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Theme

Green synthesis of Nanomaterials from plant sources
Cucurbitaceae family "Pumpkin"

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



Abstract

The aim of our study is to conduct green synthesis of zinc oxide and silver nanoparticles from natural plant sources, specifically from pumpkin (*Cucurbita moschata*) belonging to the Cucurbitaceae family, due to its therapeutic potential and biological activities, and its applications in various fields.

In our experimental study, after extracting oil from pumpkin seeds with a yield of 1.611%, we assessed its value for the presence of biologically active compounds that give it high therapeutic potential, nutritional value, and health benefits such as linoleic acid, palmitic acid, and oleic acid.

We characterized the materials synthesized from pumpkin seeds and leaves using color change, UV-VIS, FTIR, EDX/SEM, and DRX. The results showed that the synthesized materials had hexagonal and cubic shapes, ranging in size from 10,000 nm to 50,000 nm, exhibiting antibacterial activity against types of bacteria: *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*.

Keywords: Green synthesis, Nanoparticles, *C. moschata*, Zinc nano-materials, Silver nano-materials, Antibacterial, fixed oil, pumpkin seeds, Oils Extraction

المخلص

هدف دراستنا هو إجراء التصنيع الأخضر لمواد أكسيد الزنك والفضة النانوية من مصادر طبيعية نباتية؛ من نبات اليقطين الذي ينتمي إلى عائلة القرعيات تحديداً، *Cucurbita moschata*، وذلك لإمكاناتها العلاجية وأنشطتها البيولوجية، و تطبيقاتها التي دخلت جميع المجالات و الميادين .

خلال دراستنا التجريبية، بعد استخلاص الزيت من بذور اليقطين بمردود تقديره 1.611 % و تثمينه لتواجد مواد فعالة

بيولوجيا به تعطيه قدرة علاجية عالية، قيمة غذائية وفوائد صحية مثل *oleic acid*، *linoleic acid*، *palmitic acid*.

كشفتنا على المواد المصنعة من بذور و اوراق اليقطين بالتغير اللوني و *DRX* و *UV-VIS*، *FTIR*، *EDX/SEM*

، بينت النتائج ان للمواد المصنعة اشكالا سداسية و مكعبة، يتراوح حجمها من 10000 nm الى 50000 nm، مظهرة نشاطا مضادا

للبكتيريا ضد انواع من البكتيريا *Pseudomonas aeruginos*، *Escherichia coli*، *Staphylococcus aureus* : واخيرا

Bacillus subtilis

الكلمات المفتاحية: التصنيع الأخضر، الجسيمات النانوية، مواد الفضة النانوية، مواد أكسيد الزنك النانوية، مضاد للبكتيريا،

الزيت الثابت، بذور اليقطين، استخلاص الزيوت، عائلة القرعيات

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Dedication

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(و ان لیس للانسان الا ما سعی و ان سعیہ سوف یری)

صدق الله العظيم

To the Creator of the soul and the pen....and the Creator of the atom and the breeze.....and the Creator of everything from nothingness

We began this journey only with His facilitation, reached the end only with His guidance, and achieved our goal only by His grace. Praise be to Allah who enabled us to complete this step in our educational journey.

To the one who conveyed the message, fulfilled the trust, and advised the nation—our Prophet of mercy and light of the worlds.

To the land that lived within us before we lived within it, known to us through the pages of sacrifice—our second home, Palestine, our first Qibla, and the destination of our beloved Prophet. May Allah gather us at Al-Aqsa, victorious and rejoicing. Indeed, nothing is difficult for Allah.

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With all my love, I dedicate my humble effort to you.

In conclusion, we ask Allah to accept our striving, bless us in it, and we praise Him with great praise. We send blessings and peace upon the intercessor of the Ummah, our Prophet Muhammad, peace and blessings be upon him.

Jinns





Dedication

Praise be to Allah. My heart overflows with gratitude for this great blessing, and I prostrate to Allah in thanks. If I have succeeded, it is by Allah's grace; if I have erred, it is from myself.

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Nomenclature

Nomenclature:

1D	One-dimensional
2D	Two-dimensional
3D	Three-dimensional
AFNOR	Association Française de AFNOR Normalisation
Ag NPs	Silver nanoparticles
ATCC	American Type Culture Collection
CIP	Ciprofloxacin
FAMES	Fatty Acid Methyl Esters
FT-IR	Fourier transform Infrared Spectroscopy
IA	Acid number
IA	Acid number
IBr	Iodine monobromide
IC	IC
ICl	Iodine monochloride
IE	Aster number
II	Iodine number
IOD	Iodine
IS	Saponification number
NaOH	Sodium hydroxide
NI	No Inhibition
NM	Nanomaterial
NPs	Nanoparticles
SEM	Scanning electron microscope
TEM	Transmission Electron Microscope
UV-VIS	Ultraviolet–visible spectroscopy
XRD	X-Ray Diffraction
ZnO NPs	Zinc oxide nanoparticles

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General Introduction

General Introduction

There is a continuous quest for reliable and environmentally friendly processes for the production of metallic and metallic oxide nanoparticles, aiming to reduce or even eliminate the utilization of hazardous chemicals. The most effective approach to enhance these "green" methods is to develop benign synthesis strategies employing mild reaction conditions and non-toxic reaction precursors [1].

In recent times, there has been a substantial surge in both commercial and research endeavors in nanotechnology, reflecting investments surpassing \$9 billion in the past few years. Nanotechnology, often described as "engineering on a small scale," finds applications across a diverse spectrum of research and development fields, ranging from medicine to manufacturing, textiles, and consumer goods [2].

In our research, we utilized residues from pumpkin seeds (originating from the Hassi El Fehl area, Meniaa), post-extraction of oil, in combination with pumpkin leaves (sourced from the Trifawi region, El Oued), along with silver and zinc metals, to synthesize nanoparticles and evaluate their antibacterial and antioxidant properties. This prompts the inquiry: Can nanoparticles produced through this method be effectively employed in practical applications such as medicine and cosmetics?

To address this question, we opted to divide the research into theoretical and practical segments as outlined below:

Theoretical Part:

Chapter One: Latest Developments in Nanoparticles

Chapter Two: Presentation of the Studied Plant and Characterization of Nanoparticles

Chapter Three: Green chemistry and Green Synthesis of Nanoparticles (NPs)

Chapter Four: fixed Oils

Practical Part:

Chapter One: Materials and Methods

Chapter Two: Results and Discussions.

Conclusion

*Chapter I: State of the art on
Nanoparticles*

I.1. Introduction:

Up till now, nanoparticles (NPs) have been standing in front of others for their hypothetical of having a positive impact on most sectors of the economy, including consumer products, pharmaceuticals, cosmetics, transportation, energy and agriculture, etc. Moreover, they are being produced for variety of new applications within the industry and are emerging rapidly in each sector that cannot be ignored; An intriguing application of NPs in the realms of life sciences as „smart drug delivery systems“ is one such excellent application. The theory of NPs was Himmelweits first described in 1906 and was later called „magic bullets“ by the Nobel laureate P. Ehrlich. New features emerged under the NPs of metal which are of great scientific significance as it bridges large order and atomic structures. In the meantime, NPs physicochemical features have unique properties including high surface area, high reactivity, as well as tunable pore size and particle morphology. The state-of-the-art in nanotechnology involves addition of metallic NPs into industrial, biomedical and domestication products such as solvents, sunscreens, paints, cosmetics etc.[1]

I.2. Nanomaterials

Nanomaterials are substances that have particles or components at the nanoscale. Nanomaterials are of interest because at this scale, specific optical, magnetic, electric, and other properties emerge. These emergent properties have the potential for tremendous effects in electronics, medicine, and other fields. [2]

The term nano derives from the Greek phrase for dwarf. It is used as a prefix for any unit, together with a second or a meter, and it method a billionth of that unit. Hence, a nanometer (nm) is a billionth of a meter or 10 meters. To get a perspective on the size of a nanometer, look at the sequence of pics shown in Figure 1 [3]

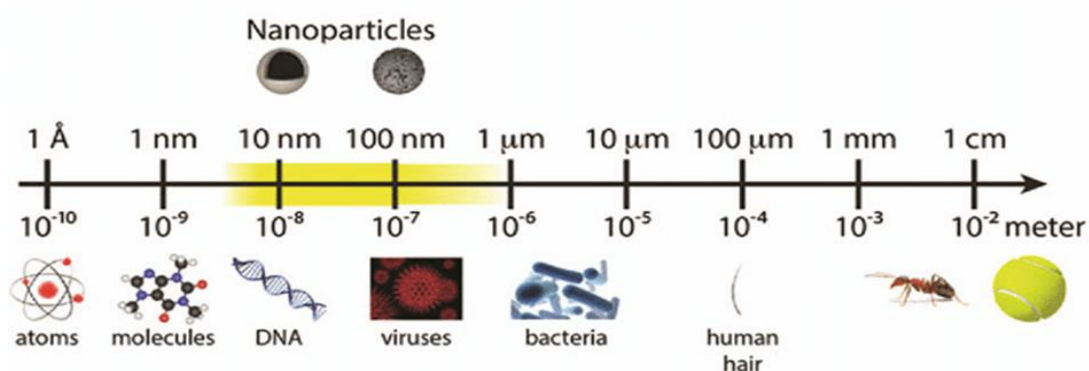


Figure I.1: Comparison of different sizes of materials with nanoscale dimensions. Modified from Bloemen 2015

I.2.1. Nanoparticles

Nanoparticles are small particles with sizes ranging from 1 to 100 nanometers. These materials have gained importance and interest in recent years owing to their large number of applications, because the matter at this scale presents a more compact arrangement of atoms and molecules, generating phenomena and acquiring or enhancing mechanical [4], electrical [[2], magnetic [3], optical [5], catalytic [6], and antibacterial [7], [8] properties that are completely different from those of their macroscopic counterparts [9]. They can be classified based on their composition, shape, and size. The most common types of nanoparticles are metals, metal oxides, carbon-based, and quantum dots. Owing to their unique sizes and properties, nanoparticles have attracted significant attention in various fields including medicine, electronics, energy, and environmental science [10], [11]. By reducing their size, nanoparticles can have a higher surface-to-volume ratio, enabling a greater number of atoms or molecules per volume, which means that less material is needed to obtain the same activity and exhibit other properties

I.2.2 Nanomaterial Classification

Nanomaterials may be classified consistent with their dimensionality, morphology, composition, uniformity, and agglomeration state, among others, as seen in Fig. 2 [5]

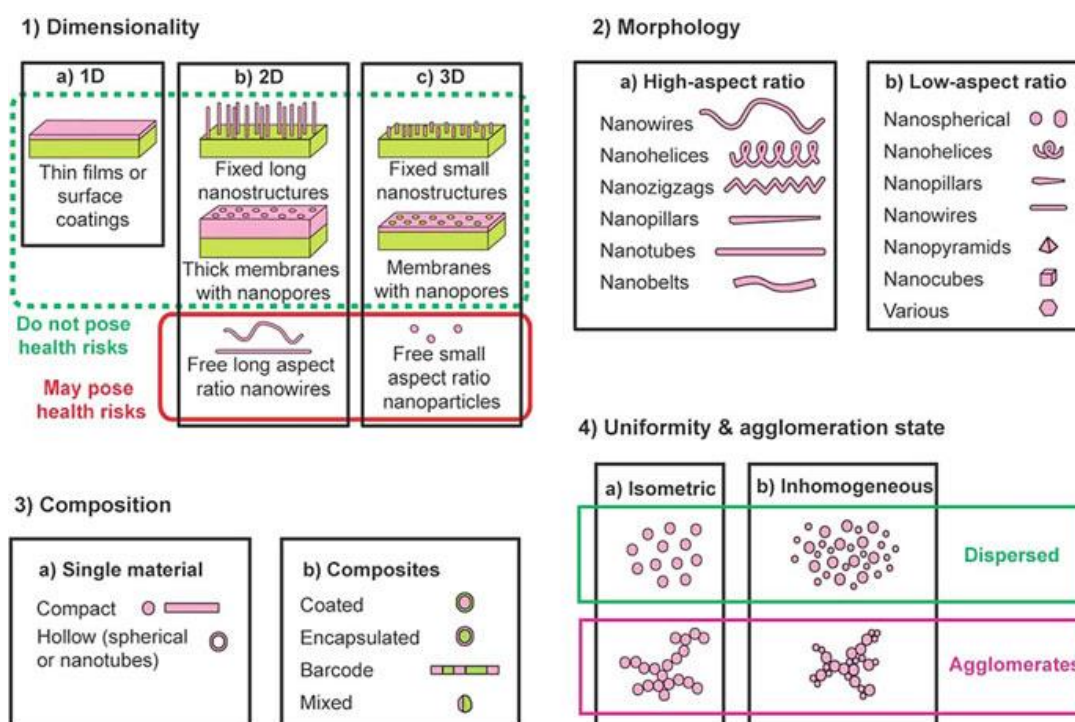


Figure I. 2: Nanomaterial classification. (Reprinted with permission from [5]. Copyright [2007], American Vacuum Society)

I.2.2.1 Nanomaterial dimensionality

The most common method of classifying nanomaterials involves categorizing them according to their dimensions. Nanomaterials can be classified as (a) zero-dimensional (0D), (b) one-dimensional (1D), (c) two-dimensional (2D), and (d) three-dimensional (3D). This classification is determined by the number of dimensions, which are not necessarily confined to the nanoscale range (<100 nm). As illustrated in the subsequent discussion, these categories of nanomaterials span from the 0D to the 3D dimensional spectrum [6].

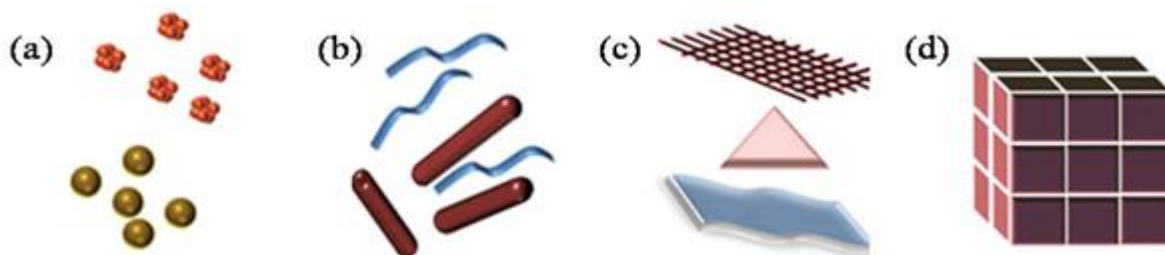


Figure I.3: Classification of Nanomaterials (a) 0D spheres and clusters; (b) 1D nanofibers, nanowires, and nanorods; (c) 2D nanofilms, nanoplates, and networks; (d) 3D nanomaterials

I.2.2.2 Nanomaterial Morphology

The ability to shape nanoparticles is not merely a scientific curiosity but a method to manipulate their inherent physical and chemical properties, thus determining their applications in optical, magnetic, or electronic devices [7].

