represents the diffraction of the zinc oxide nanoparticles sample obtained after the heat treatment. The identification form used to identify the compound was Joint Committee on Powder Diffraction Standards (JCPDS) 01-076-0205 and the diffraction peaks are located at 31.71^{o}, 34.51^{o}, 36.07^{o}, 47.29^{o}, 56.48^{o}, 62.86^{o}, and 67.85^{o} characteristic of the hexagonal type crystal structure wurtzite [183]. The evaluated average crystallite size of the nanoparticle was 30.49 nm. The diffraction shows no contaminating peaks.

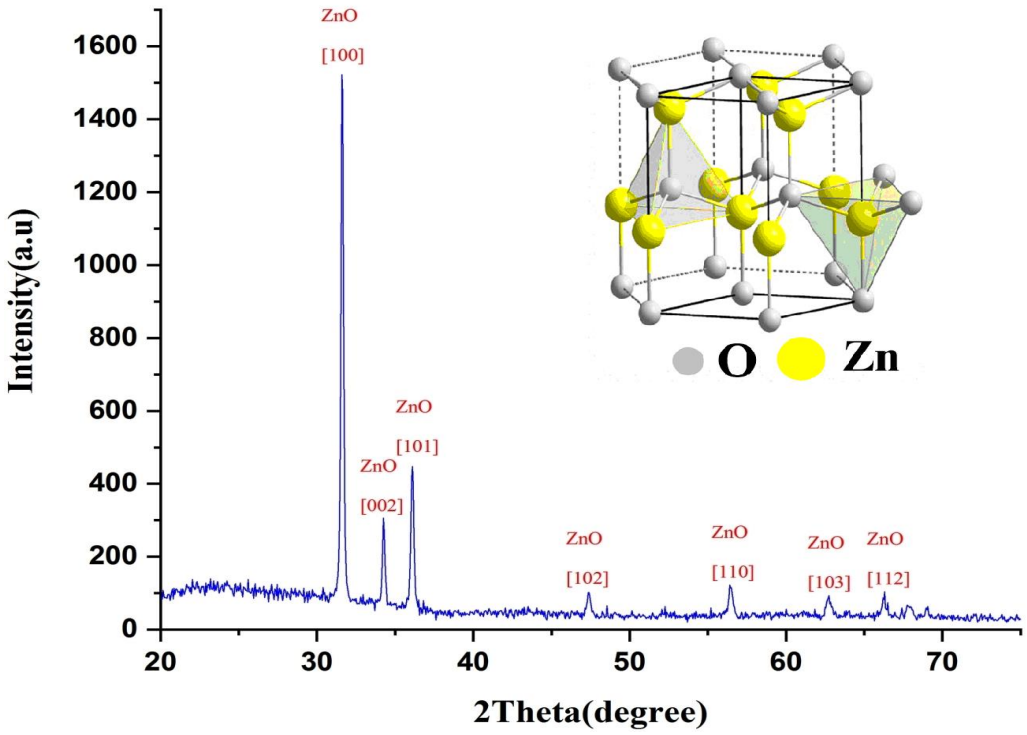


Figure (3-4): X-ray diffraction of ZnONPs

3.3.4 Atomic force microscope analysis (AFM)

AFM measurements have been obtained to determine the morphological features of ZnONPs. Information about the height, length and width of the surface and other features, such as surface texture determined the functions.

Characterization of ZnONPs has also been checked by AFM as shown in Figure (3-5). The Figure shows for the synthesized ZnONPs 2-dimensional and 3-dimensional images. The images indicated that ZnONPs have small size distribution with a diameter of 46.52 nm in dimension.

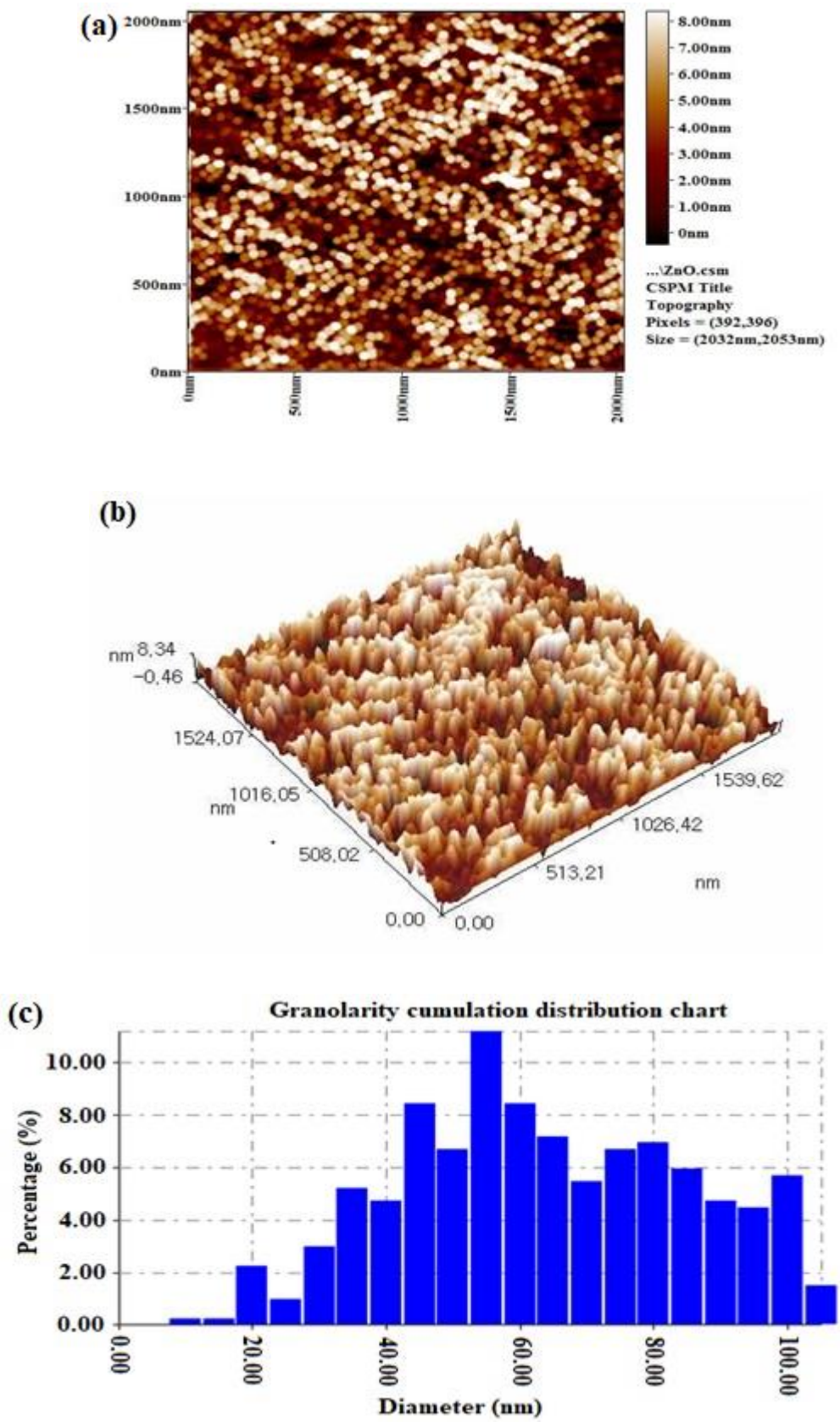


Figure (3-5): AFM analysis shows (a) 2-dimension (b)3-dimension and (c) Avarage distribution for ZnONPs of diameter 46.52 nm

3.3.5 Scanning electron microscope analysis (SEM)

SEM analysis was performed to determine the shape and surface morphology of ZnONPs prepared from aqueous grape extract with diameters ranging from 40 nm to 60 nm as shown in the SEM images as shown in Figure (3-6).