From a morphological perspective, nanomaterials can be categorized based on their aspect ratio, either high or low. The primary morphological distinction between high- and low-aspect-ratio nanomaterials lies in the ratio between their longest and shortest dimensions, hence their designation, as depicted in Fig. 2.

Low-aspect-ratio Nanomaterials exhibit a ratio between their longest dimension and their smallest dimension of at least 1-2. They can be polycrystalline or composed of a single crystal. Furthermore, the morphological classification of low-aspect-ratio nanoparticles includes spherical or oval nanoparticles, nanopyramids, nanocubes, short nanopillars, short nanohelices, hexagonal plates, octahedral plates, and disks [7].

I.2.2.3 Composition, Crystallinity, Uniformity, and Aggregation

The composition and crystal structure play a pivotal role in establishing chemical bonds between atoms, thereby rendering each nanoparticle distinct from another. Nanomaterials can be composed of single materials or multiple substances; the latter being referred to as composites. These materials encompass a variety of substances, including organic and inorganic compounds, as well as hybrids. They can range from metals and their oxides, metal compounds, and carbon-based materials such as nanotubes, and ceramic materials, to polymers.

From a uniformity perspective, nanoparticles may possess identical sizes, in which case they are termed isometric. Conversely, if they exhibit different sizes, they are termed inhomogeneous. [8]

When nanoparticles aggregate, they are classified as agglomerates. Conversely, if nanoparticles disperse and do not clump together, they are described as dispersed [8].

I.3. Unique properties of nanoparticles

The structural features of nanomaterials are in the territory defined by the behavior of atoms and that of bulk materials. It is a fact that most of these microstructured materials behave in the same manner as the bulk ones, but when you talk about a nanoscale material, its unique characteristics are discernably different from possessing a bulk and an atomic scale of materials. This phenomenon can be primarily attributed to the nanoscale dimensions of the materials, which result in One of the key features of the material being that a considerable part of its atoms lies on the face, and some of them have very great surface ener showing show spatconfineme nine, e,t aa add even comprifeweress defects, what commonoccursur in the bulk materials.[3]

I.3.1 Physical properties:

The crystal shape of nanomaterials (NM) mirrors that of bulk substances, yet it diverges in lattice parameters, leading to unique and distinctive properties that can significantly differ from those of bulk materials. While some of these extraordinary characteristics are recognized, many are still being explored.[12]

The physical properties of nanomaterials originate from the extensive atomic distribution on the material's surface and the resulting interactions with the external environment. This is attributed to the large surface energy arising from the increased surface area and the spatial confinement of electrons due to the small dimensions. Consequently, this spatial confinement restricts the freedom of electron movement [12].

I.3.2 Chemical properties

The applications of exercise are controlled by the chemical properties of the nanoparticles, such as reactivity with target molecules, stability, and sensitivity to environmentally surrounding factors like moisture, and light. N heat, and light various forms like flammability, corrosiveness anti-corrosiveness ,oxidative potential, and reductive potential, all determine which engineered nanomaterials can be applied. Nanomaterials possess or even newly discovered catalytic functions which include reactivity, selectivity, and catalysts among others, largely as compared to those of their bulk status[13].

I.3.3 Magnetic properties

Magnetic nanoparticles (NPs) represent a significant curiosity for researchers from a selective range of specialties, including heterogeneous and homogeneous catalysis, biomedicine, magnetic fluids, magnetic resonance imaging for data storage, and environmental applications such as water pollution removal. Literature has revealed that NPs perform best when the size is below a critical value, typically 10-20 nanometers. In such a low range, the magnetic properties of the NPs are effectively controlled, making these particles invaluable and usable in various applications. The uneven electronic distribution in NPs leads to their magnetic property. These properties also depend on the synthetic protocol, and various synthetic methods such as thermal solvents, co-precipitation, micro-emulsion, thermal decomposition, and flame spray synthesis can be used for their preparation.[14] .

I.3.4 Optical properties:

Localized Surface Plasmon Resonance (LSPR) is an optical property of nano-sized particles. Some studies have shown that the linewidth is affected by the size of the nano-particles. For instance, by reducing the size of the nano-particles, the emitted light shifts from the Near Infrared (NIR) region to the Ultraviolet (UV) region. Due to their small size, nano-particles may lose their LSPR property and become optically bright. As a result of quantum confinement in nano-materials, the emission of visible light can be tuned by changing the dimensions of the nano-scale. It has been discovered that with decreasing size of nano-materials, the emission peak shifts towards shorter wavelengths. The material can change its color at the nano-level; for example, gold nano-spheres can shift from yellow at 100 nanometers, to greenish-yellow at 50 nanometers, to red at 25 nanometers, while silver can also shift to orange at 200 nanometers, light blue at 90 nanometers, and blue at 40 nanometers in thickness of a thin spherical shell.[13]

I.3.5 Electrical properties

Nanomaterials, in the case of ceramics, will increase conductivity considerably, whereas with metal they will reduce electric resistance. In bulk materials, the π -carrier is regarded as delocalized, enabling the carrier to move in the face of all directions. Tiny structures have quantum features on the nanoscale at the same time. The axis of nanotubes, nanorods, and nanowires (Andersson and articles) lets electrons become delocalized. The influence of electron confinement results in energy bands being replaced by discrete energy states which makes nanomaterials firstly act as semiconductors and secondly as insulators. This means that the probability of the electron being in the semiconductor side of the metal is significantly increased. Carbon nanotubes, for instance, can be either metallic or semiconducting. The diameter of a wire, the number of electron wave-functions contributing to the conductance is defined steps. [15]

I.3.6 Mechanical Properties :

It is known that the hardness of crystalline materials generally increases as the crystalline size decreases, and the mechanical strength of materials significantly increases through grinding the structure of metallic and ceramic materials or by composing them in the nanoscale range. Additionally, since ceramic materials have a crystalline size smaller than several hundred nanometers, the unique phenomenon of super plasticity is observed, which extends several to several thousand times the original size at elevated temperatures exceeding 50% of the melting point. This may provide the possibility of shaping and processing ceramics like metallic materials. [16] .

I.4. Synthesis Methods of Nanomaterials:

We are working with exceedingly fine structures, a nanometer is a billionth of a meter. This indeed permits us to consider both the ‘bottom-up’ or top-down methods to synthesize nanomaterials, i.e. either to together or to dis-assemble (break, or dissociate) bulk solids into tiny fragments until they are composed of only a few atoms.[17]

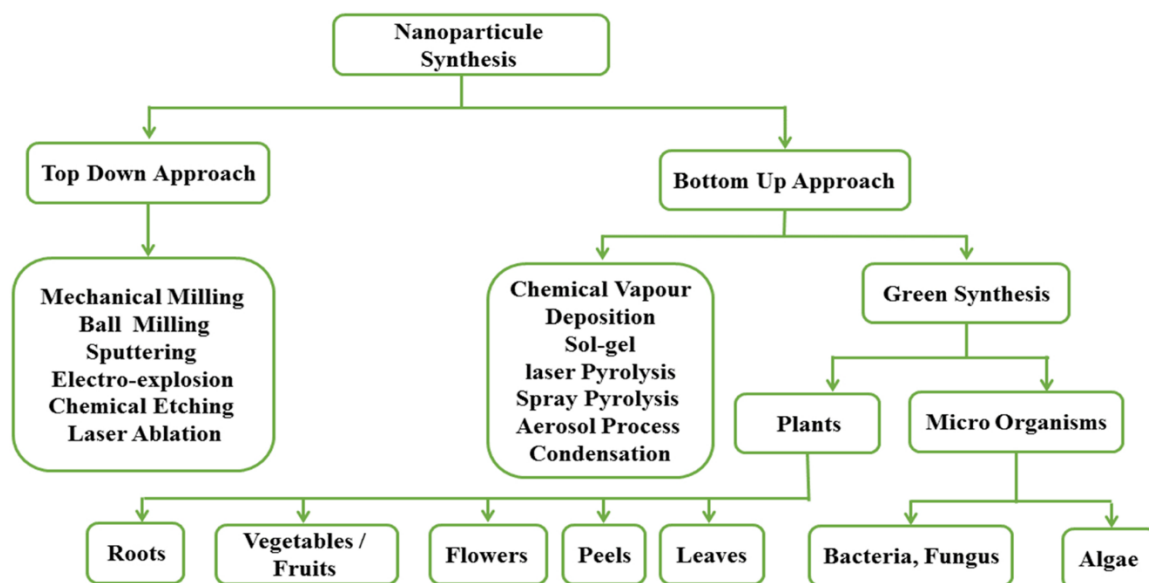


Figure I.4. Different methods of the nanoparticle synthesis [18].

I.4.1. Physical Methods for Synthesis Nanomaterials:

Physical methods for preparing NPs are considered among the oldest methods used, especially those relying on manual grinding. These physical methods apply mechanical pressure, high-energy radiation, thermal energy, or electrical energy to induce material corrosion, melting, evaporation, or condensation to generate NPs. These physical methods operate from top-down systems and are beneficial due to their solvent-free nature and production of uniform monodisperse NPs.[10]

From an economic and operational standpoint, physical methods are cost-effective. There are several techniques including high-energy ball milling, electron beam lithography, gas-phase synthesis, physical vapor deposition, and laser ablation synthesis.[10]

I.4.2. Chemical Methods for Synthesis Nanomaterials:

Chemical methods primarily rely on chemical reactions that generate mechanical or thermal forces capable of forming molecules or assembling atoms to produce materials at nanoscale sizes.

The raw chemical materials consist of a mixture of chlorides, oxides, and metals, all of which react through grinding or thermal processing to produce a powder where ultra-pure particles are dispersed within a stable salt matrix. By using appropriate solvents for each washed compound to

recover these particles with selective removal of the base material, the most important chemical methods include the sol-gel method, hydrothermal synthesis, polyol synthesis, fine emulsion technique, and microwave-assisted synthesis[10].

I.4.3. Biological methods for Synthesis Nanomaterials

Alternative synthesis approach is found be appropriate instead of the common methods which are involved in the synthesis of nanoparticles at manufacture level. Green synthesis method is environmentally friendly procedure and compatible with drug and nonmedical product introduction, since we do not involve the toxic chemicals here [19] . Additionally, this process will consume neither the high pressure nor the high temperature. Application of the microorganisms- synthetic method is quite prevalent in pollution control due to eco-friendly reasons. The employment of green materials such as plant leaf extract, microbes, fungi, and enzymes for zinc nanoparticles fabrication offers manifold advantages including, mainly easy accessibility and support of pharmaceutical and biomedical uses. [20]

I.5. Characterization of Nanomaterials

Many cutting-edge dimensional characterization strategies are constantly being developed to identify and detect the presence of metal within composite nanomaterials.

Characterization of nanomaterials can be carried out using the basic strategies of spectroscopic and microscopic analysis.[10]

I.5.1. Microscopy Techniques

Scanning electron microscope (SEM)

Transmission electron microscope where the object is bombarded by a beam of high-energy electrons that can reveal the chemical make-up of surface elements and can magnify the surface at higher magnification, higher resolution and high-depth of focus. SEM (Scanning electron microscope) uses cathode filaments to generate beam of electrons (1–30 keV) and they are restricted by low energy. The objects for SEM are put on discs made of aluminum or carbon - they can exist in liquid or solid form. Unlike TEM, in SEM, the samples can be prepared in bulk form which lessens the need to section it; which therefore, allows the samples to undergo imaging directly. When it comes to sample preparation, SEM focuses more on the point rather than the criterion with which TEM is more complex to use. Liquid samples in this regard can be directly transferred to the sample stub in SEM, which can be treated with special chemical options. Then, these options will undergo cutting at the notch.[21]

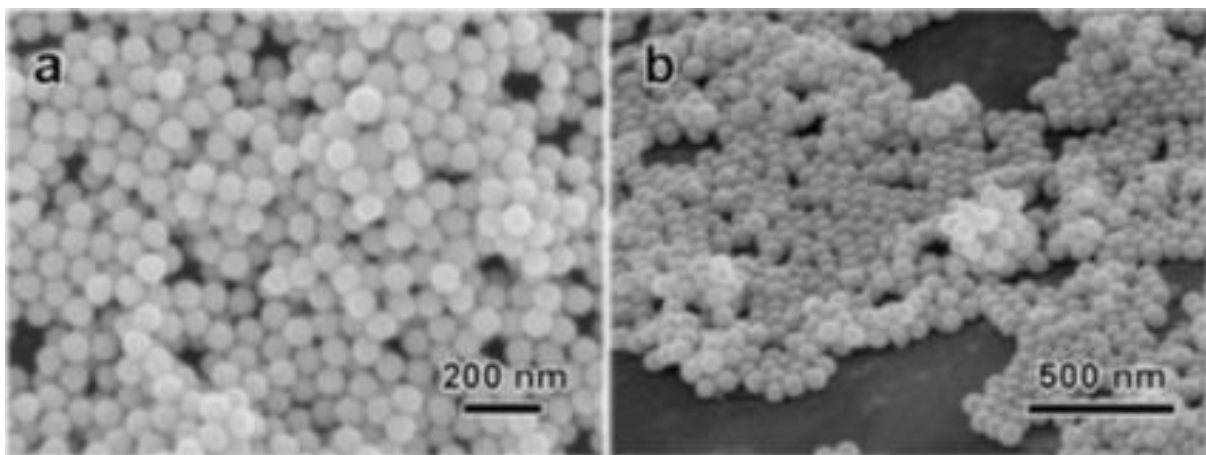


Figure I.5: SEM for Au nanospheres (a) top view, (b) tilted view (Liu et al., 2015a, 2015b).