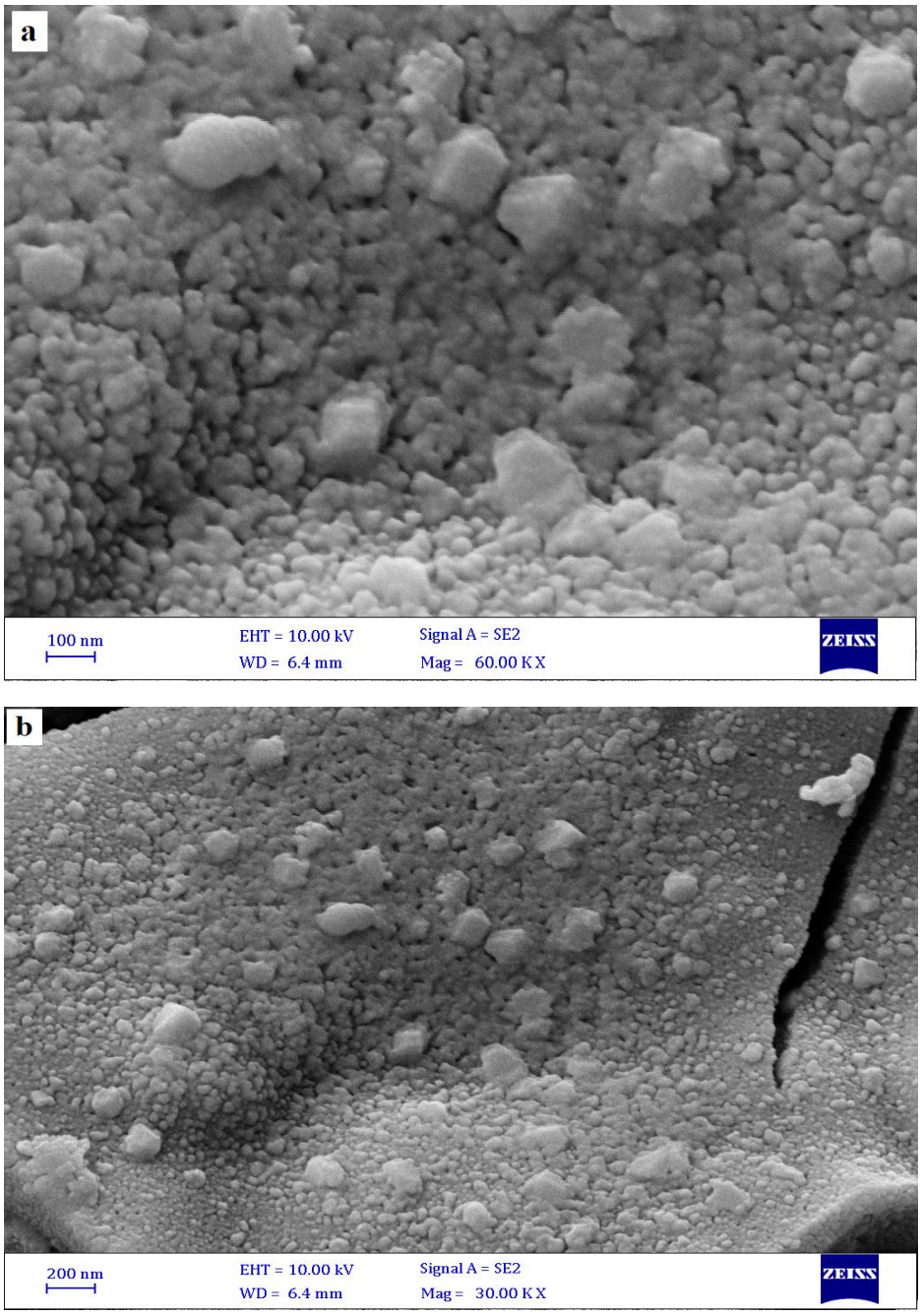
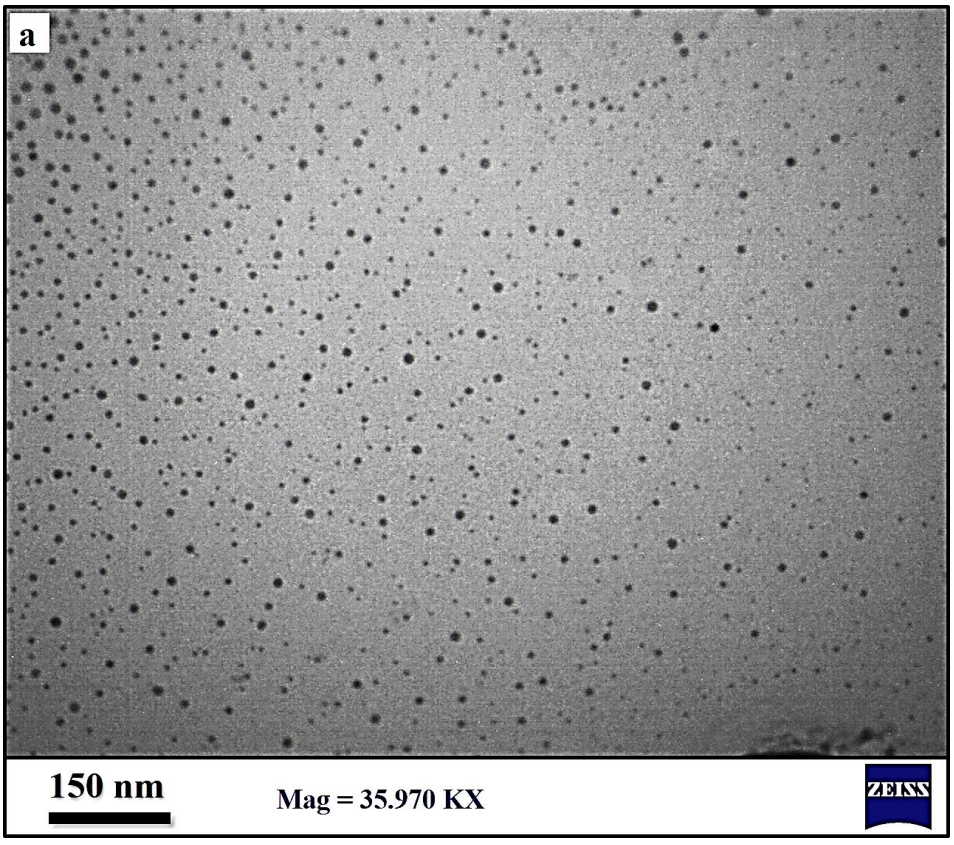


Figure (3-6): SEM micrograph ZnONPs (a) 100 nm (b) 200 nm

3.3.6 Transmission electron microscope analysis (TEM)

A morphological characterization study and determination of the size and Structure of ZnONP have been recorded for TEM analysis. The images show that most nanomaterials contain zinc oxide which have spherical forms, as demonstrated in Figure (3-7). The nanoparticles can be seen to be arranged approximately parallel and the small amounts of nanoparticles is very high. Further, the synthesized ZnO is obviously a large quantity of nanoparticles. The exhibited ZnO nanoparticles have a diameter ranging from 40 nm to 60 nm, which are consistent with the X-ray result.



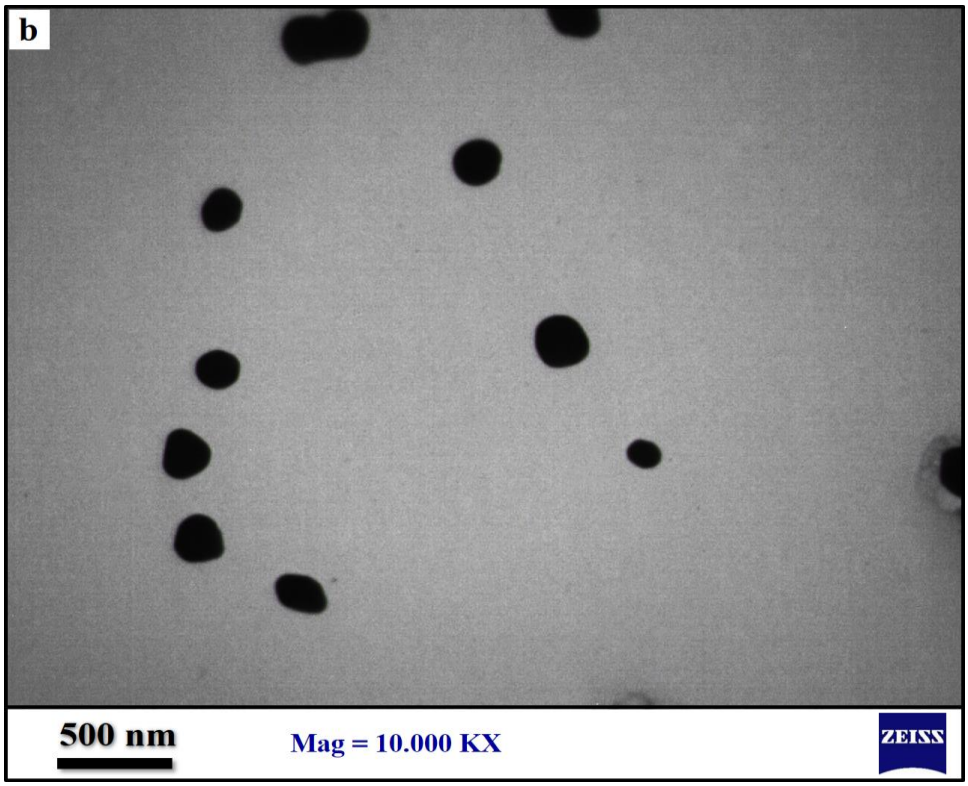


Figure (3-7): TEM micrograph ZnONPs **(a)** 150 nm and **(b)** 500 nm

3.4 Synthesis of nickel oxide nanoparticles

Green synthesis of NiONPs was made from the aqueous extract of grapes (*Vitis vinifera*). During the reaction process, nickel chloride ($NiCl_2.6H_2O$) was dissolved in the water and then decomposed the phytochemicals by heating to generate oxygen. Finally, the oxygen binds to nickel to form nanoparticles.

3.5 Mechanism of NiONPs formation

The phytochemicals reduce metal ions into metal atoms. The metal salts, such as nitrates, chlorides, oxides and sulphates, have a high reduction potential because of their connection to metal and tendancy to donate electrons to a metal with parts of chloride, oxide and sulphides. The electronic density of connective metal salts increases due to these two causes.

By considering of paragraph the following three stages, we can identify the mechanism of the plant that mediated the synthesis of metal oxide NPs:

1- The process of activation includes reduce both of the metal ions and metal atoms in the nucleation phase of metals.
2- The development step entails the spontaneously coalescence of small neighboring NPs into larger NPs, i.e. the producing of Ostwald (a mechanism in which NPs are directly produced by heterogeneous nucleation and growth and further metal ion reduction), the thermodynamic stability of NPs is improved by this method.
3- For metal oxide NPs, the final form of NPs is determined by the termination process. The last product is then dried or calcinated into the air to obtain final metal oxide NPs, as shown in Figure (3-8) [184,185].

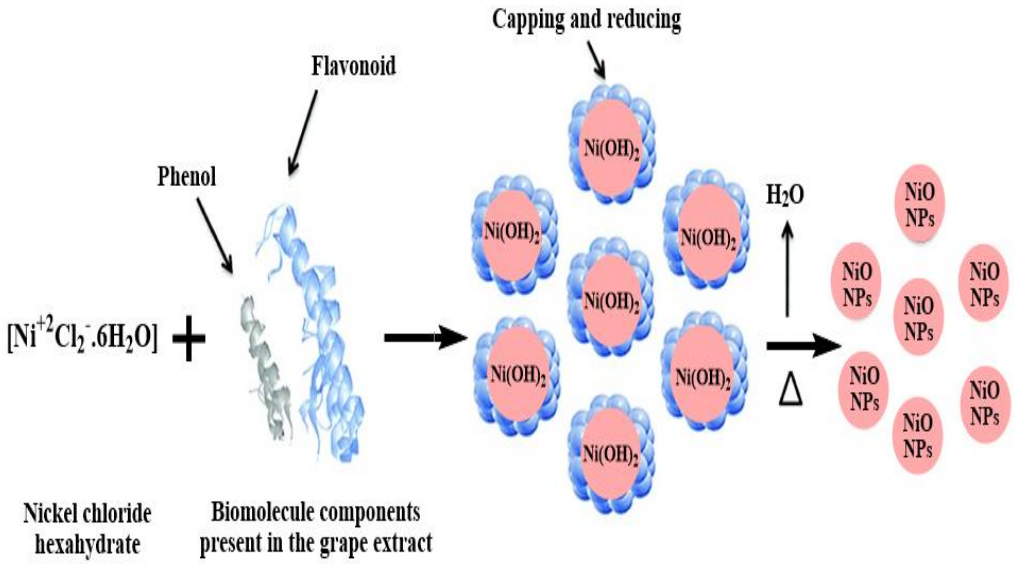


Figure (3-8): Mechanism of formation of NiONPs [185]

3.6 Characterization of NiONPs

3.6.1 Measurement of UV-Visible spectroscopy

The UV-Visible spectrum of NiONPs is shown in Figure (3-9). A broad spectrum at 328 nm (λmax) was shown in the NiONPs photo-spectrometrical analysis, indicating a NiONPs shape and validating the optical observation [154].