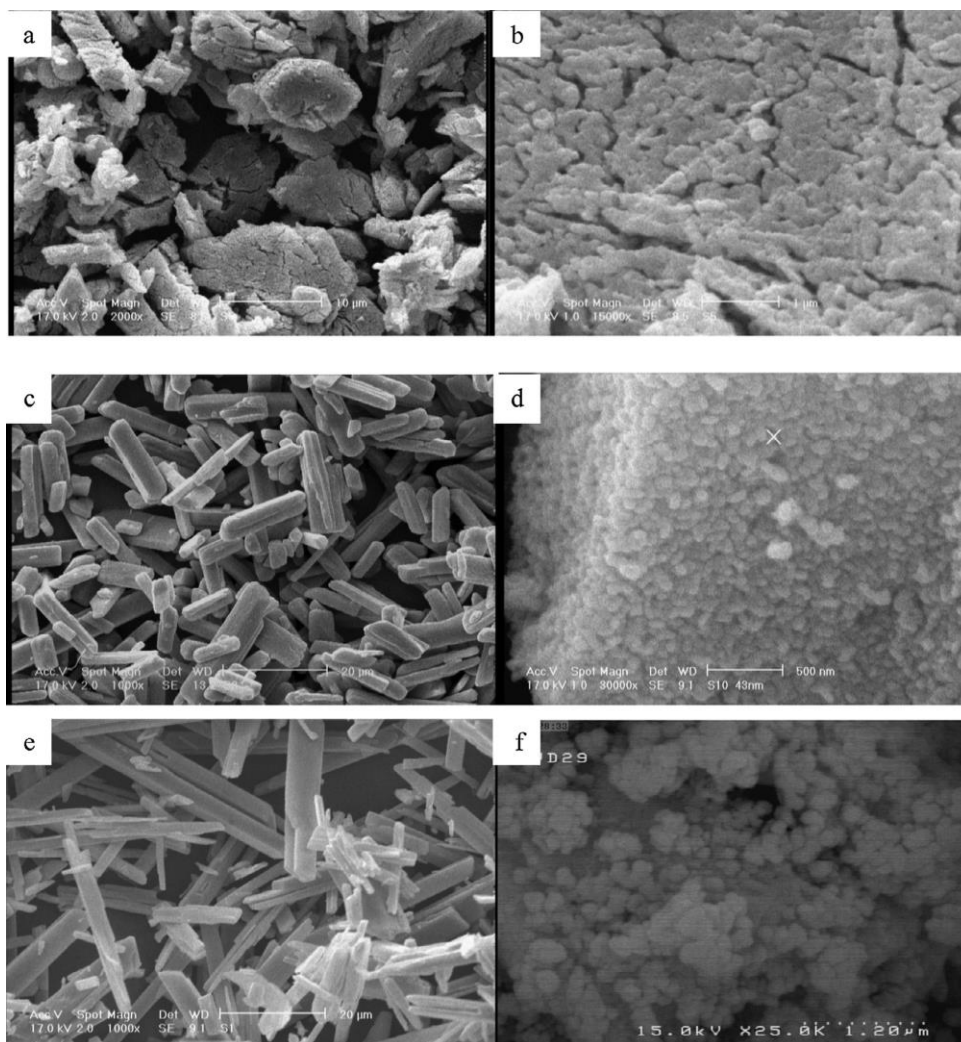


Figure I.6: SEM images of ZnO modified MOFs at different temperatures (Mirzadeh and Akhbari, 2016).

Transmission Electron Microscope (TEM)

Transmission electron microscope proves to be the most suitable tool to examine the nanomaterials, since its very high resolution, which is at least 0.5 nm or better, can be achieved using it. The very reason why it is referred to as the transmission electron microscope (TEM) is that the electrons are carried through the specimen. These are electrons with huge kinetic energies (≥ 50 keV) which undergo series of magnetic lenses, where the work is being done in a way similar to optical or SEM, which has been already addressed before.[22]

The most important parts of a TEM are electron source, condenser lens, and specimen that follow sequence or objective lens, diffraction lens, intermediate lens, projector lens, and a fluorescent screen. Additionally of the objectives may be used with other microscopes to enhance the image quality as well as resolution. The light rays, which are electromagnetic, pass through lenses with multiple focal lengths so that the images can be well focused without moving the lenses. Unlike in an optical microscope, they are left to stand at one location. [22]

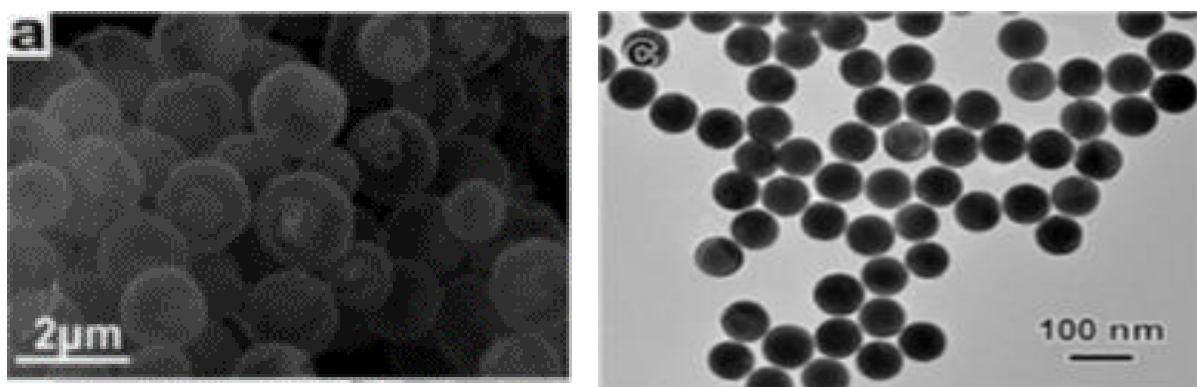


Figure I.7 :(c) TEM image of Au nanospheres (Liu et al., 2015a, 2015b).

I.5.2. Spectroscopic Techniques

Ultraviolet-Visible Spectroscopy

Nanoparticles from metals together with gold and silver engage strongly at particular wavelengths of mild (500–six hundred and 400–450 nm) based on their optical homes and surface plasma resonance. [10].

Such traits of the nanoparticles can be attributed to loose-drift electron oscillation within the elements composing the nanoparticles being precipitated by using the electrons to magnetic subject [10]. However, those techniques are used only to detect metallic nanoparticles and are frequently not well-suited to other nanomaterials.

A change in color of the preliminary approach to pink or violet for gold nanoparticles shows the presence of synthesized nanomaterials inside the extract, and the solution turns brown in the case of silver nanoparticles (Noruzi et al. 2012). Thus, coloration change suggests and

impacts the absorption sample, which confirms the presence of synthesized nanomaterials within the extract. The plasma resonance belongings have been determined to vary with the scale and form of nanoparticles an alternate in floor resonance because of variation in particle size may also result in a shift in wavelength from pink (increase in particle length) to blue (discount in particle length). However, in several cases, unfavorable results on the position of plasma resonance have been located because of factors such as environmental dielectric houses, interactions that can be bodily or chemical and appear at the particle floor, floor fee, interparticle distance, and aggregation. Asymmetrical and vast plasma resonance bands suggest that numerous orientations fabric frequently result in the formation of anisotropic nanoparticles [9]

X-Ray Diffraction (XRD)

XRD is one of the most effective techniques for analyzing the shape of nanomaterial which offers us facts regarding the width and form of the nanomaterials (size of crystalline, lattice, dislodgment of particle systems, and many others.). Samples are frequently prepared as smears or compact flat or in capillary and are uncovered to a monochromatic beam of X-ray which is diffracted using the nanomaterial and facts at the diffracted beam are accumulated at an angle (2θ) visit appreciation the incident beam. A powder diffraction pattern supplies data from the nanomaterial on levels gift, section concentrations, and structure, as well as the diploma of crystallinity or amorphous content observed using details on crystallite length/strain. The top widths in the given segment pattern present statistics at the average crystallite length where big crystallites supply rise to sharp peaks and any boom inside the top width shows a discounted crystallite length.[23]

Fourier transform Infrared Spectroscopy

FT-IR is by and large used to decide the useful organizations of the protein sure over the surface of nanomaterials or useful agencies of exopolysaccharides, which act as capping retailers and thereby improve stability in the course of organic synthesis of nano-materials. Identifying such biomolecules is important to trade or decorate the chemical structural composition which might also end in recognizing the development of their residences and in all likelihood future programs. These styles of biomolecules in organic suspension fall into the absorption spectrum inside the range of $1000\text{--}1800\text{ cm}^{-1}$ (C=C, C=O, C-N, C-O), which implies the possible position of these compounds in retaining the nanoparticle stability. The stretching height for amine and amide corporations throughout an FT-IR analysis falls within the variety between $3200\text{ and }3500\text{ cm}^{-1}$ (N-H and O-H) [11]

I.6. Application of Nanoparticles

NPs can be used in variety of applications. Some important of these are given below.

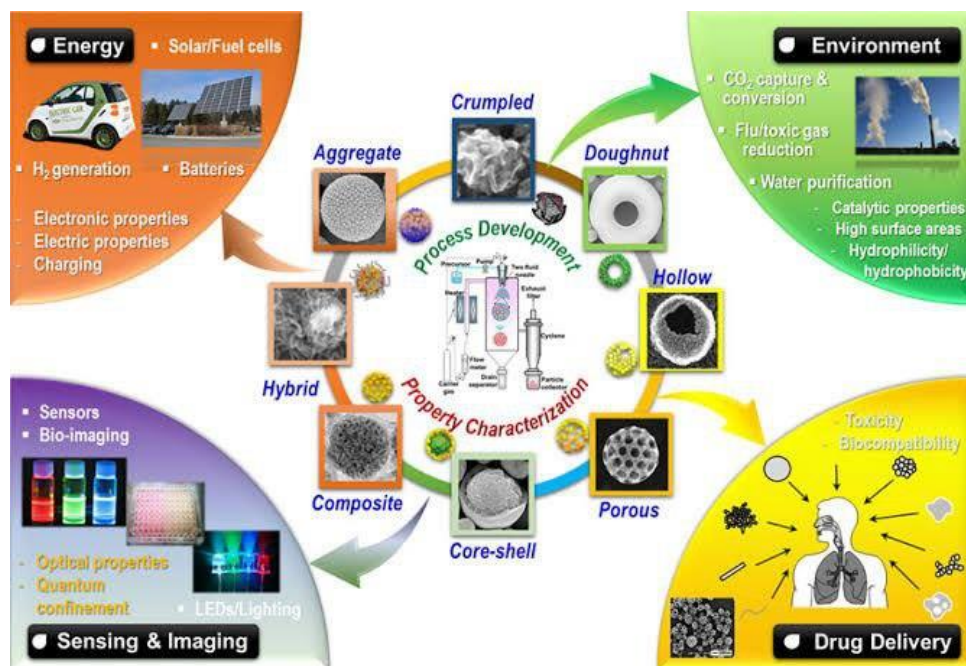


Figure I.8: General scheme of the synthesis and application of nanoparticles in cancer diagnosis and treatment.[24]

I.6.1. Applications in drugs and medications

Nano-sized inorganic particles of either simple or complex nature, display unique, physical and chemical properties and represent an increasingly important material in the development of novel nanodevices which can be used in numerous physical, biological, biomedical and pharmaceutical applications [25]. NPs have drawn increasing interest from every branch of medicine for their ability to deliver drugs in the optimum dosage range often resulting in increased therapeutic efficiency of the drugs, weakened side effects and improved patient compliance [26]). Iron oxide particles such as magnetite (Fe₃O₄) or its oxidized form maghemite (Fe₂O₃) are the most commonly employed for biomedical applications ([27]). The selection of NPs for achieving efficient contrast for biological and cell imaging applications as well as for photo thermal therapeutic applications is based on the optical properties of NPs. Mie theory and discrete dipole approximation method can be used to calculate absorption and scattering efficiencies and optical resonance wavelength for the commonly used classes of NPs i.e. Au NPs

I.6.2. Applications in manufacturing and materials

Nanocrystalline materials provide very interesting substances for material science since their properties deviate from respective bulk material in a size dependent manner. Manufacture NPs display physicochemical characteristics that induce unique electrical, mechanical, optical and imaging properties that are extremely looked-for in certain applications within the medical, commercial, and ecological sectors [28], [29], [30] NPs focus on the characterization, designing and engineering of biological as well as non-biological structures < than 100 nm, which show unique and novel functional properties. The potential benefits of nanotechnology have been documented by many manufacturer at high and low level and marketable products are already being mass-produced such as microelectronics, aerospace and pharmaceutical industries [31]. Among the nanotechnology consumer products to date, health fitness products from the largest category, followed by the electronic and computer category as well as home and garden category. Nanotechnology has been touted as the next revolution in many industries including food processing and packing. Resonant energy transfer (RET) system consisting of organic dye molecules and noble metals NPs have recently gamed considerable interest in bio photonics as well as in material science [32]. The presence of NPs in commercially available products is becoming more common.

Metals NPs such as noble metals, including Au and Ag have many colors in the visible region based on plasmon resonance, which is due to collective oscillations of the electrons at the surface of NPs [33], [34][35]The resonance wavelength strong depends on size and shape of NPs, the interparticle distance, and the dielectric property of the surrounding medium. The unique plasmon absorbance features of these noble metals NPs have been exploited for a wide variety of applications including chemical sensors and biosensors [35]

I.6.3. Applications in the environment

The increasing area of engineered NPs in industrial and household applications leads to the release of such materials into the environment. Assessing the risk of these NPs in the environment requires on understanding of their mobility, reactivity, Eco toxicity and persistency [36], [37]. The engineering material applications can increase the concentration of NPs in groundwater and soil which presents the most significant exposure avenues for assessing environmental risks [38], [39]. Due to high surface to mass ratio natural NPs play an important role in the solid/water partitioning of contaminants can be absorbed to the surface of NPs, co-precipitated during the formation of natural NPs or trapped by aggregation of NPs which had contaminants adsorbed to their surface. The interaction of contaminants with NPs is dependent on the NPs characteristics, such as size, composition, morphology, porosity, aggregation/disaggregation and aggregate structure. The luminophores are not safe in the environment and are protected from the environmental oxygen when they are doped inside the silica network [40].

Most of environmental applications of nanotechnology fall into three categories:

- 1.Environmentally benign sustainable products (e.g. green chemistry or pollution prevention).
- 2.Remediation of materials contaminated with hazardous substances
- 3.Sensors for environmental stages [41].

The removal of heavy metals such as mercury, lead, thallium, cadmium and arsenic from natural water has attracted considerable attention because of their adverse effects on environmental and human health. Superparamagnetic iron oxide NPs are an effective sorbent material for this toxic soft material. So, for no measurements of engineered NPs in the environment have been available due to the absence of analytical methods, able to quantify trace concentration of NPs [42]. Photodegradation by NPs is also very common practice and many nanomaterials are utilized for this purpose. used NiO/ZnO NPs modified silica in the tandem fashion for photodegradation purpose. The high surface area of NPs due to very small size (<10 nm), facilitated the efficient photodegradation reaction [43]. The same group has reported the synthesis of variety of NPs and reported their optical, florescence and degradation applications [44], [45], [46].

I.6.4. Applications in electronics

There has been growing interest in the development of printed electronics in last few years because printed electronics offer attractive to traditional silicon techniques and the potential for low cost, large area electronics for flexible displays, sensors. Printed electronics with various functional inks containing NPs such as metallic NPs, organic electronic molecules, CNTs and ceramics NPs have been expected to flow rapidly as a mass production process for new types of electronic equipment [47].

Unique structural, optical and electrical properties of one dimensional semiconductor and metals make them the key structural block for a new generation of electronic, sensors and photonic materials [48], [49], [50].

The good example of the synergism between scientific discovery and technological development is the electronic industry, where discoveries of new semiconducting materials resulted in the revolution from vacuumed tubes to diodes and transistors, and eventually to miniature chips [51].

The important characteristics of NPs are facile manipulation and reversible assembly which allow for the possibility of incorporation of NPs in electric, electronic or optical devices such as “bottom up” or “self-assembly” approaches are the bench mark of nanotechnology [52].

I.6.5. Applications in energy harvesting

Recent studies warned us about the limitations and scarcity of fossil fuels in coming years due to their nonrenewable nature. Therefore, scientists shifting their research strategies to generate renewable energies from easily available resources at cheap cost. They found that NPs are the best candidate for this purpose due to their, large surface area, optical behavior and catalytic nature. Especially in photocatalytic applications, NPs are widely used to generate energy from photoelectrochemical (PEC) and electrochemical water splitting. Beside water splitting, electrochemical CO₂ reduction to fuels precursors, solar cells and piezoelectric generators also offered advance options to generate energy. NPs also use in energy storage applications to reserve the energy into different forms at nanoscale level Recently, nanogenerators are created, which can convert the mechanical energy into electricity using piezoelectric, which is an unconventional approach to generate energy shows some energy generating devices, and uses NPs.[14]

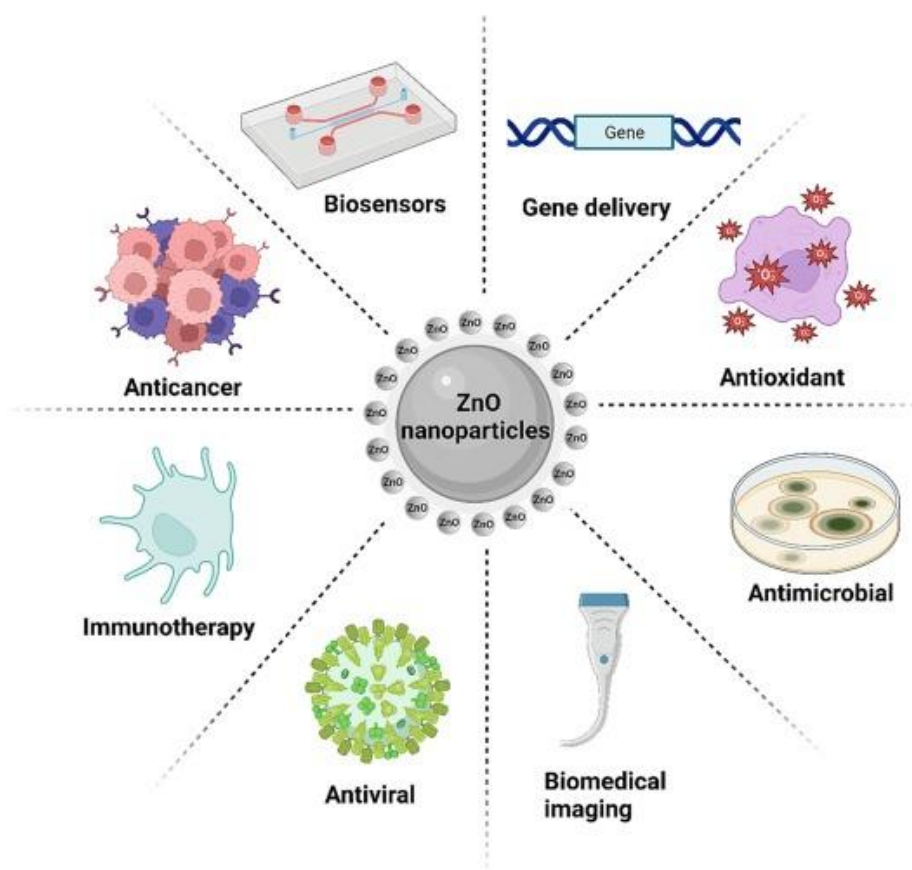


FIGURE I.9: application of ZnO NPs.[53]

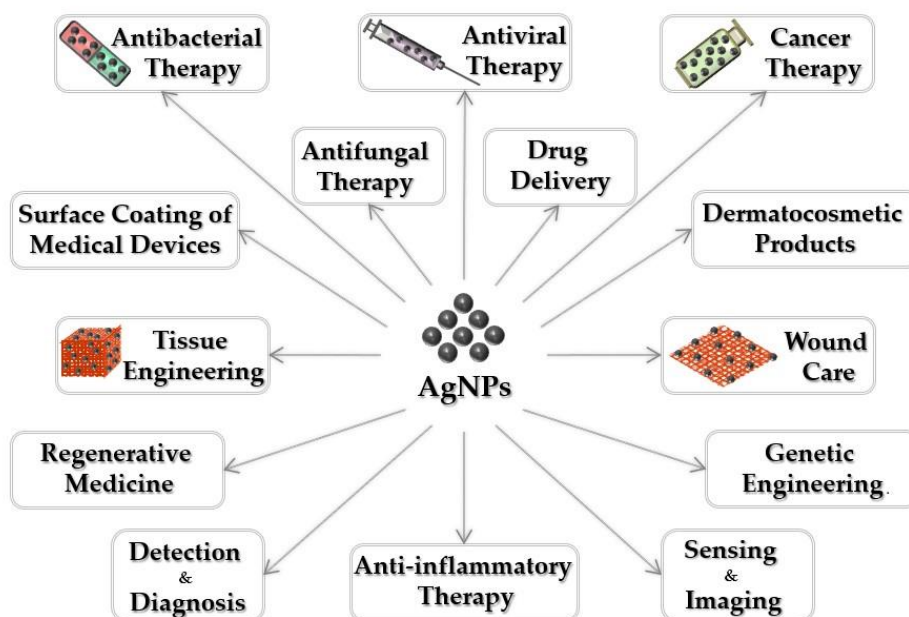


FIGURE I.10: application of Ag NPs.[54]

I.7. Toxicity of nanoparticles

In addition to the multiple industrial and medical applications of Nanoparticles and other nanomaterials, Nano-safety is of primary importance because these harmful effects need appropriate mechanisms for increasing their awareness. The side effect of nanotechnology is nanoparticles being stirred up through water, soil, and air by various human activities. Nevertheless spilling or deliberately injecting manufactured Nanoparticles in the soil or water bodies can be considered as a misuse because these NPs can pollute the environment also. Thus, the leaders from all sectors have taken serious steps and action as they are now worried partners. The merits of magnetic nanoparticles (NPs), being composed of extremely small particles, active and highly reactive, and having a great capacity, may degenerate into potentially hazardous factors, as they cause cellular toxicity and harm health. Unlike their counterparts (micron-sized), NPs have a unique capacity. Essentially, numerous studies reveal that Nanoparticles can locate their thought organisms during ingestion or inhalation and can translocate, along with Nanoparticles, to many organs and tissues, which puts the Nanoparticles in a position of reactivity and toxicological consequences. The feasibility of uncovering the toxicological effects of Nanoparticles on the cells of animals and plants has been investigated in many studies, but the studies of similarly toxicological studies on plants that have been carried out to date are still scarce. The application of Ag Nanoparticles in several people's items generates their emission into the aquatic environment. As the contamination of Ag makes way for a dissolved Ag, it has harmful consequences for the bacteria, algae, daphnia, and fish. The respiratory system assumes special importance and is

probably a target of the potential toxicity of Nanoparticles among multiple organs because, in addition to being accessible for inhaled particles, it receives the full cardiac output.[14]

The results of nanoparticle usage in various bio-applications globally are highly optimistic but still, it is unclear about the toxic effects of nano-sized particles left in human beings and the surrounding environment at different concentrations, following long-term exposure to them. Nevertheless, it can be safely assumed that the environmental effects of Nanoparticles are going to increase in the future. One of the nanoparticle toxicity phenomena is organizing around protein concentration that is largely governed by the size of particles, their crookedness, shape, electrical charge, and functional groups such as fascinated chemical groups and free energy of restructuring. This interaction could lead to negative biological outcomes because it changes the protein bonding, creation of fibrils, disulfide links, and deactivation of the enzyme with oligomers. Another case is the mobility of toxic ions that a reacting atmosphere causes when these elements commute with the environment into a liquid or biological disolvent. The nanoparticle cells go into a hard water and seawater aggregation forming a particular nanomaterial (made of organic matter and other natural particles that form colloids in freshwater) inescapably influencing their properties. The challenges of dispersion will make formulating ecotoxicology required to be structured, but the behavior of abiotic factors like pH, salinity, and the presence of empty substances may have to be thoroughly studied before.[14]

***Chapter II: Presentation of the plant
studied***

II.1. Introduction:

The vegetable crop pumpkin, the pumpkin belongs to the family of cucurbitaceae. The family contains chemicals, in the process amino acids, saponins, proteins, mucilage, polysaccharides and minerals (iron, zinc, manganese, copper, etc) [55]. Family of plants is probably the largest one in the universal plant kingdom, as it comprises the highest number of edible plant species among different families in the plant realm. Easily recognizable by their citrusy taste, these seeds irrevocably look similar to one another due to the fact that they are embedded in a bright yellow fibrous shell. Apart from that, these fairly big seeds are non-endospermic; furthermore, they have a dark red color. It cannot replace staples, which provide the particles and compounds essential for survival, but it should provide the minerals and vitamins missing from staple diets. This is specifically a characteristic of such cultures because they yield more nutrients than staple foods per they use land area. Pumpkin oil usually is a highly unsaturated oil, which is characterized by the fact that linoleic and oleic acids it is constituted of form the major part. Low endogenous amounts of linolenic acid or other similar fatty acids are present in it, which gives the pumpkin seed oil high oxidative stability for storage and processing purposes, as well as a low level of free radical formation in the human diet.[56]

The squash is counted among the most common vegetables resulting in its extension to different parts of the world. It falls in the Cucurbita genus and the Cucurbitaceae family, which is stated as the extensive plant family of the kingdom of plants. Although a great number of types exist across the world, only a the small palm inspired variety can be seen in Sri Lanka. At present, eating nuts and berries in pumpkins belong to a category of most significant sells because of numerous nutrition and health properties these gardening products have.[56]

II.2. Cucurbitaceae family:

The Cucurbitaceae family includes many well-known vegetable plants, such as cucumbers, melons, squashes, and pumpkins. These plants are generally annual or perennial herbaceous vines with climbing stems equipped with tendrils to attach themselves. Their leaves are large, alternate, palmate or deeply lobed.[57]

The Cucurbitaceae comprise about 120 genera and nearly 1,000 species distributed mainly in the tropics and warm temperate regions. Among the most important genera are Cucumis (cucumbers, melons), Citrullus (watermelons), Cucurbita (squashes, pumpkins), Luffa (luffas), Lagenaria (gourds) and Sechium (chayotes).[57]

A striking feature of this family is the separation of male and female flowers on different plants (monoecious plants). The fruits are fleshy berries, often large in size like squashes, melons or watermelons. They have a highly variable shape and contain numerous flattened seeds.[57]

Beyond their food uses, some Cucurbitaceae also have recognized medicinal properties dating back to antiquity (e.g. Citrullus seeds against intestinal worms). Other tropical species are used for their fibers, such as the luffa, known as the "vegetable sponge." [57]

This family plays an important role in agriculture and human nutrition around the world, especially in warm regions favorable to the cultivation of these plants. [57]

II.2.1 Cucurbita moschata Duch:

The origin of the word "squash" has been attributed to the native American word "Wata hti" (true squash) - "Wata miha sned" (long squash), etc. Fewkes [56]; according to whom one of the southern sides of the Hopi Indian people is identified with the Patun family, originated from the equines seeds that were found in the mortuary bowls. [55]



FIGURE II.1: Cucurbita moschata

Squashes and pumpkins, with a strong preference for gourds are the fruits most frequently depicted in ancient iconography (Whitaker and Davis 1962); only maize, of course, surpasses them in the riches of relics found. The bottle gourd dates back thousands of light years and was one of the first agricultural plant crop farming of the Incas as well as the Mexicans. Europeans thought that it was a native to the old world which is considered to be Africa. [58]

Cucurbita moschata is well known archaeologically in both Mexico (5000 B.C.) and Peru (3000 B.C.). [58]