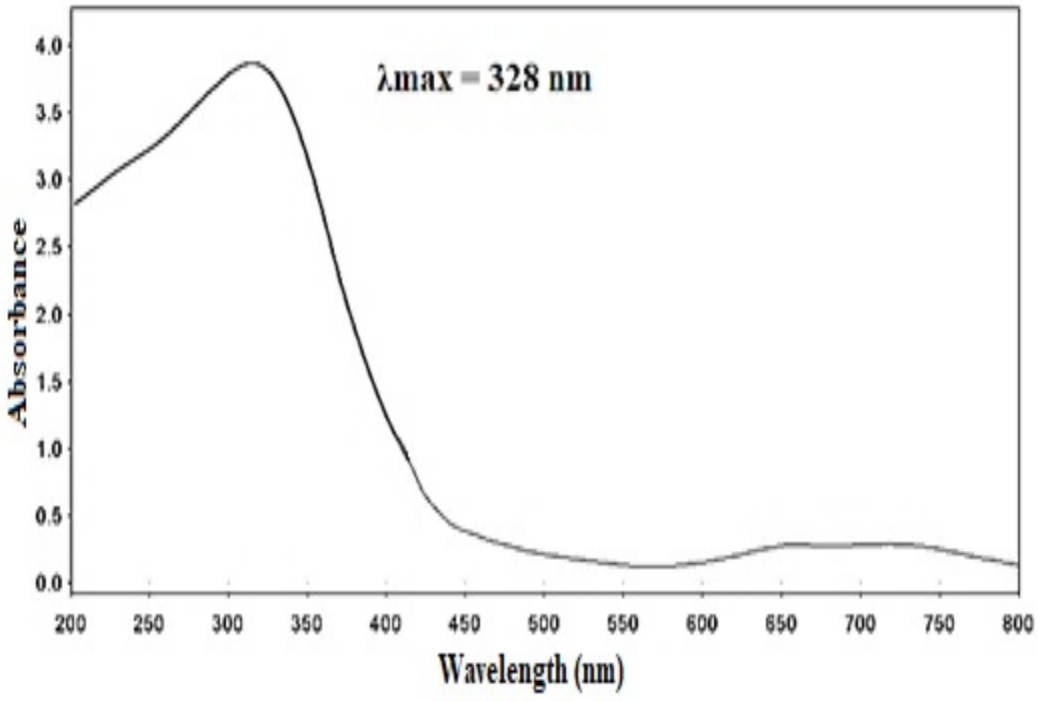


Figure (3-9): UV-Visible spectrum of NiONPs

3.6. 2 Fourier transforms infrared spectroscopy analysis (FT-IR)

Figure (3-10) shows the FT-IR results, it shows the absorption band that appeared at ~2918.30 cm^{-1}, it represents the characteristic vibrations of aliphatic C–H groups [186]. The wide band at 3593.38cm^{-1} may be related to the stretching vibrations of O–H [187], and the weak band at ~1647.21 cm^{-1} is assigned to the bending vibrations of C=O and H–O–H [188]. In hydroxyl groups which might be due to the employment of the KBr pellet technique as KBr is highly hygroscopic.

The bands at ~2385 cm^{-1} are assigned to the stretching vibrations of C=C or C=N because of the absorption of CO_2 from the atmosphere [189,190]. The band at approximately ~1035.77 cm^{-1} is associated with the asymmetric stretching of the resonance interaction between vibration modes of oxide ions in the nanoparticles. The vibrations of nickel species are usually active in the wavenumber range of (400-700) cm^{-1}. A close observation of this region result in two absorption bands at ~600 and ~464.84 cm^{-1} and shoulder at ~542 cm^{-1}, which can be easily deconvoluted to obtain Gaussian absorption bands. The shoulder at ~542 cm^{-1} may be ascribed to the bond with bending vibrations of Ni–O units, whereas the last band at ~427 cm^{-1} is associated with the stretching vibrations of Ni–O units. The position of stretching vibrations of Ni–O units confirmed the formation of nanoparticles [191].

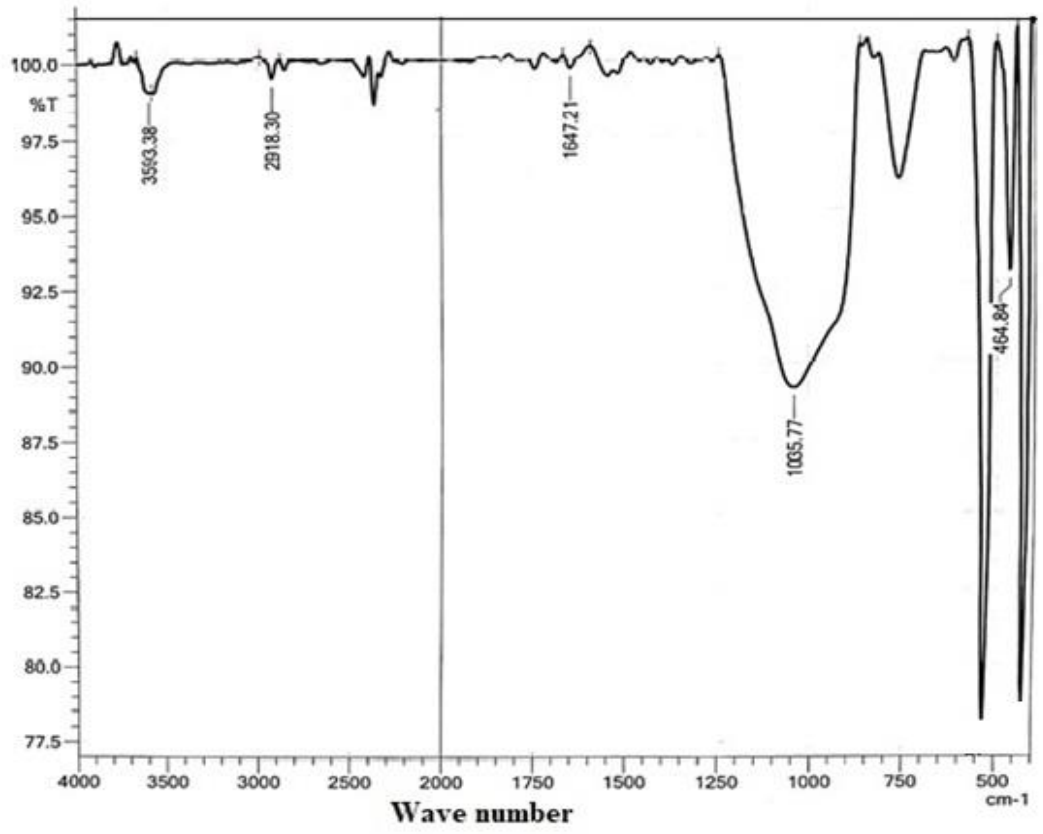


Figure (3-10): FT-IR spectrum of NiONPs

3.6. 3 X-ray diffraction analysis (XRD)

XRD study was carried out for biogenic NiO nanoparticles, as shown in Figure (3-11) The XRD peaks appeared at angles (2θ) of 37.29°, 43.45°, 63.11° and 75.35° corresponding to the (111), (200), (220) and (311) planes, which result in high arrange agreement with the single and pure step of a cubic NiO crystal structure (JCPDS Card No. 78-0643). The NiONP XRD pattern is consistent with some previous reports [192-194].

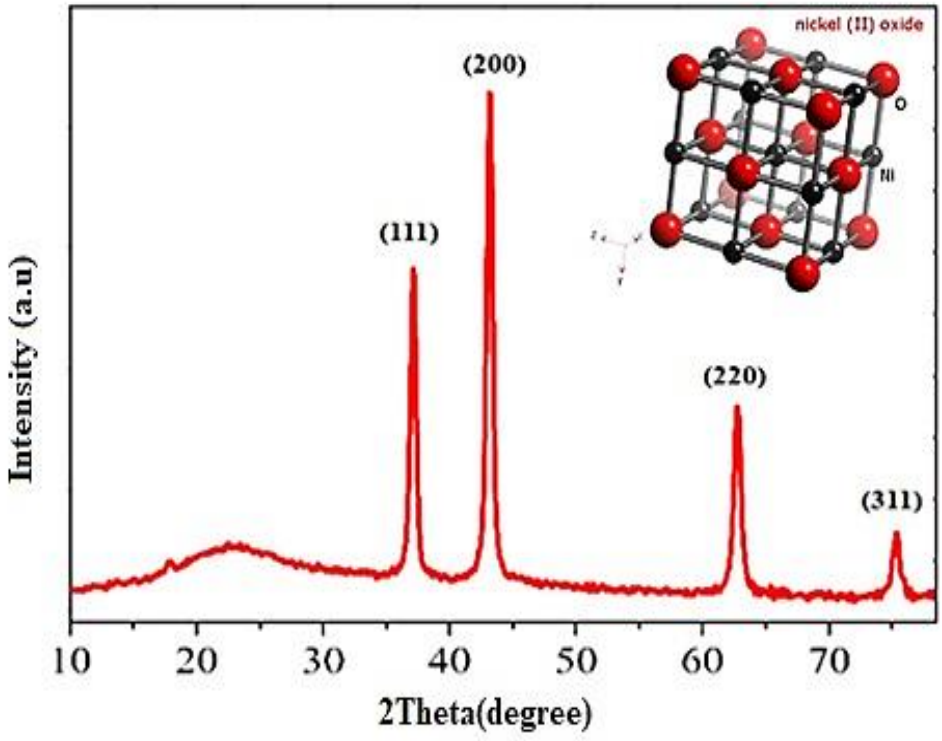


Figure (3-11): XRD of NiONPs

3.6.4 Atomic force microscope analysis (AFM)

The characterization of NiONPs was evaluated using AFM, as shown in Figure (3-12). The figure displays representations of the synthesized NiONPs in 2-dimension and 3-dimension. The images revealed that the distribution of NiONPs was small with a diameter of 61.19 nm.