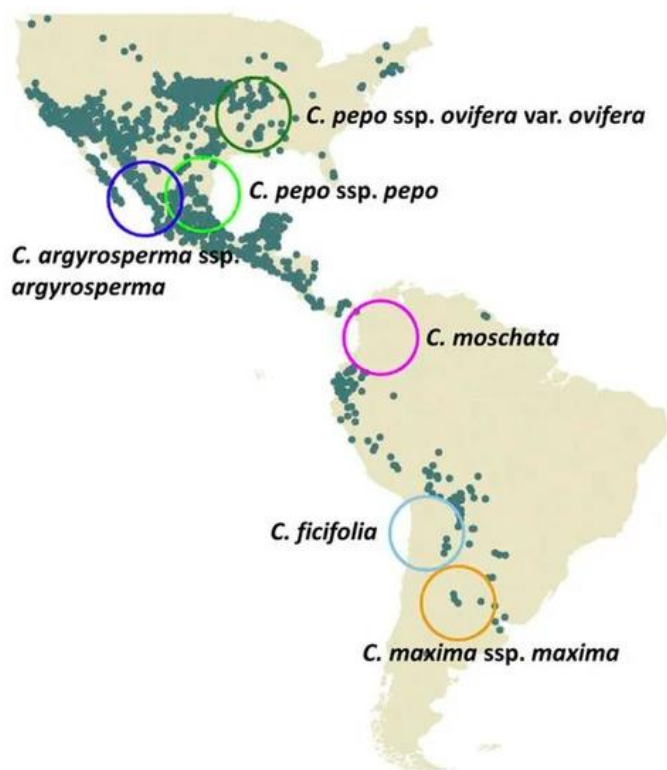


FIGURE II.2: . Distribution map of wild species of Cucurbita with occurrences based on 7000 records from GBIF

(www.gbif.org, accessed on 15 November 2020). Hypothesized origins of domestication for the five Cucurbita species are indicated by open circles. C pepo was split into the two cultivated spp. C. pepo ssp. ovifera: dark green; C. pepo ssp. pepo: light green; C. argyrosperma ssp. argyrosperma: blue; C. moschata: magenta; C. ficifolia: light blue; C. maxima ssp. maxima: orange Copyright 2021 Kates, Lopez-Anido, Sánchez-de la Vega, Eguiarte, Soltis and Soltis[59]

II.3. Plant description :

However, as in any member of the C. Pepo or C. maxima, this one is much more variable among different groups and it is hard to infer the particular traits of each variety. The plants carry tubular stems (rarely cylindrical) with a design of five chevrons occurred in the most instances, yet in a few of cases it was rather cylindrical having narrow ridges and grooves. Leaves and stems usually contains small hairy & rarely rough hairy, and apart from exceptions completely covered with little white spots created in places where veins merge. Although the spots borne here are not like those which are sometimes visible on C. Pepo leaves, they may sometimes themselves be confused with them. Whereas other species' flowers are delicate and feeble, this species' blossoms have a roughness yet resilience. Cucumis melo var. Ditches yellow with the tube a bit flaring with middle lobes that is neither as pointy as C. Pepo nor as rounded as C. maxima. The peduncle is pentahedral or otherwise often with or without fissures. The anthers projecting a bit out of something like the mouth of Thor. This comprises of staminate or pistillate flowers, and consists of highly variable calyx tubes; covered at the top by its associating petals

forming a disc-like structure, that may have the pentagonal shape and the edge flanges in both staminate and pistillate flowers; sepals may be long and short with or without a tomato leaf resemblance on the end. For example, in the evening before opening, the flowers of *C. Baccata*, where the tip of the flower will be pointed rather like *C. maxima*. Covercraft (one and only exception) is relatively thin of soft. And the cucumber stalk is usually five-sided, clearly ruffled, and markedly bulged at fruit attachment point. Conversely, in some cases of some of these categories, these characters are somewhat modified as follows: circular about of the outline, planned with rather even checks, and neither flaring nor noticeably enlarged at attachment to fruit. Fruit poles are hard or medium hard, anyhow, they do not get soften as in *C. maxima*. Stem elongated or not, vertical or obtect. Scar mark slanting, horizontal or circular. It seeds are from a semi-white to chocolate-brown with the edge of thin lines tracing the contour of the seed but with a different texture from the body of the seed.[55]

II.4.Morphological characteristics

TABLE II.1: Morphological characteristics of pumpkin plants[60]

Lifestyle:	annual creeping herb;
Stem:	Stems often rooted, densely covered with white bristles;
Leaves:	petioles 8-19 cm long, covered with bristles; Leaves broadly ovate or ovate, slightly soft, with 5 angular or 5 shallowly lobes, sparsely obtuse, 12-25 cm long, 20-30 cm wide, densely toothed; tendrils 3-5;
Flowers:	female flowers solitary; Ovary room 1;
Fruit:	The fruit stalk is thick, ridged and grooved, 5-7 cm long, and the guati expands into a trumpet; The shape of the gourd fruit is varied, depending on the variety, often with several longitudinal grooves or none;
Seeds:	Seeds oblong or oblong, grayish-white, with thin margins, 1-1.5 cm long;

II.5. Sciences classification:

It is essential to comprehend how plants are categorized scientifically in order to recognize their traits and their uses. This section covers the taxonomy of *Cucurbita moschata*, sometimes referred to as pumpkin, which is a member of the Cucurbitaceae family. In order to investigate the species' potential contribution to the environmentally friendly synthesis of nanomaterials, it is crucial to have a framework for comprehending the biological and ecological elements of the species.

TABLE II.2: Scientific classification of pumpkin plants[61]

Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Subclass	Magnoliidae
Order	Cucurbitales
Family	Cucurbitaceae
Genus	Cucurbita
Species	Cucurbita moschata

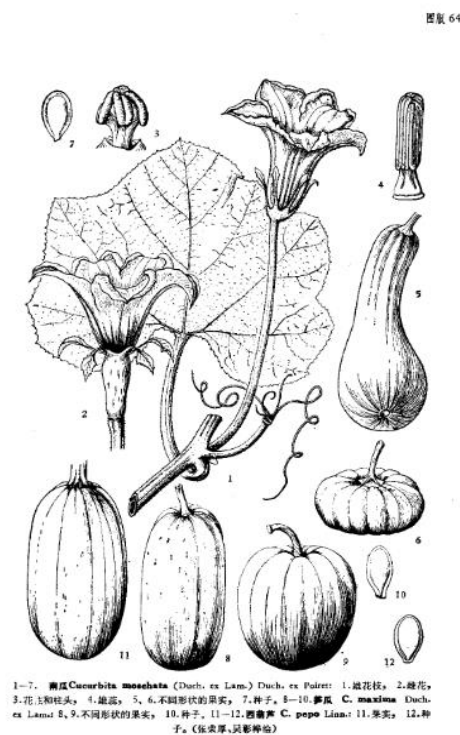
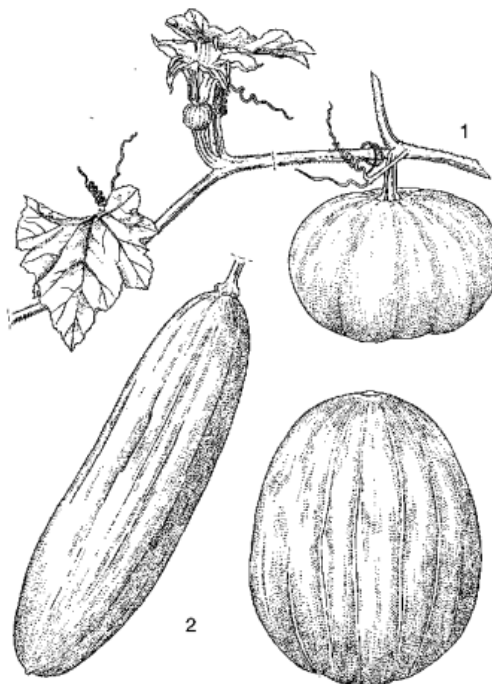


Figure II.3: *Cucurbita moschata* (Duch. ex Lam.) Duch. ex Poiret,[60]



Cucurbita moschata (Duchesne ex Lamk) Duchesne ex Poiret - 1, flowering and fruiting shoot; 2, fruit types.

FIGURE II.4: *Cucurbita moschata* (Duchesne ex Lamk) Duchesne ex Poiret - 1, flowering and fruiting shoot; 2, fruit types.[62]

II.5. Uses:

The native Americans were there first and these seed and therapeutic vegetables were considered. The Aztecs relied largely on the seeds to treat red worm infestation which is recorded all along their history.[63]

During the 1800s and 1900s, the USA contracted for the supply of pumpkin and their seeds as remedies for parasites. Next to that, the worldwide spread of the enteric fever is related to the usage of the feces and urine of the cowbeing. A particular colony became responsible for transporting this fruit in Europe and other destinations on the earth. At the moment, global leading pumpkin producing countries include Mexico, India, the United States, and China. The pumpkin is an essential component not only in the fruits, but also in many different recipes.[63]

Cultivated pumpkin such as *Cucurbita* was the main staple for the Indians as the source of food. There are only some examples of food Indians' meals with boiled or fried flowers in modern days. Many evidences remain, fortified by the regularity of green fruits stems (peduncles) presentation. Furthermore, the adoption of young fruits demonstrate that the use of green fruits is very old. Ripe fruit was eaten as fresh, or stored for a later use. A tribe in South, Central, and North America, including the Indians, do report about the storing the entire fruits, and also slices of dried or cooked strips or rings of the flesh.[64]

Perhaps of little importance, their Cucurbita were used at that time as pots and ornaments, rattles, and an attachment of musical instrument.[64]

The extracted oil from *C. maxima* seeds could be potentially utilized as a preservative and functional ingredient in foods and cosmetics.[65]

II.6. Benefits of Pumpkin:

Pumpkin becomes famous for its nutritional value because of the multiple biologically active elements that it contains, such as polysaccharides, para-amino benzoic acid, fixed oils, sterols, proteins and peptides. The fruit has carotenoids and gamma-aminobutyric acid which are its crucial ingredients. Protein is the one of the most important properties of pumpkin seeds and one can also get a useful amount of linoleic acid from them. Not only that, there are many health benefits from eating pumpkins such as improved eye sight, reduced cholesterol levels, protection against cancer, and a clearer skin all these health benefits can be achieved by eating pumpkins regularly.[63]

Carotenoids- rich group of bioactive agents of which the importance is unseen by the ordinary man, performs multiple duties of general importance. The key carotenoids present in pumpkin are: zeaxanthin, lutein, β -carotene and retinol equivalents are responsible for the vitamin A activity.[65]

II.7. Chemical composition and bioactive components

Compounds and the whole range of nutrient profiles of the pumpkin depend on the type of the species or the variety. The moisture content of the fried pumpkin pulp ranged between 75.81 and 91.33% while the crude protein was between 0.2 and 2.7%, 0.47 and 2.1% crude ash, and 3.91 and 13% carbohydrate [26]. Pumpkin-based products or fruits are filled with plenty of different nutrients such as polysaccharides, proteins, important amino acids, provitamin A preparations, carotenoids and minerals. Seeds of pumpkin are very fat rich, and it may be because of their broad genetic diversity which may vary the oil content. Pumpkins can save on oil costs due to its high nutritive values (in table 1) and may also serve as a good source of protein. This type of oil is almost uniquely suited for enriching the nutritional values of other foods because of having a high content of iron, zinc, and linoleic and alpha-linoleic acids.[66]

II.7.1. Pumpkin Seeds

Pumpkin seeds contain phytoestrogens (lignans and other plant compounds) that have estrogenic properties. They are also rich in fatty acids, proteins, and vitamins A&E. Thus, the benefits are manifold, to include anti-inflammatory properties. The seeds contain alkaline and are used in a variety of food products which is directly we can add sweetness in our food items. Moreover, they have mainly easily digested by the digestive system of the the body. Pumpkin seeds come in green color and they are wrapped with protective white coverings. The outcomes of which different research has become evident some health benefits of the seeds such as lowering risk or type 2 diabetes, weight loss and cholesterol level, and improving brain healthy. These polyunsaturated fatty acids are abundant in this food, and they can reduce blood pressure levels. Nowadays, the scientific studies of the health protective value of protein and oil from the seeds and polysaccharides from the pulp of the pumpkin fruit are contributory to the interest of such food processing industries as feed, pharmaceutical, and even the pumpkin fruit producers to the pumpkin derived products in the modern times.[63]

TABLE II.3: Bioactive components and their percentage in Pumpkin seed (nutritive value per 100 g).[66]

Components	nutritive value	Percentage of RDA
Energy	559 kcal	28
Carbohydrates	10.71 g	8
Protein	30.23 g	54
Total fat	49.05 g	164
Cholesterol	0 mg	0
Dietary fibre	6 g	16
Vitamins		
Folate	58 µg	15
Niacin	4.987 mg	31
Pantothenic acid	0.750 mg	15
Pyridoxine	0.143 mg	11
Pyridoxine	0.143 mg	11
Riboflavin	0.153 mg	12
Thiamine	0.27 mg	23
Vitamin A	16 IU	0.5
Vitamin C	1.9µg	3
Electrolytes		
Sodium	7 mg	0.5
Potassium	809 mg	17
Minerals		
Calcium	46 mg	4.5
Copper	1.343 mg	159
Iron	8.82 mg	110
Magnesium	592 mg	148
Manganese	4.543 mg	19
Phosphorus	1,233 mg	176
Selenium	9.4 µg	17
Zinc	7.81 mg	71
Phytonutrients		
Carotene-b	9 µg	-
Cryptoxanthin-b	1 µg	-
Lutein-zeaxanthin	74 µg	-

Chapter III: synthesis of NPs (...)