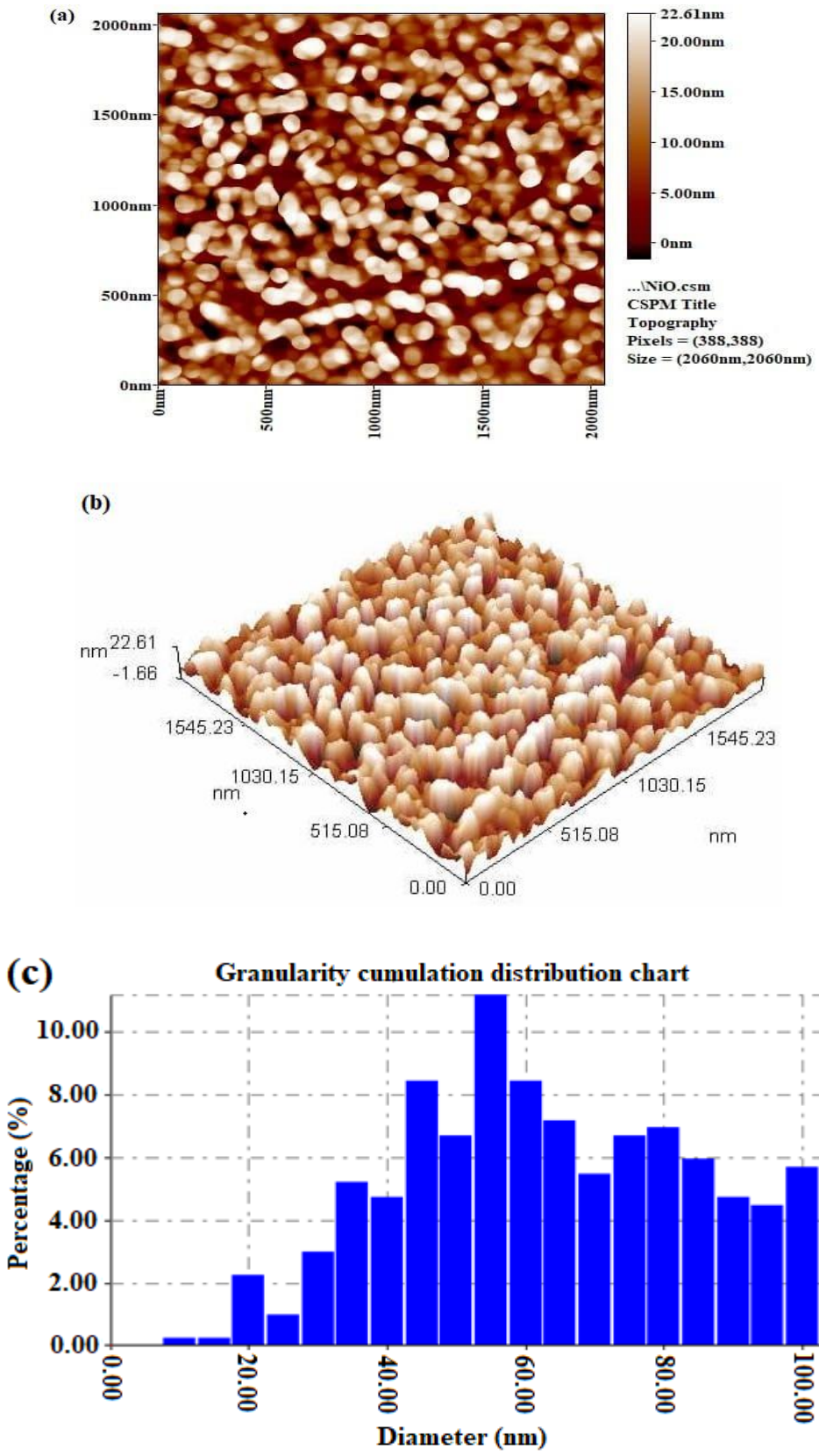


Figure (3-12): AFM analysis for NiONPs **(a)** 2-dimension and **(b)** 3-dimension structure and **(c)** Avarage distribution for NiONPs of diameter 61.19 nm

3.6.5 Scanning electron microscope analysis (SEM)

SEM was used to visualise the scale and the shape of the compounds. The SEM images of the synthesised nanoparticles of NiONPs are shown in Figure (3-14). The SEM images showed a comparatively spherical outline of the nickel oxide nanoparticle shaped with a diameter of (50-60) nm.

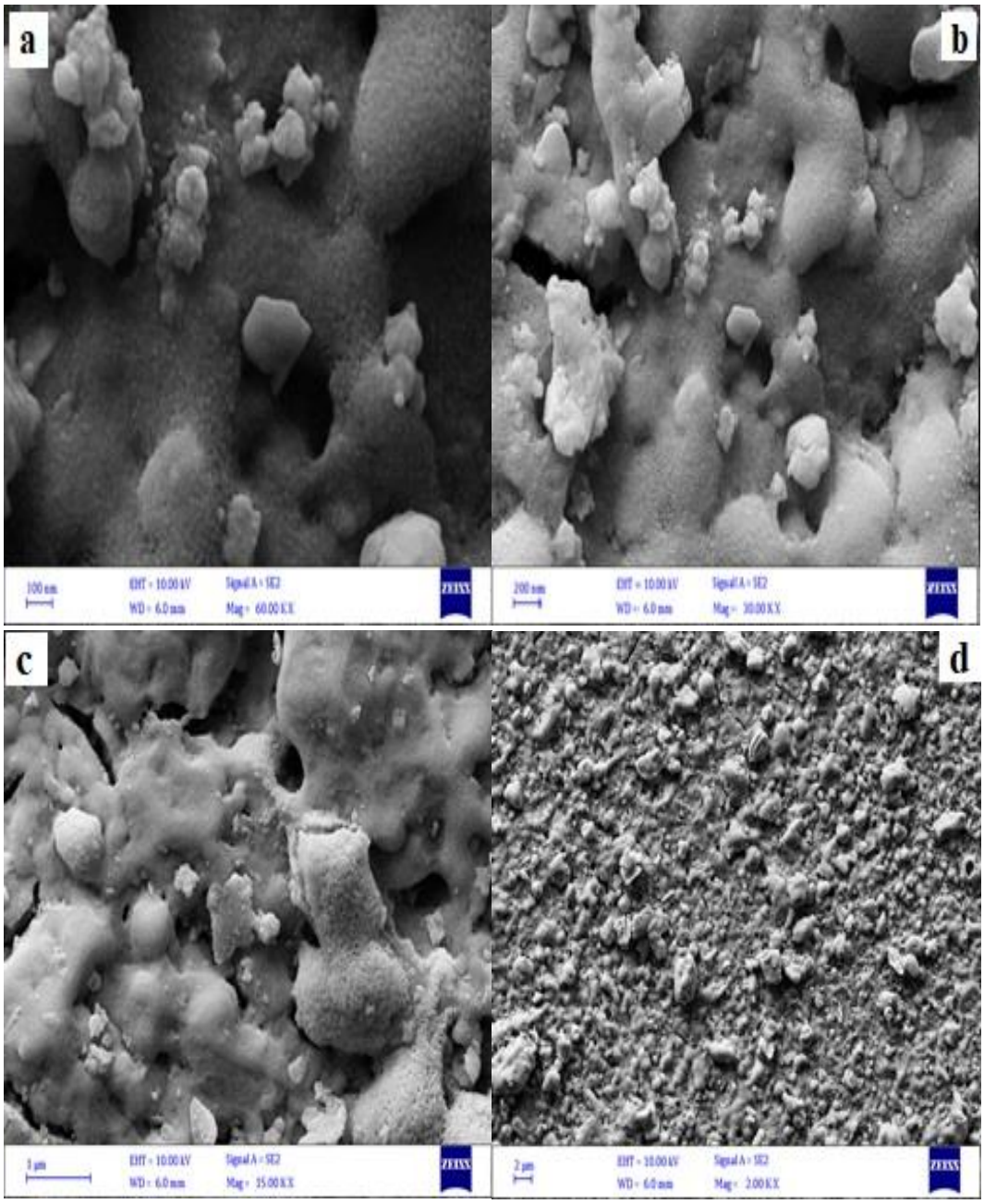


Figure (3-13): SEM for NiONPs **(a)** 100 nm, **(b)** 200 nm, **(c)** 1 µm and **(d)** 2 µm

3.6.6 Transmission electron microscope analysis (TEM)

TEM revealed the morphology of NiONPs. Typical TEM representations of NiONPs are shown in Figure (3-13). TEM images showed the spherical morphology of the nanoparticles with an average diameter of 60 nm.

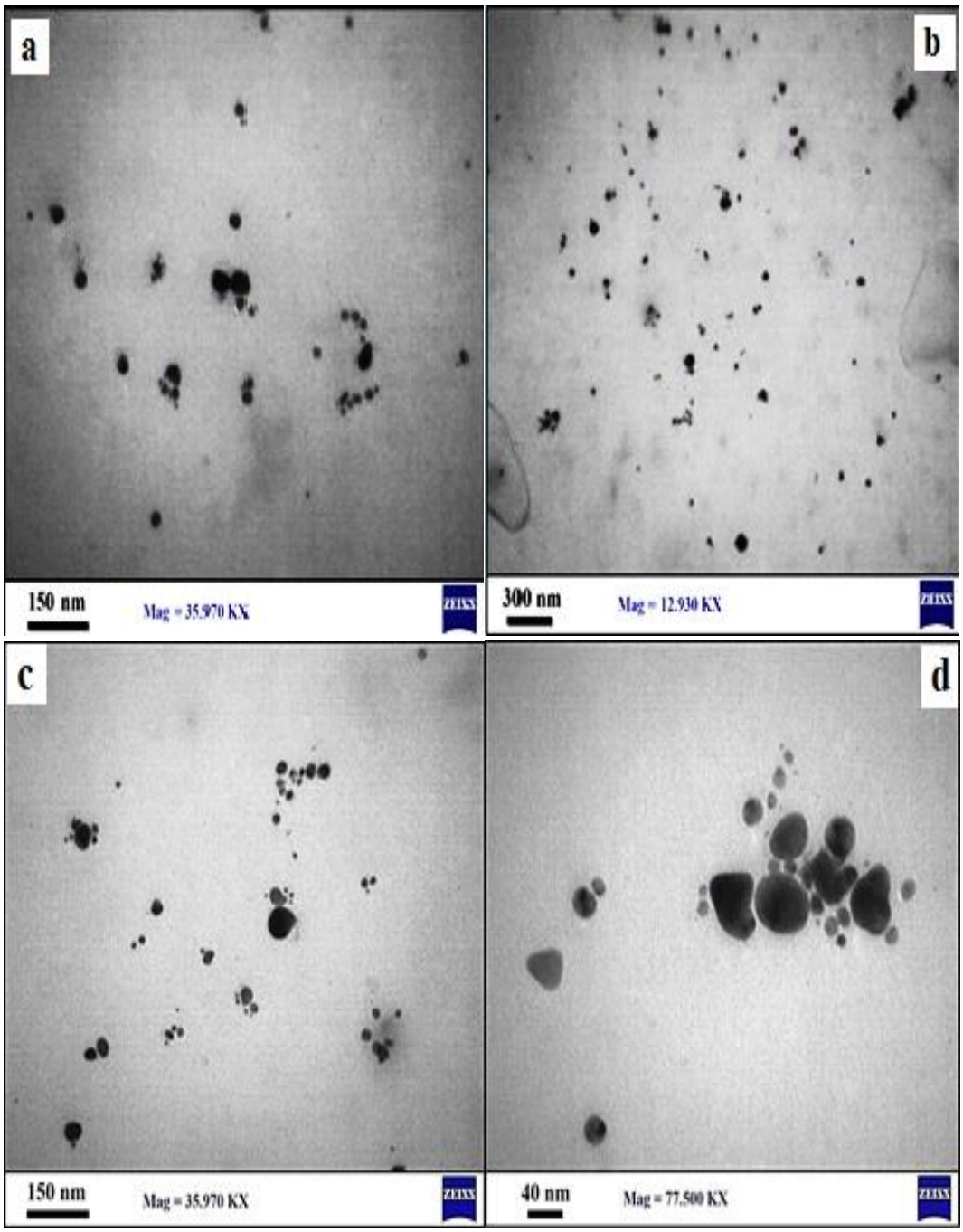


Figure (3-14) TEM of NiONPs **(a)** 150 nm, **(b)** 300 nm, **(c)** 150 nm and **(d)** 40 nm

3.7 Anti-cancer activity

Anti-cancer agents have to destruct cancer cells without damaging to the normal cells. MTT was used to investigate the impact ZnONPs and NiONPs against an MCF-7 and AMGM5 cell lines, which showed a significant cytotoxic effect against these cell lines. These nanoparticles may also be used as a development technique for effective therapeutic anti-cancer agents.

3.8 Thiazolyl blue tetrazolium bromide (MTT) assay

MTT test is a colorimetric process, which is based on the metabolic activities of active cells to convert yellow tetrazolium salt to purple formazan crystals. An MTT trial was tested the cytotoxic activity of ZnONPs and NiONPs for 72 hours on the viability of two types of human cancer cell lines, MCF-7 and AMGM5.

3.8.1 Anti-cancer of ZnONPs

The electrostatic properties of ZnO NPs were important for biomedical applications. ZnO NPs have also been used as a convenient alternative material in cancer and drug delivery applications. Several experiments have shown that ZnO NPs have substantial cytotoxicity against different types of cancer cells compared with macro-scale ZnO. Thus, ZnO NPs under physiological conditions have a positive charge. Considering that cancer cells also have high anionic phospholipid concentrations on their outer membrane, electrostatic bonds that increases cellular uptake and cytotoxicity can direct contact with positively charged ZnO NPs [(195)]. The cytotoxic effect of ZnO NPs against human breast cancer MCF-7 cell line and human brain cancer AMGM5 cell lines was studied. by its ability to inhibit the proliferation of cancer cells. The results

showed a significant cytotoxic activity of ZnO NPs against MCF-7 and AMGM5 cell lines. The results suggest the ability of ZnONPs to suppress the growth of these cell lines, and this effect is concentration dependent.

The figure (3-1) shows the inhibition rate of cell line of MCF-7 and AMGM5 cell linesby ZnONPs in dose dependent manner.

Table (3-1): Inhibition rate of ZnONPs on AMGM5 and MCF-7 cell line

Concentration	% Mean inhibition rate of AMGM5 cell line	SDM	% Mean inhibition rate of MCF-7 cells line	SDM
Control (untreated cells)	---	---	---	---
0.02 M	78 %	± 0.83	80 %	± 0.91
0.01 M	65 %	± 0.57	70 %	± 0.79
0.005 M	53 %	± 0.78	59 %	± 0.87
0.0025 M	25 %	± 0.98	28 %	± 0.60
0.00125 M	15 %	± 1.53	16 %	± 0.68

3.8.2 Anti-caner of NiONPs

Cancer is a debilitating illness, which is a major cause of death in the world wide. By 2030, the number of cases is expected to reach approximately 21 million [(149)]. The MTT assay was conducted following exposure to NiONPs for 72 hours against the MCF-7 cell line of human breast cancer and AMGM5 cell lines of human brain cancer. Cytotoxicity in MCF-7 and AMGM5 cells was caused by varying concentrations of NiONPs [(196)]. The dose-dependent inhibition of cancer cells was achieved by cancer cells treated with varying concentrations of NiONPs (0.00125 - 0.02) M. Our results have shown a good anti-cancer activity of NiONPs with the maximum inhibition recorded at 0.02 M, a dose-dependent inhibition with 75%

mortality was obtained. This finding suggests the therapeutic potential and the ability of nanoparticles to suppress the growth of cancer cell lines [149].

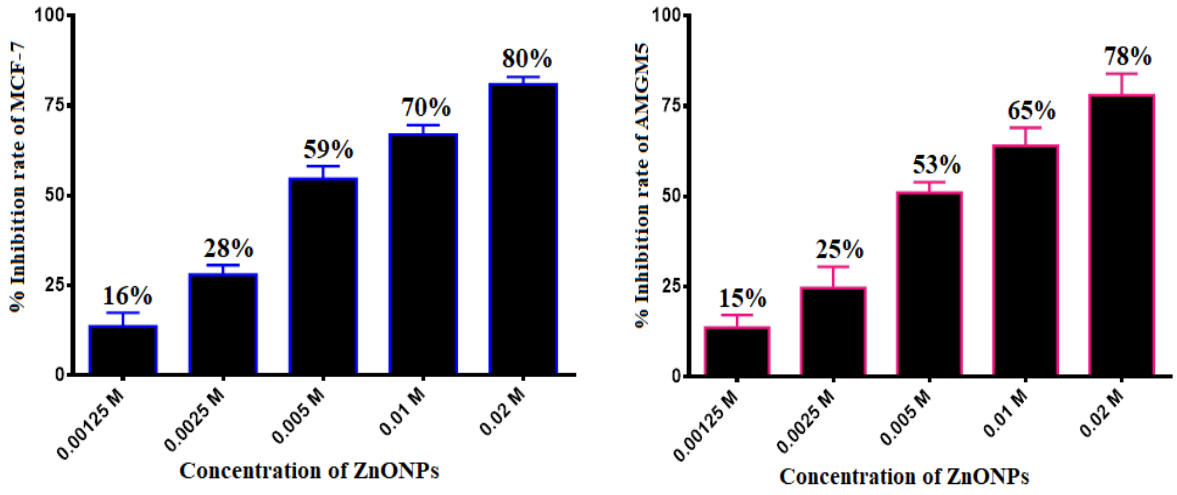
The figure (3-2) shows the inhibition rate of cell line of MCF-7 and AMGM5 cell linesby NiONPs in dose dependent manner.

Table (3-2): Inhibition rate of NiONPs on AMGM5 and MCF-7 cell line

Concentration	% Mean inhibition rate of AMGM5 cell line	SDM	% Mean inhibition rate of MCF-7 cells line	SDM
Control (untreated cells)	---	---	---	---
0.02 M	72 %	± 0.85	75 %	± 0.63
0.01 M	56 %	± 0.75	67 %	± 0.86
0.005 M	44 %	± 0.67	48 %	± 0.97
0.0025 M	24 %	± 0.95	25 %	± 1.12
0.00125 M	14 %	± 0.57	16 %	± 1.22

The AMGM5 and MCF-7 cell lines were morphologically examined to assess the apoptogenic benefit of the active compounds. Inverted phase contrast microscopes have been visualized in order to determine morphological modulations to clarify apoptosis. Morphological differences in the AMGM5 and MCF-7 cell lines are shown in the figures (3-15 a and b), (3-16), (3-17 a and b) and (3-18) compared with the control cells, that are shown after the incubation with examined compounds for 72 hours.

Visualizes of the untreated cells showed that their initial morphology were preserved, with the majority of control cells were sticking to the tissue culture template. In the meantime, ZnONPs and NiONPs have shown high productivity for cancer cell lines proliferation and morphology for 72 hours after treatment.