III.1. Introduction

Green chemicals are sustainable chemicals; Its mission is to develop chemical processes that are efficient, safe, economical and renewable. In 1998, Paul Anastas and John Warner outlined the principles that currently guide the use of green chemistry practices in industry, government, and academia. Although we cannot easily replace all fossil fuels, by applying the principles of green chemistry, as Coates et al did, it is possible to use fossil fuels more efficiently and reduce dependence.[67]

III.2. Green Chemistry

Green chemistry involves creating chemical products and processes that minimize or eliminate the utilization or generation of harmful substances. It encompasses all stages of a chemical product's life cycle, from conception and fabrication to application and eventual disposal.[68]

III.2.1. The four basic concepts of green chemistry are:

1- **Better use of raw materials:** Maximizing the use of raw materials, which when transformed, should be widely present in the final product, thus limiting the consumption of raw materials. This involves extracting or transforming the maximum amount of valuable material to obtain a residue with the lowest possible volume or mass. This also means that the final product should contain all the compounds of interest. In practice, how can we impoverish the raw material? For example, designing and using sophisticated and efficient filtration systems, even at low temperatures, to concentrate and fractionate milk, blood, fruit juices, or products from fermentation and biotechnologies, in order to recover the most interesting compounds (proteins, hormones, antioxidants, etc.) while maximizing the depletion of the raw material used and the resulting residual waste. Once impoverished, the residual waste is likely to be inert and harmless to the environment.[68]

2- **Using clean solvents, non-toxic and environmentally compatible:** As we have seen previously, the use of chemicals in daily life involves the use of organic solvents. We now know that some of these organic solvents are not very friendly to our health and the environment. Therefore, it becomes urgent to be able to abandon organic solvents such as benzene and hexane, or organochlorine solvents such as dichloromethane and chloroform, in favor of solvents that are inert towards operators, consumers, and the environment.[68]

3- **Optimizing energy use in terms of efficiency, savings, sources, and emissions.** Currently, transportation and housing are considered the two sectors that predominantly consume energy and are major contributors to greenhouse gas emissions. We need energy, and the lifestyles we adopt, as well as the increasing demands of countries like China and India, new players in the

energy market, will not help matters. One solution is to save energy; the cheapest energy is the energy we don't use.[68]

4- **Producing minimal quantities of waste** in suitable forms (solid, liquid, or gas) that limit their potential dispersion and facilitate recycling.[68]

III.2.2. 12 principals of green Chemistry

The twelve principles put forward by the American chemists Paul Anastas and John Warner in the year 1998 to lay the foundation for green chemistry are listed below.[68]

Waste Prevention: Preventing the generation of waste is always preferable to removing it after it has been generated.[68]

Atomic Economics: Synthetic processes and methods used in green chemistry should always aim to maximize the consumption and integration of all raw materials in the final product. This must be strictly adhered to in order to minimize the waste generated in each process.[68]

Avoiding the formation of hazardous chemicals: Reactions and processes by which certain toxic substances that pose a threat to human health are synthesized must be optimized to prevent the formation of such substances[68]

Developing safe chemicals: When developing chemical products that perform specific functions, care must be taken to ensure that the chemicals are as non-toxic to humans and the environment as possible.[68]

Safe auxiliary and solvent design: The use of auxiliaries in processes should be avoided whenever possible. Even if you must use it, you should optimize it to be as harmless as possible.[68]

Energy efficiency: The energy consumption of a process should be kept as low as possible.

Inclusion of renewable raw materials: The use of renewable raw materials must be prioritized over the use of non-renewable raw materials. [68]

Reducing the formation of derivatives: The use of unnecessary derivatives should be minimized as they require the use of additional reagents and chemicals and tend to lead to excessive waste generation.[68]

Introduction to catalysis: To reduce the energy requirements of chemical reactions in a process, the use of chemical catalysts and catalytic reagents should be encouraged.[68]

Designing chemicals with degradation in mind: When designing a chemical product to perform a specific function, care must be taken during the design process to ensure that the chemical does not become an environmental pollutant. This can be achieved by ensuring that chemicals are broken down into non-toxic substances.[68]

Incorporating real-time analytics: Processes and analytical methods must be developed to the point where they can provide real-time data for monitoring. This allows parties to stop or control processes before toxic/hazardous substances are produced.[68]

Including safe chemicals to prevent accidents: When designing a chemical process, it is important to ensure that the materials used in the process are safe to use. This helps prevent certain workplace accidents such as explosions and fires. Additionally, this helps create a safer environment for the process.[68]

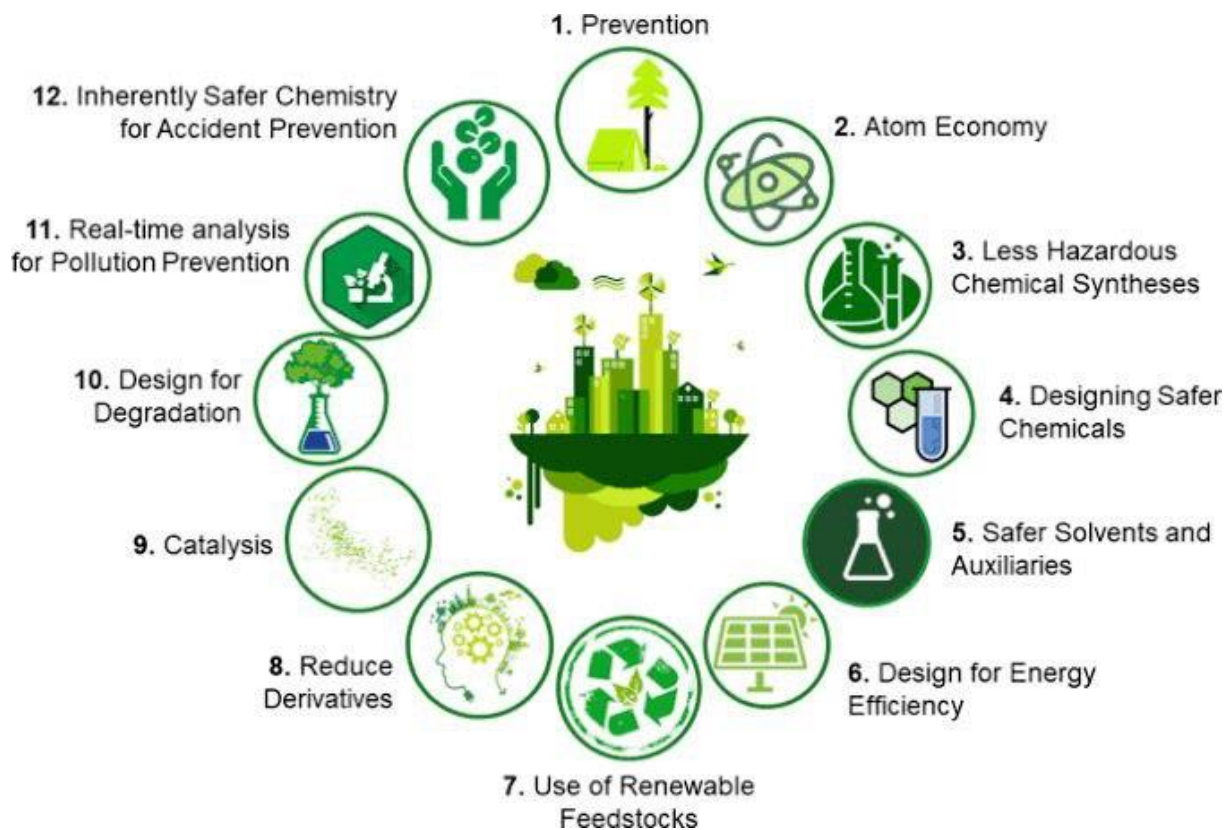


FIGURE III.1: The twelve principles of green chemistry [69]

III.3. Green nanotechnology and their synthesis

The fourth objective is "sustained" by green nanotechnology. Green nanotechnology helps nanotechnology itself to grow in a more sustainable and responsible way by either reducing or eliminating harmful polluting substances in the synthesis of nanomaterials or using the products of nanotechnology to reduce or eliminate these pollutants in current chemical processes.[70]

Green synthesis is a method of creating nanomaterials that are safe, economical, ecologically benign, and clean. Microorganisms that serve as substrates for the environmentally friendly production of nanomaterials include bacteria, yeast, fungus, algal species, and certain plants. The ultimate form and size of the nanoparticle are determined by several active molecules and precursors, including metal salts. Furthermore, the process of green synthesis yields nanomaterials with natural reducing, stabilizing, and antibacterial characteristics. [71]

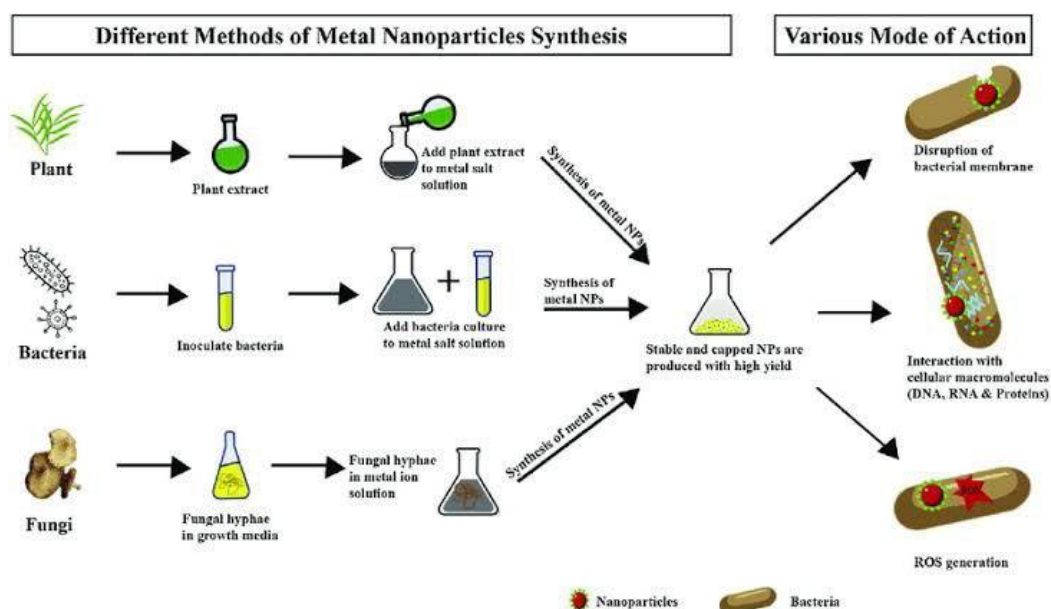


FIGURE III.2: green synthesis of nanoparticles.

Chapter IV: Fixed oils

IV.1. Introduction:

Vegetable oils that are solid are distinctive in that they do not evaporate or volatilize unlike volatile oils. These solid fatty oils are closely related to animal fat in their chemical composition, consisting of glycerin combined with a fatty acid. Typically containing oleic acid, these oils are insoluble in water but dissolve in various organic solvents. Fatty oils are produced in many plant families and are stored in large quantities in seeds, and to a lesser extent in fruits, tubers, and roots (Bernhard, 1997).[72]

IV.2. Definition:

Vegetable Oils Vegetable oils are hydrophobic substances derived from the seeds of plants like soybeans, rapeseed (or canola), or coconut, to name a few. They primarily consist of triacylglycerols, commonly known as triglycerides. Triglycerides result from the reaction between one molecule of glycerol and three molecules of fatty acids, making them glycerol (glycerin) triesters of fatty acids. Vegetable oils typically contain around 95% triglycerides, with the remaining portion comprising mono- and diglycerides, along with unsaponifiable compounds such as alcohols, carotenoids, chlorophyll, hydrocarbons, sterols, and tocopherols.[73]



FIGURE IV.1 : Collection of fixed oils

IV.3. Benefit of seeds oil:

Pumpkin seed oil is a nutritional powerhouse with numerous health benefits. It is rich in essential fatty acids, which support heart and brain health, and contains high levels of antioxidants like vitamin E that protect and nourish the skin. This oil is also a good source of magnesium, aiding muscle function and calming the nervous system, and zinc, which boosts immune health and metabolism. Furthermore, phytosterols in pumpkin seed oil can help lower cholesterol and support prostate health.

Incorporating pumpkin seed oil into a balanced diet can contribute to overall well-being, although it should be used in moderation due to its high-calorie content. For those with sensitivities to squash or gourds, it is advisable to consult a healthcare provider before using the oil.[74]

IV.4. Physical and chemical constants for oils

IV.4.1. Natural (physical) constants for oils:

The inherent factors dictate the oil's category and purity level. As oils contain diverse fatty acids and triglycerides, they lack natural uniformity, consistently falling within specified boundaries rather than fixed values. However, these traits are commonly referred to as oil constants.[75]

Relative Density

It's described as the ratio of the weight of a specific volume of oil at a given temperature to the weight of an equivalent volume of water at the same temperature (or at 15.5°C) (or at 20°C). Knowing the density allows for the estimation of:[76]

- The oil or fat's purity level

Determining the oil weight in vessels of known volume Practically, Relative Density is determined by measuring the mass of a certain volume of oil and comparing it with the mass of the same volume of water at the identical temperature.[76]

And the Relative Density is determined practically by calculating the mass of a certain volume of oil and we also calculate the mass of the same volume of water at the same temperature[76]

If using a temperature θ higher than the standard temperature we use the following relationship:

$$d_4^{20} = d_4^t + (\theta - 20) \times 0.00068$$

d_4^{20} : Density is at 20 C°

d_4^t : Density at laboratory temperature

θ : laboratory temperature

0.00068: coefficient of change of density when changing the temperature by 1C°.

Refractive index n_D^{20} :

Also referred to as the refractive index, it represents the ratio between the sine of the angle of incidence and the sine of the angle of refraction as light of a wavelength of 589.3 nm transitions from air into oil at a specific temperature. For oils, the refractive index is typically estimated at 20°C, while for solid fats, it's estimated at 40°C. [77]

A refractometer, a device used to measure the refractive index, directly reads the index of refraction when a liquid sample is sandwiched between two glass sheets. [78]

IV.4.2. Chemical constants for oils

Acid number:

It represents the quantity of potassium hydroxide in milligrams required to neutralize free fatty acids within one gram of oil or fat. This measurement provides insight into the percentage of free fatty acids present and the extent of glyceride decomposition within the oil. In general, it offers an indication of the suitability of oils for consumption. [79]

The acid number is determined practically following the AFNOR NFT 60-204 standard. This involves dissolving 0.2 grams of oil in 10 milliliters of hexane, adding phenolphthalein reagent drops, and titrating with a standard potassium hydroxide solution (N 0.01) until the color shifts from transparent to violet, recording the amount of base required.[80]

The acid number is calculated from the following relationship:

$$IA = \frac{V \times N \times 56.1}{m}$$

IA: the acid number

V: is the volume of potassium hydroxide solution needed for titration, in milliliters

N: standardized potassium hydroxide solution

m: the mass of the oil sample in grams

56.1: Molecular weight of potassium hydroxide.