(a) **(b)**

Figure (3-15): **(a)** Inhibition rate of ZnONPs against MCF-7 cell line, **(b)** Inhibition rate of ZnONPs against AMGM5 cell line

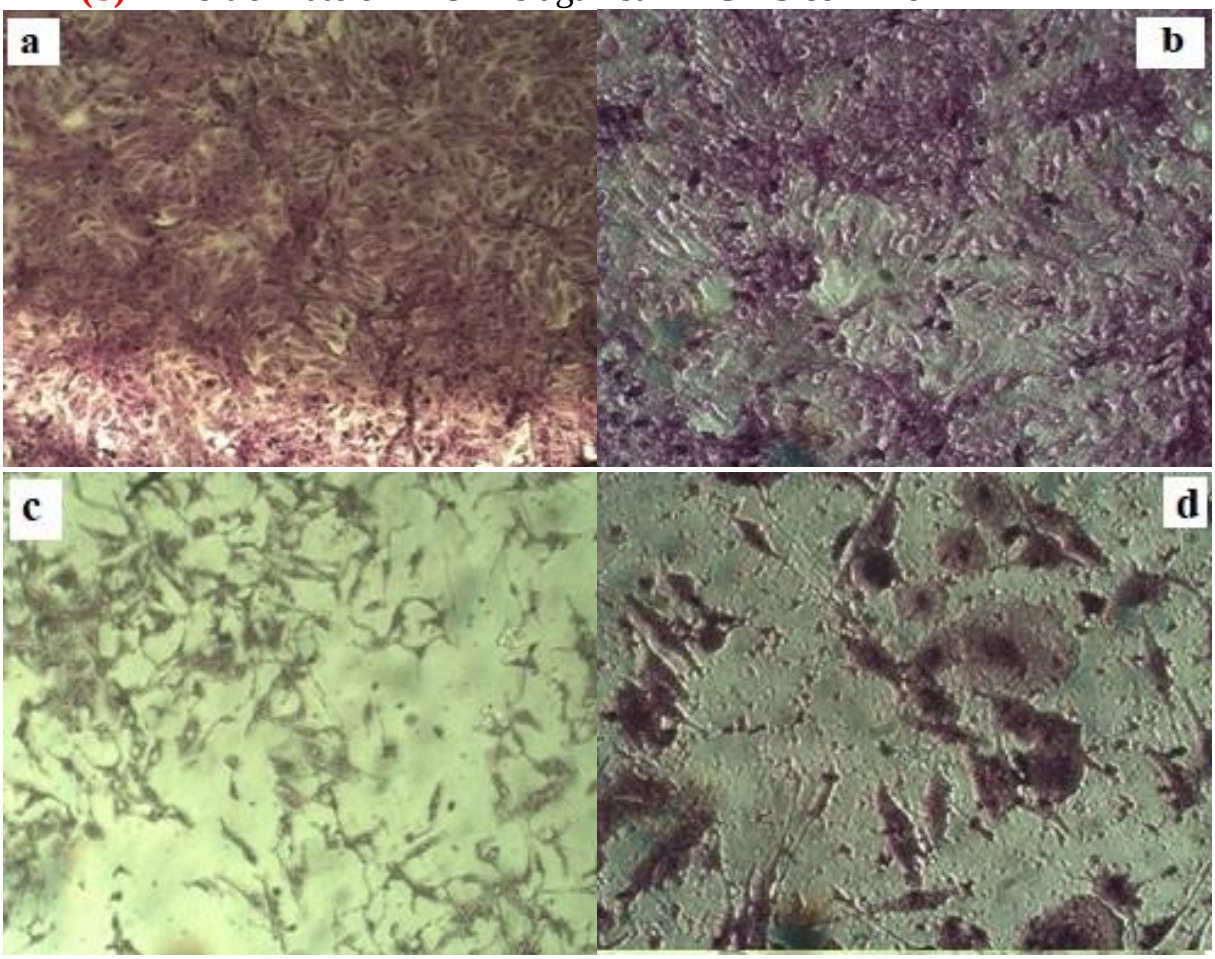
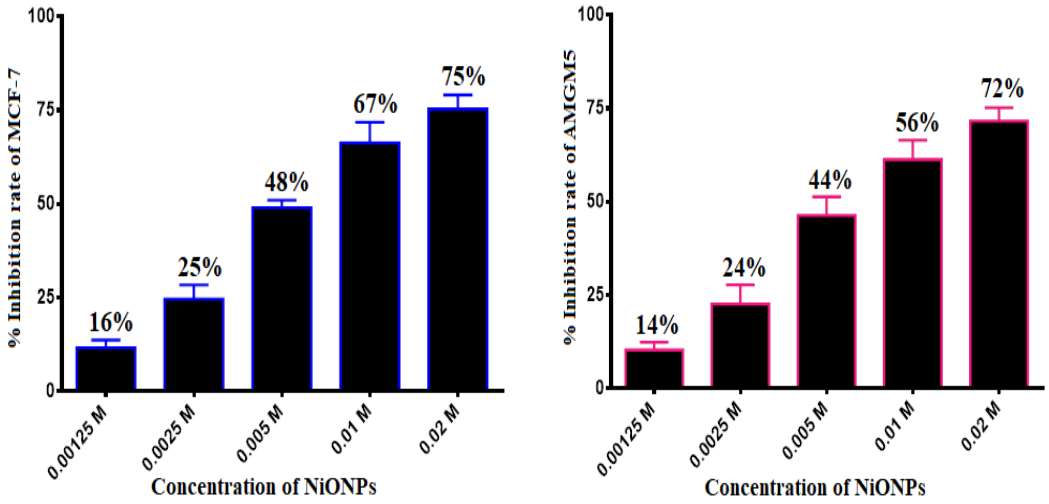


Figure (3-16): **(a)** Control MCF-7 cell line, **(b)** Control AMGM5 cell line, **(c)** Morphological changes in MCF-7 cell line after treated with ZnONPs, **(d)** Morphological changes in AMGM5 cell line after treated with ZnONPs



(a) **(b)**

Figure (3-17): **(a)** Inhibition rate of NiONPs against MCF-7 cell line**,** **(b)** Inhibition rate of NiONPs against AMGM5 cell line

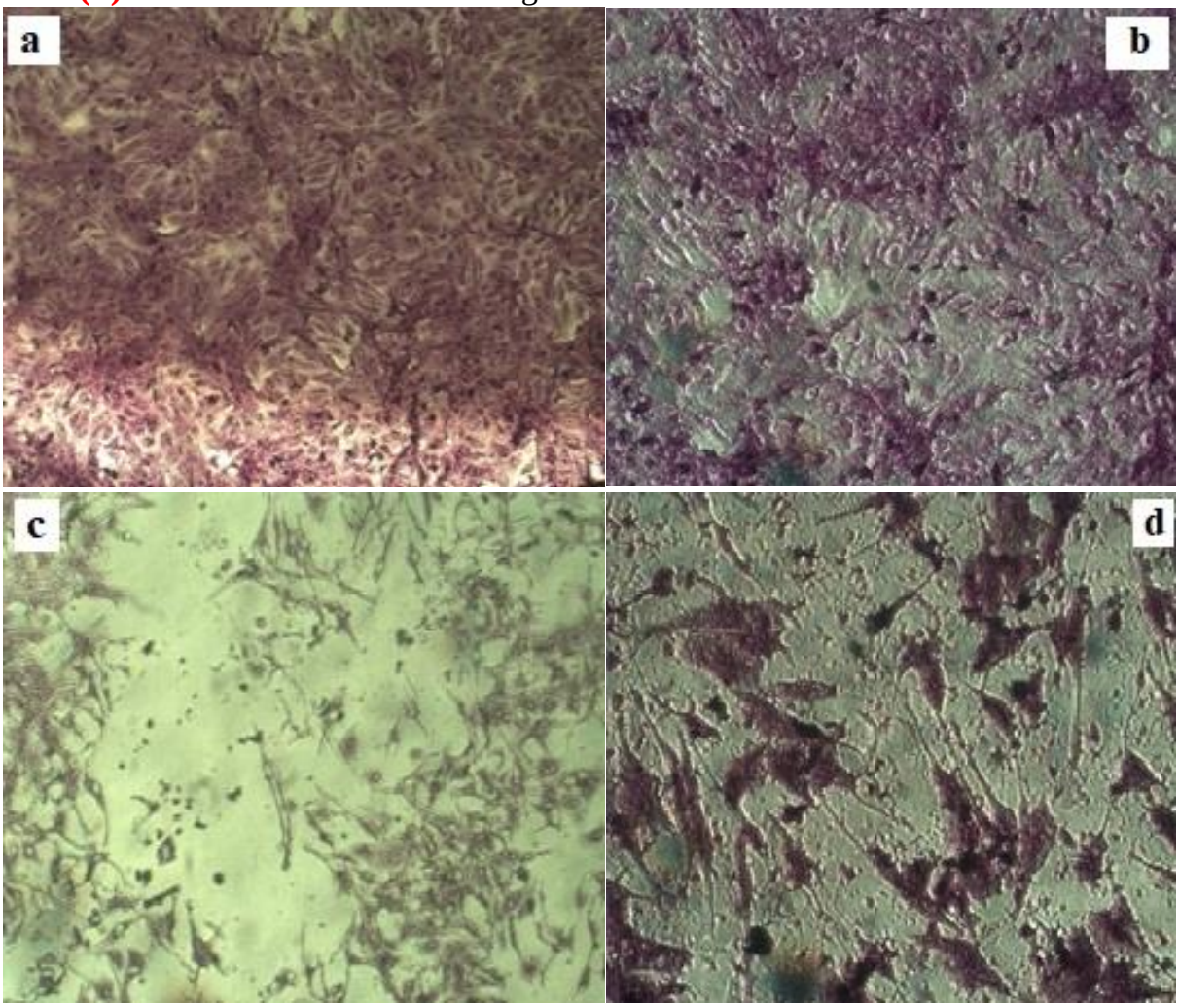


Figure (3-18): **(a)** Control MCF-7 cell line, **(b)** Control AMGM5 cell line, **(c)** Morphological changes in MCF-7 cells after treated with NiONPs, **(d)** Morphological changes in AMGM5 cells after treated with NiONPs

3.9 Anti-bacterial activity

Bacterial sensitivity test toword ZnONPs and NiONPs. The anti-bacterial efficacy of ZnONPs and NiONPs was tested against *K. pneumoniae* (G^-) and against *S. aureus* (G^+). It can be clearly observe that these nanoparticles have great anti-bacterial activity.