Saponification number

The saponification number denotes the quantity of potassium hydroxide necessary to saponify one gram of fat or oil. This figure can be estimated based on the average molecular mass of triglycerides and fatty acids present in the oils, providing insights into the length of the fatty acid carbon chain.[79], [81]

The saponification number for oils is typically determined according to a specific protocol (AFNOR NF T 60-206).[80]

This involves placing 0.4 grams of oil in a 100 ml beaker, adding 20 ml of KOH alcohol (N0.2), and heating the mixture until boiling with condensation for 30 minutes, ensuring the oil drops vanish (transforming into soap). After a brief period, phenolphthalein reagent drops are added, and the soap solution is titrated with a 0.2 N aqueous solution of HCl (hydrochloric acid).[81]

The saponification number is calculated from the following relationship:

$$IS = \frac{(V_0 - V) \times N \times 56.1}{m}$$

IS: saponification number

V₀: Volume of HCl used in the comparison experiment, in milliliters (without using oil)

V: the volume of HCl in milliliters needed to adjust the soapy solution

N: standardized HCl solution

m: the mass of the oil sample in grams

56.1: Molecular weight of potassium hydroxide.

Iodine number:

The iodine number characterizes the amount of iodine (or an equivalent halogen) absorbed per 100 grams of oil or fat, serving as an indicator of the quantity of double bonds present and thus reflecting the degree of unsaturation.[79], [80], [81]

This assessment is conducted through two methods:

Wijs method, which utilizes an iodine monochloride solution (ICl).

Hanus method, employing an iodine monobromide solution (IBr).

A rise in the iodine number corresponds to an increase in double bonds, indicating heightened unsaturation. Consequently, this suggests a higher proportion of unsaturated fatty acids or the liquid state of the oil at room temperature. [79], [82]

An iodine number surpassing 130 signifies dryness, while a range between 90 and 130 indicates partial dryness. Conversely, a value below 90 indicates lack of dryness.[79], [82]

Practically, the iodine number is determined following the (AFNOR NF T 60-203) standard.[83]

In a 250 ml Arlen Meyer flask, 0.2 grams of oil are placed and dissolved in 15 ml of chloroform (CHCl₃). Subsequently, 15 ml of Wijs solution (0.1M ICl solution) are added, and the solution is left in darkness for an hour. Afterward, 10 ml of 10% potassium iodide solution (KI) and 150 ml of distilled water are introduced. The mixture is titrated with a solution of Na₂S₂O₃ (N 0.4) until the red color begins fading and transitions to a pale yellow, with starch employed as a reagent.[84]

The iodine number is calculated from the relationship:

$$II = \frac{(N_0V_0 - N_1V_1) \times 12.69}{m}$$

II: the iodine number

N₀: standardized wjis solution

V₀: Volume of wjis solution in milliliters

N₁: standardized sodium thiosulfate

V₁: The volume of sodium thiosulfate in milliliters

m: the mass of the oil sample in grams

Ester number:

The ester number represents the quantity of potassium hydroxide in milligrams required to saponify one gram of neutralizing oil, which consists solely of triglycerides without any fatty acids. [84]

This parameter is derived from the following formula:

$$IE = IS - IA$$

IE: the ester number

IS: saponification number

IA: the acid number.

IV.5. Extraction:

IV.5.1. Definition:

Extraction is defined as the process of separating active components from inactive components in biological tissues using suitable solvents based on the polarity and chemical nature of the component, to obtain the crude extract

IV.5.2. Oils Extraction:

A specific method for extracting oils from a particular plant organ is tested or preferred based on several considerations, including:

-The chemical composition of the essential oil. When extracting oil from a plant, the method must be chosen to ensure obtaining it in its natural state, without any decomposition or alteration in its chemical properties, thus preserving its scent and taste.[85]

-The part of the plant containing the essential oil, the location of oil-containing cells, and the thickness of these cell walls. The method of extracting oil from flowers, for example, differs from extracting it from fruits, leaves, or roots.[85]

-The quantity of oil present in the plant determines the extraction method to be used. If the oil content is low, it should be extracted using solvent extraction to ensure maximum yield, especially in commercial operations, where economic aspects are crucial.[85]

-Economic considerations in the extraction method, particularly at the commercial level, where obtaining the maximum amount of oil from the plant with minimal costs is essential.[85]

-The process of harvesting aromatic plants, their treatment, and preparation before extraction significantly impact the final oil quality. Aromatic plants from which oil is extracted from leaves and flowers can tolerate longer storage periods before extraction compared to plants where oil is extracted from seeds or fruits, which may withstand storage for up to six months.[85]

These are the types of extraction, and we will explain the methods used in our research

Classic Methods :

a. Water Distillation

- Hydrodistillation
- Water and Steam Distillation

b. Solvent Extraction

- Solid-Liquid Extraction
- Maceration
- Decoction
- Infusion
- Liquid-Liquid Extraction

The liquid-liquid extraction method depends on distributing the solute between two immiscible solvents. One is aqueous and the other is organic. In our research, we choose hexane³¹

- Conditions of the Solvent Used

The solvent used must meet the following conditions:

- The oil dissolves easily in the solvent of choice, leaving the other plant components behind without dissolving it or dissolving the smallest amount of it.
- The solvent must not enter into interactions with the essential oils to be extracted or other materials present. In the plant.
- The solvent must have a low boiling point as much as possible, so that it does not leave any traces after evaporation.[86]

- Pressure or Mechanical Expression

The principle of this method is very simple: the secretory pockets, which are located in the layers of the peel or in bags inside the juicy lobes, are torn (destroyed), either by manual squeezing or using mechanical squeezing machines, and then the essential vegetable oils are directly collected after removing the solid remains. This method is commonly used on citrus fruits and aromatic plant sources.

- **Enzymatic Hydrolysis**
- **Cold-Fat Extraction**
- **Hot-Fat Extraction**
- **Soxhlet Extraction**
- **Aqueous Diffusion**

B. Modern Methods for Extraction:

- . Solid-Phase Extraction
- . Supercritical Fluid Extraction
- . Subcritical Fluid Extraction
- . CO₂ Extraction
- . Microwave-Assisted Extraction
- . Solvent-Free Microwave Extraction (SFME)
- . Microwave-Assisted Extraction with Aqueous Diffusion and Gravity
- . Microwave Steam Distillation without Solvent (MASD)
- . Microwave-Assisted Water Distillation (MAHD)
- . Ultrasound-Assisted Extraction

TABLE IV.1 :Standards applied in extraction processes [87]

Extraction technique	basis of separation
evaporation	Volatility
distillation	
Co-distillation	
Solid-liquid extraction	solubility
Liquid-liquid extraction	
Recrystallization	
Grinding/Sieving	
Compression/filtration	The size and shape of the constituent molecules
Separation by membranes	
Chromatographic separation	
	adsorption

Practical Part





*Chapter I: Tools, Materials , equipment
used And Methods*

In this study, we are focusing on the green synthesis of compounds using plant-based sources. The objective is to utilize environmentally friendly methods and materials in our chemical processes. Below is a detailed list of the tools, equipment, and chemicals used in our experiments.

I.1. Used Tools:

Tool name	The Properties
Conical flask	250 ml, 500 ml
Becher	500 ml, 50 ml, 100 ml
graduated cylinder	100 ml, 50 ml, 25 ml, 10 ml
funnel	
filter papers	N: 01
pestle and mortar	
Magnetic stirring bars	
metal spoon,	
Special tubes	centrifuge 50 ml
Petri dish	
Separatory funnel	
Rotary evaporator	

I.2. Chemicals used:

Chemical name	The Properties
Distilled water	Nassah
Double distilled water	
Silver nitrate (AgNO_3)	SIALCHIM: MW: 169,87 g/mol D:4,35 g/cm ³ 
Zinc acetate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$)	RECTAPUR: MW: 219,50 g/mol 
SODUIM hydroxide (NaOH)	MW:39,997 g/mol 
N, Hexane	MW: 86,175 g/mol 

I.2. Used equipment:

Equipment name	The Properties
Magnetic Stirrer with Heater	
Centrifuge	
Oven	
PH Meter,	
Thermometer	
Analytical balance	
FTIR	Shimadzu-00463
UV/VIS	
DRX	
GC-MS/MS	Nexis GC-2030
Seed pressing machine	

I.4. Sample collection and preparation:

I.4.1. Seeds:

I.4.1.1. Washing and drying:

Pumpkin fruits were acquired from the daily market of the city of Metlili, Ghardaïa Province, and it is known that they are from an agricultural area in the city of Hassi El Fahal, Meniaa Province. All their seeds were collected, washed well with water, then with distilled water, and prepared for cold pressing to extract the oil from them.

After pressing, the remains were collected and dried in the open air for 15 days

I.4.1.2. Preparing the extract:

After drying the sample, the extract was prepared by soaking 10 g of seed residue in 100 ml of double-distilled water. The solution was shaken at a temperature of 80 degrees Celsius for a period of 25-28 minutes at 400 rpm.

After shaking, the solution was left to cool at room temperature and then filtered.



Figure I.1: Seed extract preparation protocol

I.4.2. Leaves:

I.4.2.1. Washing and drying :

The leaves were brought from the agricultural area, the city of Tarifawi, El Oued Province. The leaves were washed well with tap water to remove all traces of dust and dirt from them, then with distilled water. They were cut into small pieces and then left to dry in the open air for 15 days.

I.4.2.2. Preparing the extract:

After drying, 25 grams of leaves were weighed and soaked in 500 ml of double-distilled water to prepare the extract. They were shaken at a temperature between 60-70 degrees Celsius for half an hour at 400 rpm.

After shaking, the solution was left to cool at room temperature and then filtered



Figure I.2 :Leaves extract preparation protocol

I.5. Oil extraction:

After washing, the seeds were dried to remove any water droplets on them in an oven at 60 degrees Celsius for 30 minutes, ensuring the pressing seeds were fresh.

After light drying, take 148.286 grams of seeds for cold pressing in an electric press.



FIGURE I.3: Electric pressing machine for extracting oils

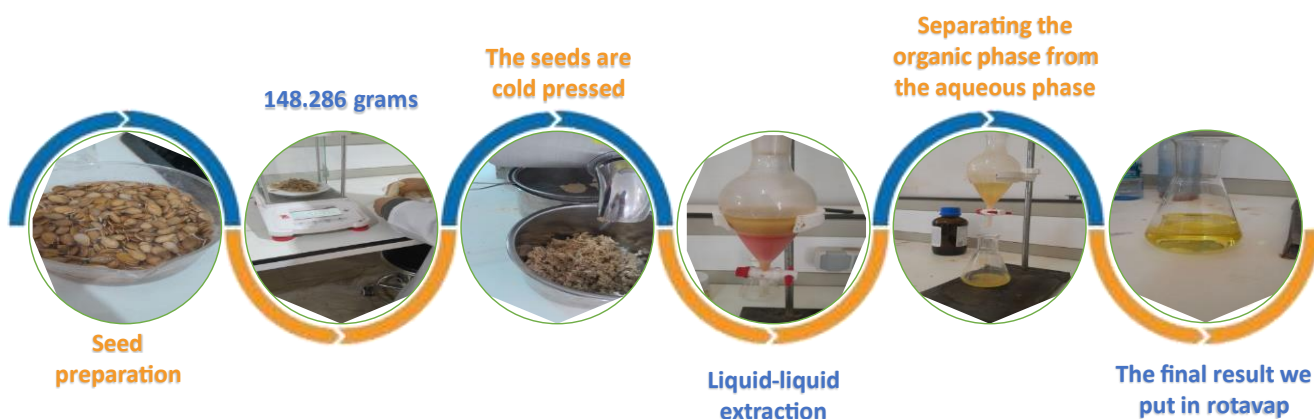


FIGURE I.4: Oil extraction Protocol

After pressing, the extract was taken to perform liquid-liquid extraction using hexane solvent.

I.6. Green synthesis of nanomaterials:

I.6.1. reparation of Solutions:

I.6.1.1. Silver Nitrate Solution :

To prepare a solution with a molarity of 0.5 M, 8.49 grams of silver nitrate were dissolved in 100 ml of double-distilled water. The solution was then transferred to a securely sealed 250 ml Erlenmeyer and wrapped in aluminum foil for storage.

I.6.1.1. Zinc Acetate Solution:

To prepare a solution with a molarity of 0.5 M, 9.17 grams of zinc acetate were dissolved in 100 ml of double-distilled water. The solution was then transferred to a securely sealed 250 ml volumetric flask for storage.

I.6.2. Silver nanomaterials:

- Take 75 ml of silver nitrate solution 0.5 M
- Add 15 ml of the extract to the solution, (working with both solutions simultaneously).
- Mix the solution at a temperature between 25-30 degrees Celsius for 24 hours at 400 rpm.
- Divide the solution equally into centrifuge tubes with a capacity of 50 ml, then centrifuge at 4000 rpm for 10 minutes. Two phases will form: precipitate and upper liquid.
- Separate the precipitate from the liquid, wash it, and dry it in an oven at 550 degrees Celsius for 1.5 hours.
- After the precipitate forms, grind it using a mortar and pestle.

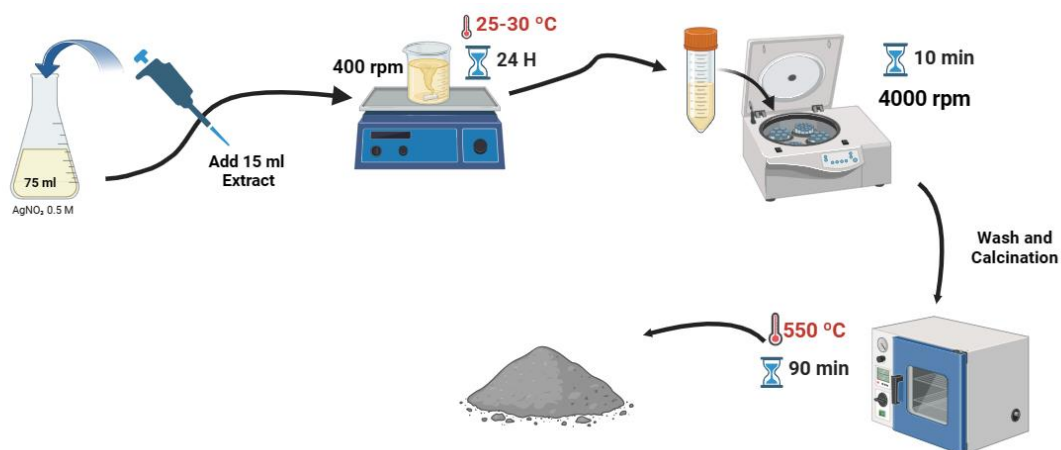


FIGURE I.5: Ag NPs synthesis protocol

Note:

- The color of the leaf extract changed from Light brown to reddish-brown.
- The color of the seed extract changed from white Grayish black.