3.10 Agar well diffusion method

In the current study, ZnONPs and NiONPs have shown an anti-bacterial role as agents for *K. pneumoniae* (G^-) and *S. aureus* (G^+), which is demonstrated by using a system of agar well diffusion.

3.10.1 Anti-bacterial activity of ZnONPs

One of the key factors for hospital-acquired infections is rise of anti-biotic resistance. Its management involve the use of medication formulations with more side effects. Thus, the researchers have focus on using a mix of low risk and successful bacterial resistance control. One of the ways to reduce hospital acquired infections (HAI) is using of nano-material and evaluate their anti-microbial effects. ZnO non-nanoparticles have been shown to be strongly resistant to microorganisms while ZnONPs exhibit strong anti-bacterial activities on broad-spectrum pathogenic bacteria including *S. aureus* and *K. pneumonia*. Anti-bacterial activity of ZnONPs was examined by the measurement of minimum inhibitory concentration (MIC) using serial dilution methods [(182)] as shown in Figures (3-19) and (3-20).

The inhibition areas diameters are shown in the Table (3-3), which represent the activity of ZnONPs as anti-bacterial in avarious concentrations against *S.aureus* . ZnONPs demonstrated considerable inhibition with increased dose concentration.

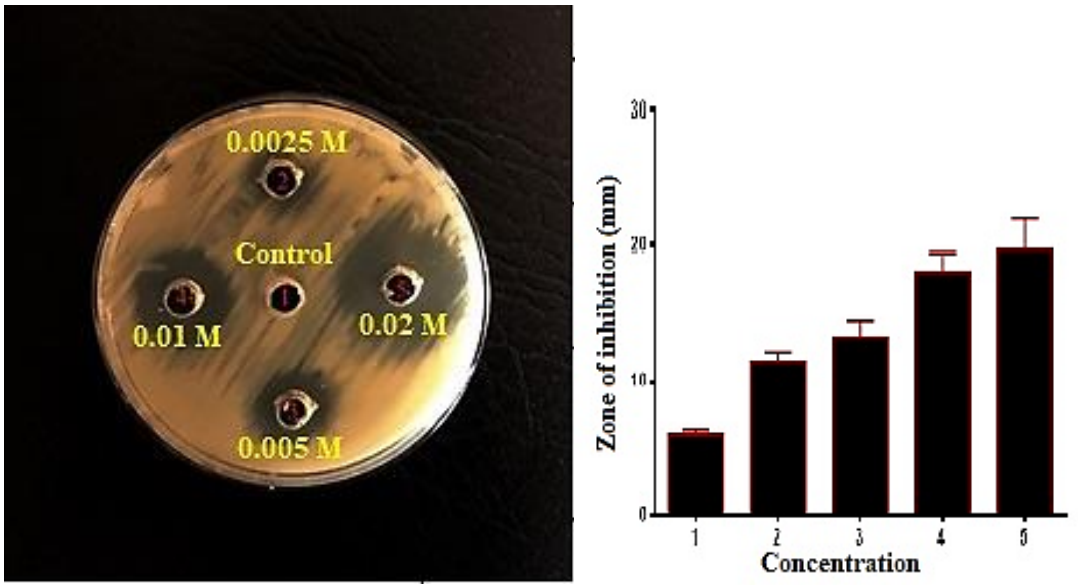
Table (3-3): Growth inhibition of *Staphylococcus aureus* by ZnONPs

Concentration	Inhibition zone (mm)	SDM
Control	---	---
0.02 M	23.85 mm	± 0.80
0.01 M	18.75 mm	± 0.32
0.005 M	15 mm	± 0.41
0.0025 M	11.8 mm	± 0.57

Zinc oxide nanoparticles displayed an inhibition zone with a diameter at various concentrations against *Klebsiella pneumonia*, as seen in the Table (3-4). ZnONPs were shown a significant inhibition with increase concentration.

Table (3-4): Growth inhibition of *Klebsiella pneumonia* by ZnONPs

Concentration	Inhibition zone (mm)	SDM
Control	---	---
0.02 M	20 mm	± 0.35
0.01 M	18 mm	± 0.21
0.005 M	13.85 mm	± 0.23
0.0025 M	11.5 mm	± 0.33

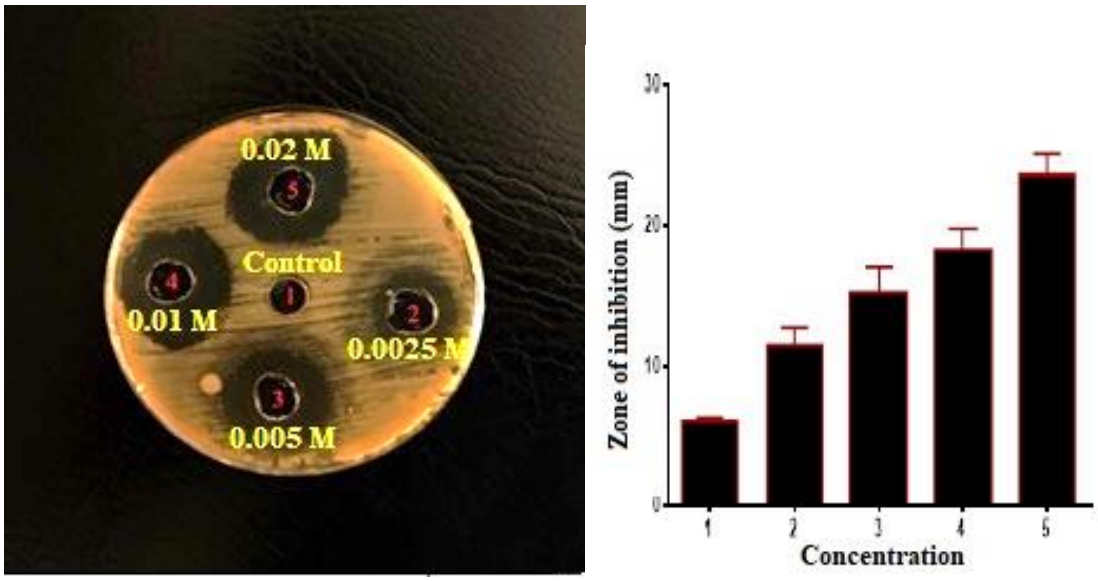


(a) **(b)**

Figure (3-19): **(a)** Anti-bacterial activity of zinc oxide NPs against *K. pneumoniae*

1. Represented control untreated bacterial strain
2. Bacterial strain treated with ZnONPs at concentration 0.0025 M
3. Bacterial strain treated with ZnONPs at concentration 0.005 M
4. Bacterial strain treated with ZnONPs at concentration 0.01 M
5. Bacterial strain treated with ZnONPs at concentration 0.02 M

(b) Zone of inhibition for zinc oxide NPs in mm



(a) **(b)**

Figure (3-20): **(a)** Anti-bacterial activity of zinc oxide NPs against *S. aureus*

1. Represented control untreated bacterial strain
2. Bacterial strain treated with ZnONPs at concentration 0.0025 M
3. Bacterial strain treated with ZnONPs at concentration 0.005 M
4. Bacterial strain treated with ZnONPs at concentration 0.01 M
5. Bacterial strain treated with ZnONPs at concentration 0.02 M

(b) Zone of inhibition for zinc oxide NPs in mm

3.10.2 Anti-bacterial activity of NiONPs

Modern nanotechnology advances would provide innovative methods for the design and manufacture of new substances with specific anti-microbial properties [197]. The anti-bacterial activity of the NiONPs was tested against Gram-positive *S. aureus* and Gram-negative pure bacterial cultures *K. pneumonia*. Figures (3-21) and (3-22) show that the anti-bacterial effect increases as the concentration of NiONPs increases from 0.0025 M to 0.02 M, and for both samples, the maximum zone of inhibition was observed at 0.02 M. The anti-bacterial findings have shown that considering the variation in the cell wall composition, Gram-positive bacteria are more sensitive to NiO nanoparticles than Gram-negative bacteria. The Gram-positive bacterial cell wall consists of a dense coating of peptidoglycan that is bound to teichoic acids, which are unique to Gram-positive bacteria and can be more easily [198]. However, in Gram-negative bacteria, the cell wall includes a thin film of peptidoglycan and an exterior membrane consisting of closely packed molecules of lipopolysaccharide [199], which are narrowly permeable and governs transfering via the plasma membrane. This structure may be decreases the resistance of Gram-negative bacteria to NiONPs [200].

Diameters of inhibition areas are shown in the Table (3-5), which are represent the activity of NiONPs as anti-bacterial at various concentrations against *S.aureus*. NiONPs demonstrated considerable inhibition of growth with increase dose concentration.

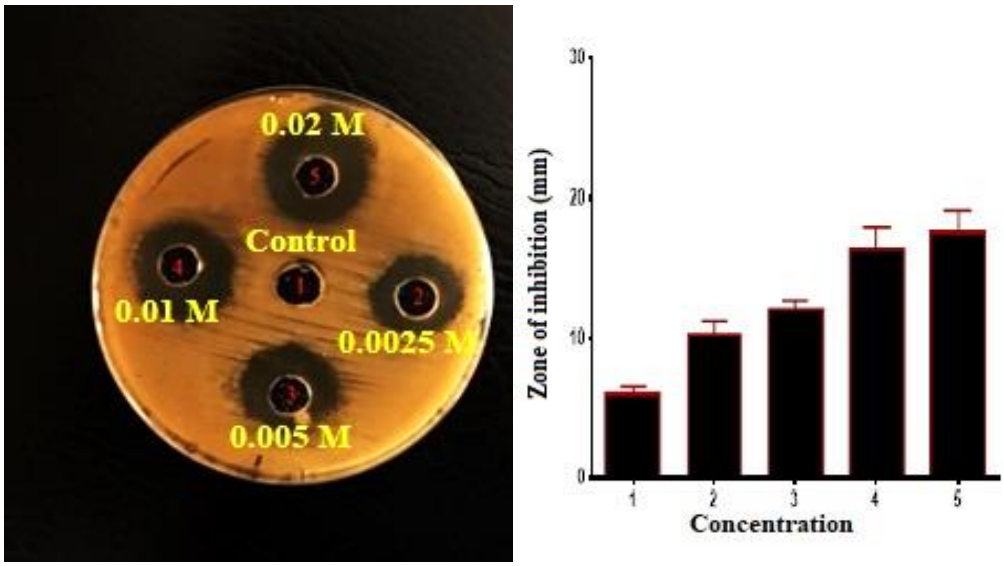
Table (3-5): Growth inhibition of *Staphylococcus aureus* by NiONPs

Concentration	Inhibition zone (mm)	SDM
Control	---	---
0.02 M	21 mm	± 0.25
0.01 M	16.25 mm	± 0.46
0.005 M	14 mm	± 0.38
0.0025 M	10.5 mm	± 0.44

Nickel oxide nanoparticles displayed diameter inhibition zones at various concentrations against *K. pneumonia*, as shown in the table (3-6). NiONPs have shown a significant inhibition with increase dose concentration.

Table (3-6): Growth inhibition of *Klebsiella pneumonia* by NiONPs

Concentration	Inhibition zone (mm)	SDM
Control	---	---
0.02 M	17.75 mm	± 0.65
0.01 M	16.15 mm	± 0.86
0.005 M	12.25 mm	± 0.55
0.0025 M	10.25 mm	± 0.73

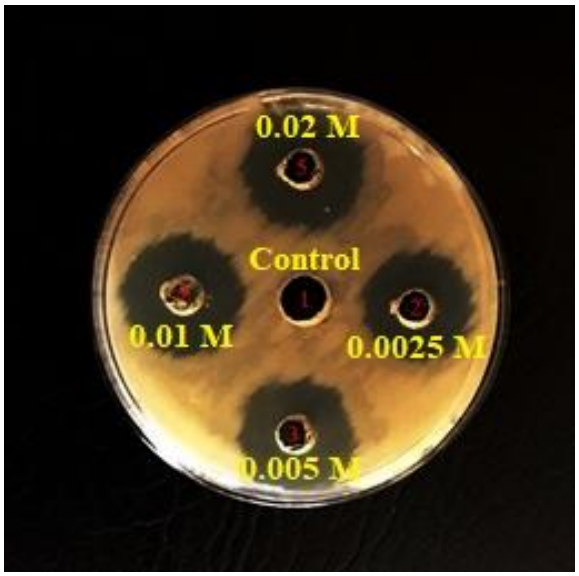


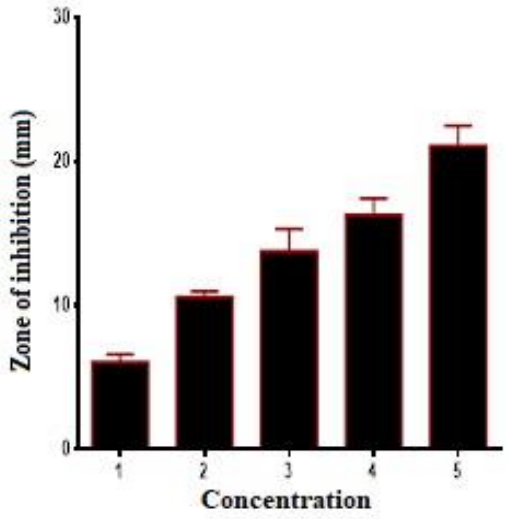
(a) **(b)**

Figure (3-21): **(a)** Anti-bacterial activity of NiONPs against *K. pneumoniae*

1. Represented control untreated bacterial strain
2. Bacterial strain treated with NiONPs at concentration 0.0025 M
3. Bacterial strain treated with NiONPs at concentration 0.005 M
4. Bacterial strain treated with NiONPs at concentration 0.01 M
5. Bacterial strain treated with NiONPs at concentration 0.02 M

(b) Zone of inhibition for nickel oxide NPs in mm





(a) **(b)**

Figure (3-22): **(a)** Anti-bacterial activity of NiONPs against *S. aureus*
1. Represented control untreated bacterial strain
2. Bacterial strain treated with NiONPs at concentration 0.0025 M
3. Bacterial strain treated with NiONPs at concentration 0.005 M
4. Bacterial strain treated with NiONPs at concentration 0.01 M
5. Bacterial strain treated with NiONPs at concentration 0.02 M

(b) Zone of inhibition for nickel oxide NPs in mm

3.11 Conclusions and recommendations

3.11.1 Conclusions

One of the innovative methods in the bio-medical area of nanotechnological is production of bio-compatible molecules as anticancer and anti-bacterial agent.

Green synthesis of nano-scale ZnONPs and NiONPs was performed by using the extract of grape juice as a non-toxic source. The physical and chemical properties of the prepared nanoparticles were identified by UV-Visible spectroscopy, FT-IR, XRD, AFM, SEM, and TEM. The cytotoxic activity of ZnONPs and NiONPs was conducted by using the MTT assay, AMGM5 and MCF-7 cell lines showed high sensitivity against ZnONPs compared to NiONPs in a

dose-dependent manner. ZnONPs and NiONPs also showed a high anti-bacterial activity with an increase of dose concentration against *Klebsiella pneumoniae* (G^-) and *Staphylococcus aureus* (G^+). The agar well diffusion approach revealed that the ZnONPs had more effect against these species of pathogenic bacteria than NiONPs. The ease of configuration of both ZnONPs and NiONPs are a feasible option for potential biomedical applications due to their biocompatibility and economic efficiency.

3.11.2 Recommendations

In the right of the present study, the following recommendations may be helpful for further investigations:

1- Study the cytotoxicity ZnONPs and NiONPs other cancer cell lines and other general of pathogenic bacterial.
2- Study effect of the ZnONPs and NiONPs on the water treatment.
3- Study the ZnONPs and NiONPs drug delivery vectors for human cancers.
4- Evaluation the pharmacological effects of the ZnONPs and NiONPs by using animal experimentation (In vivo).

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الخلاصة

ظهرت تقنية النانو مؤخراً في العديد من التطبيقات، بما في ذلك الاستخدام الطبي. حيث توفر بدائل لتطوير أنظمة الإطلاق الخاضعة للرقابة لعلاج الأمراض المختلفة وتقليل الآثار الجانبية للأدوية. تركز التكنولوجيا الحيوية النانوية على تخليق الجسيمات النانوية وتطبيقها. حيث تلعب دوراً مهمًا في علم الأورام السرطانية. من جهة اخرى، طورت العديد من انواع البكتيريا المسببة للامراض مقاومة عالية للمضادات الحيوية التقليدية، وبالتالي، يمكن استخدام المواد النانوية لمنع نموها. ان مستخلص العنب غني بالمواد الكيميائية النباتية مثل البولي فينول والفلافونويد ومضادات الاكسدة، القادرة على اختزال أملاح المعادن. حيث تتمتع المواد الكيميائية النباتية الموجودة في مستخلص العنب بفوائد علاجية عالية، مما يجعلها بديلاً مغرياً لعوامل اختزال السموم التقليدية.

في هذه الدراسة، تم تحضير الجسيمات النانوية لأكسيد الزنك (ZnO) وأكسيد النيكل (NiO) بنجاح بطريقة صديقة للبيئة (Green biosynthesis) من مستخلص العنب المائي (*Vitis vinifera*) لتحضير جزيئات نانوية لأكاسيد المعادن متعددة الوظائف. لذلك، تهدف هذه الدراسة إلى تقييم كفاءة ZnONPs و NiONPs في المختبر لتدمير خط خلايا سرطان الثدي البشري (MCF-7) وخط خلايا سرطان الدماغ البشري (AMGM5)، وكذلك دراسة فعالية هذه الجسيمات النانوية في قمع نمو بكتيريا *Staphylococcus aureus* و*Klebsiella pneumonia*.

لفحص خواص الجسيمات النانوية لأكاسيد الزنك والنيكل، تم استخدام تقنيات التشخيص الفيزيائية مثل طيف الأشعة فوق البنفسجية المرئية (UV-Visible) و طيف الأشعة تحت الحمراء (FT-IR) و حيود الأشعة السينية (XRD) و مجهر القوة الذرية (AFM) والمجهر الإلكتروني الماسح (SEM) والمجهر الإلكتروني النافذ (TEM)، والتي أثبتت أن هذه الجسيمات النانوية لها خصائص كيميائية وفيزيائية فريدة. تم تحديد الخواص المضادة للسرطان لـ ZnONPs و NiONPs ضد خطوط الخلايا البشرية MCF-7 و AMGM5 باستخدام تقنية MTT السامة للخلايا. كما تم تحديد الخواص المضادة ضد بكتريا *S. aureus* (G^+) و *K. pneumonia* (G^-) باستخدام تقنية انتشار حفر الآبار. وقد أظهرت الجسيمات النانوية المُصنّعة حيوياً كفاءة عالية كعوامل مضادة للسرطان ومضادة للبكتيريا بطريقة تعتمد على الجرعة.

في الختام، كشفت النتائج التي توصلنا إليها أن التصنيع الحيوي بطريقة (Green synthesis) لـ ZnONPs وNiONPs من مستخلص العنب المائي (*Vitis vinifera*) أعطت نتائج أولية مشجعة للعمل المستقبلي لاستخدامها في النهج العلاجي كأدوية مضادة للسرطان ومضادة للميكروبات.

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قسم الكيمياء

تحضير الجسيمات النانوية لأكاسيد الزنك و النيكل ودراسة تطبيقاتها الحيوية

رسالة

مقدمة إلى كلية العلوم- جامعة الأنبار

وهي جزء من متطلبات نيل شهادة الماجستير في علوم الكيمياء

من قبل الطالب

براء يوسف حسين التميمي

بكالوريوس كيمياء ٢٠١٦م

بأشراف

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