I.6.3. Zinc nanomaterials:

- Take 150 ml of zinc acetate 0.5 M.
- Add 60 ml of the extract, (working with both extracts simultaneously).
- Add a quantity of NaOH to raise the pH to approximately 6.5-7.
- Mix the solution at a temperature of 40 degrees Celsius for one hour at 400 rpm.
- Divide the solution equally into centrifuge tubes with a capacity of 50 ml, then centrifuge at 4000 rpm for 10 minutes. Two phases will form: precipitate and upper liquid.
- Separate the precipitate from the liquid, wash it, and dry it for one and a half hours in an oven at 550 degrees Celsius.
- After the precipitate forms, grind it using a mortar and pestle.

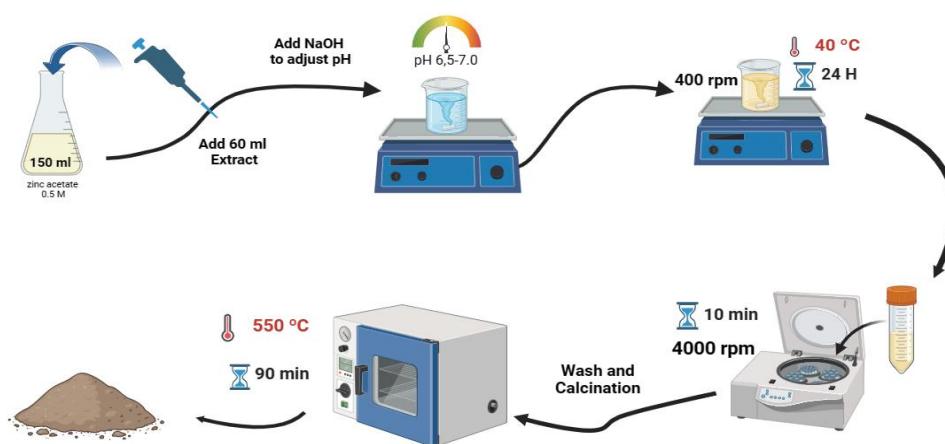


FIGURE I.5: ZnO Nps synthesis protocol

Note:

- The color of the leaf extract changed from reddish-brown to green.
- The color of the seed extract changed from light white to white.

I.6.4. The mixture between Silver and Zinc:

- In an Erlenmeyer flask, place 90 ml of the extract.
- Add 9.17 grams of zinc acetate, (working with both extracts simultaneously).
- Stir the solution for 30 minutes at a temperature ranging from 30 to 40 degrees Celsius.
- Add 10 ml of silver nitrate solution.
- Stir the mixture again for another half an hour.
- Halfway through, add a quantity of NaOH to raise the pH to approximately 6-6.5.
- After the specified time, store the solutions for 24 hours in darkness at a temperature of 25-30 degrees Celsius.
- Divide the solution equally into centrifuge tubes with a capacity of 50 ml, then centrifuge at 4000 rpm for 10 minutes. Two phases will form: precipitate and upper liquid.
- Separate the precipitate, wash it, and dry it in the oven at 550 degrees Celsius (calcination).

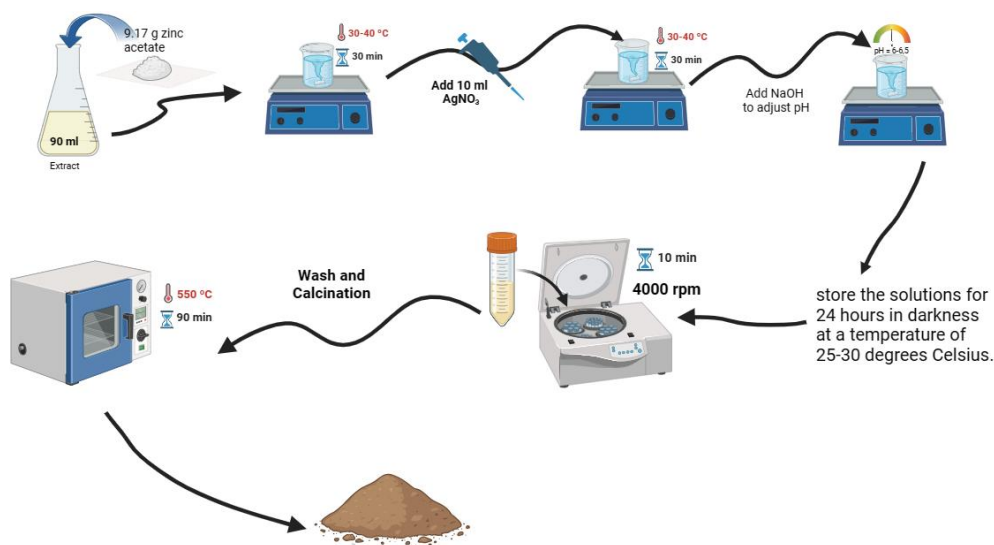


FIGURE I.6: Ag/ZnO Nps synthesis protocol

Note:

- The color of the seed extract changed from white-yellowish in the initial stage to a darker yellow, then to brown after the addition of silver nitrate, and finally to reddish-brown after the addition of NaOH.
- The color of the leaf extract changed from brown to green in the initial stage, then to a darker green after the addition of silver nitrate, and finally to reddish-brown after the addition of NaOH.

Chapter II: Results and Discussions.

II.1.Fixed Oil:

II.1.1. Analysis of Pumpkin Seed Oil: GC-MS/MS Results

Sample and Method Information

- **Analysis Date:** March 20, 2024
- **Analyst:** Admin
- **Sample Type:** Unknown
- **Instrument:** GCMS-TQ Series (GC-2030 with AOC-20i autosampler)
- **Injection Volume:** 1.00 μ L
- **Column Oven Temp:** 40°C to 250°C with specified program

Chromatographic Conditions

- **Initial Temp:** 40°C, hold for 3 minutes
- **Ramp 1:** 40°C/min to 170°C, hold for 10 minutes
- **Ramp 2:** 40°C/min to 180°C, hold for 8 minutes
- **Ramp 3:** 5°C/min to 250°C, hold for 5 minutes
- **Carrier Gas:** Helium
- **Flow Rate:** 2.20 mL/min

Device Information

- **GC-MS Model:** GCMS-TQ Series
- **Autosampler:** AOC-20i
- **Column:** Specifics not provided, assumed standard for FAME analysis
- **Carrier Gas:** Helium

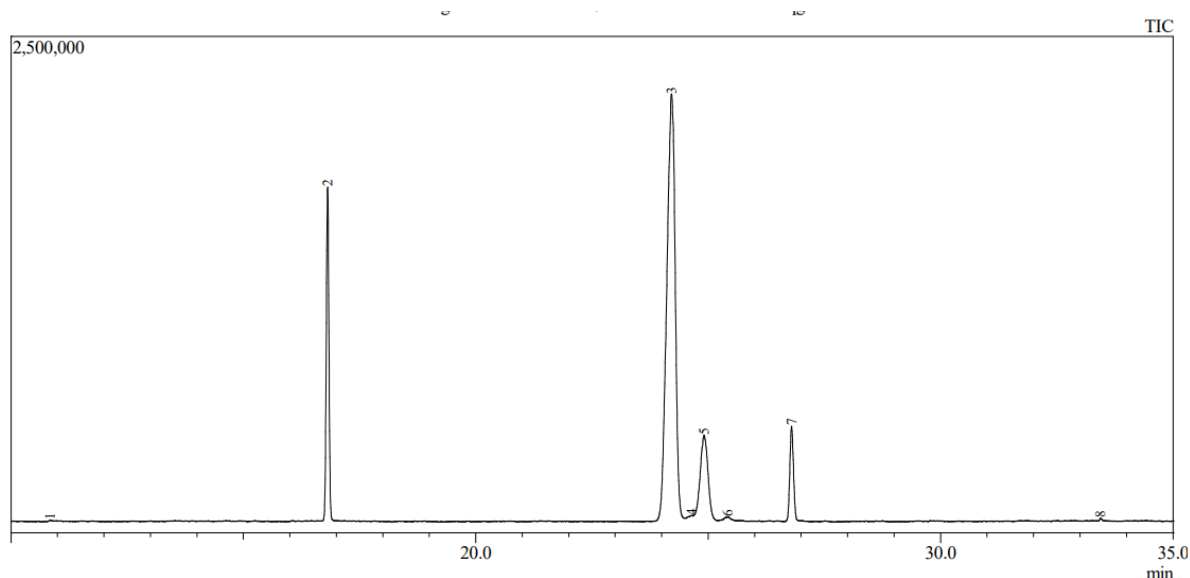


FIGURE II.2 : GC-MS chromatogram of pumpkin seed oil

II.1.2. Compounds Identified:

The following table summarizes the fatty acid methyl esters (FAMES) identified in a pumpkin seed oil sample using gas chromatography-tandem mass spectrometry (GC-MS/MS). The analysis was performed on March 20, 2024, utilizing a GCMS-TQ Series instrument with an AOC-20i autosampler. Key chromatographic parameters included a column oven temperature program ranging from 40°C to 250°C and helium as the carrier gas. The table details the retention times, peak areas, peak heights, and compound identities, providing a comprehensive profile of the fatty acid composition in the sample.

TABLE II.1: Fatty Acid Methyl Esters (FAMES) Identified in Pumpkin Seed Oil by GC-MS/MS

Peak#	Retention Time (min)	Area	Area%	Height	Height%	Similarity	Compound Name	CAS#
1	10.842	24,995	0.06	8,226	0.17	87	Methyl tetradecanoate	124-10-7
2	16.813	6,753,130	15.87	1,714,032	35.03	96	Hexadecanoic acid, methyl ester	112-39-0
3	24.213	27,595,052	64.87	2,194,968	44.86	96	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	112-63-0
4	24.648	79,172	0.19	13,608	0.28	69	9,12,15-Octadecatrienoic acid, methyl ester (Z,Z,Z)	301-00-8
5	24.912	5,186,008	12.19	438,536	8.96	96	9-Octadecenoic acid, methyl ester (E)	1937-62-8
6	25.423	180,648	0.42	20,001	0.41	93	9-Octadecenoic acid (Z)-, methyl ester	112-62-9
7	26.795	2,674,961	6.29	488,846	9.99	96	Methyl stearate	112-61-8
8	33.44	46,374	0.11	14,198	0.29	90	Eicosanoic acid, methyl ester	1120-28-1

• **Dominant Compounds:**

- **9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS: 112-63-0):**
 - **Area%:** 64.87%
 - **Height%:** 44.86%
 - **Similarity:** 96
- **Hexadecanoic acid, methyl ester (CAS: 112-39-0):**
 - **Area%:** 15.87%
 - **Height%:** 35.03%
 - **Similarity:** 96
- **9-Octadecenoic acid, methyl ester (E) (CAS: 1937-62-8):**
 - **Area%:** 12.19%
 - **Height%:** 8.96%
 - **Similarity:** 96

II.1.3. Trace Compounds:

- Methyl tetradecanoate, Eicosanoic acid, methyl ester, and others present in minor amounts.

II.1.3. Interpretation

The analysis identified several key fatty acid methyl esters (FAMES) in the pumpkin seed oil. The major components are unsaturated fatty acids, specifically 9,12-Octadecadienoic acid (linoleic acid), which is the most abundant, followed by Hexadecanoic acid (palmitic acid) and 9-Octadecenoic acid (oleic acid). These findings are consistent with the typical fatty acid profile of pumpkin seed oil, which is known for its high content of linoleic and oleic acids, contributing to its nutritional value and health benefits.

II.1.4. Conclusion

The GC-MS/MS analysis of the pumpkin seed oil sample confirms the presence of major fatty acids, with linoleic acid being the predominant component. The analytical method used, including the specific temperature program and flow rates, successfully identified and quantified the compounds in the sample.

II.2. characterisation of NPs :

II.2.1. Color Change :

Ag NPs leaves Ex :

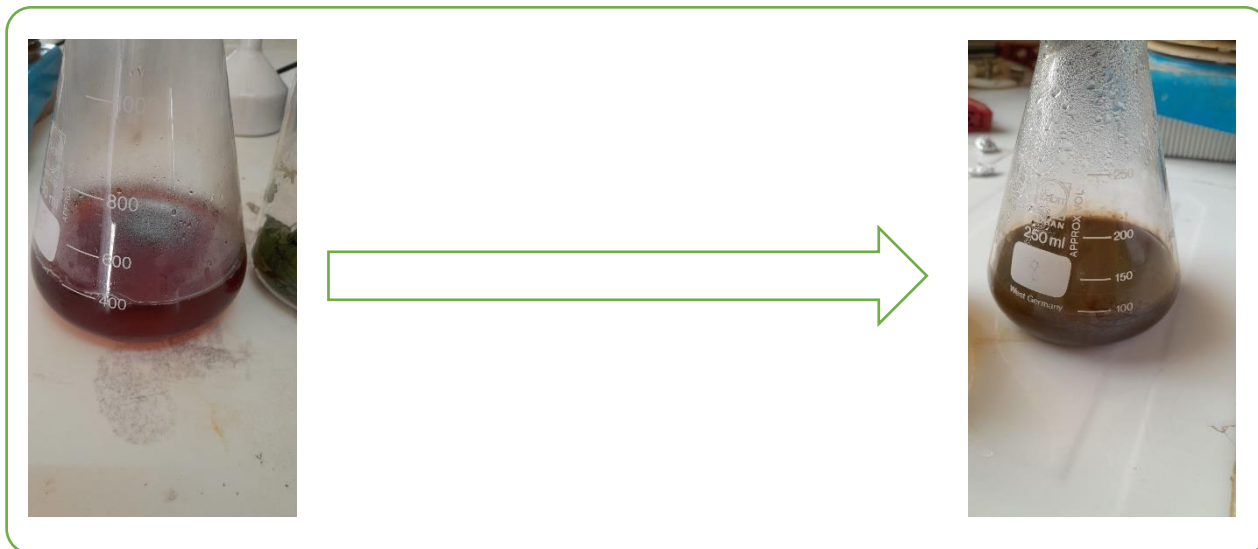


FIGURE II.3. Photo showing the color change during preparation for: Ag NPs leaves Ex

Ag NPs seeds Ex:

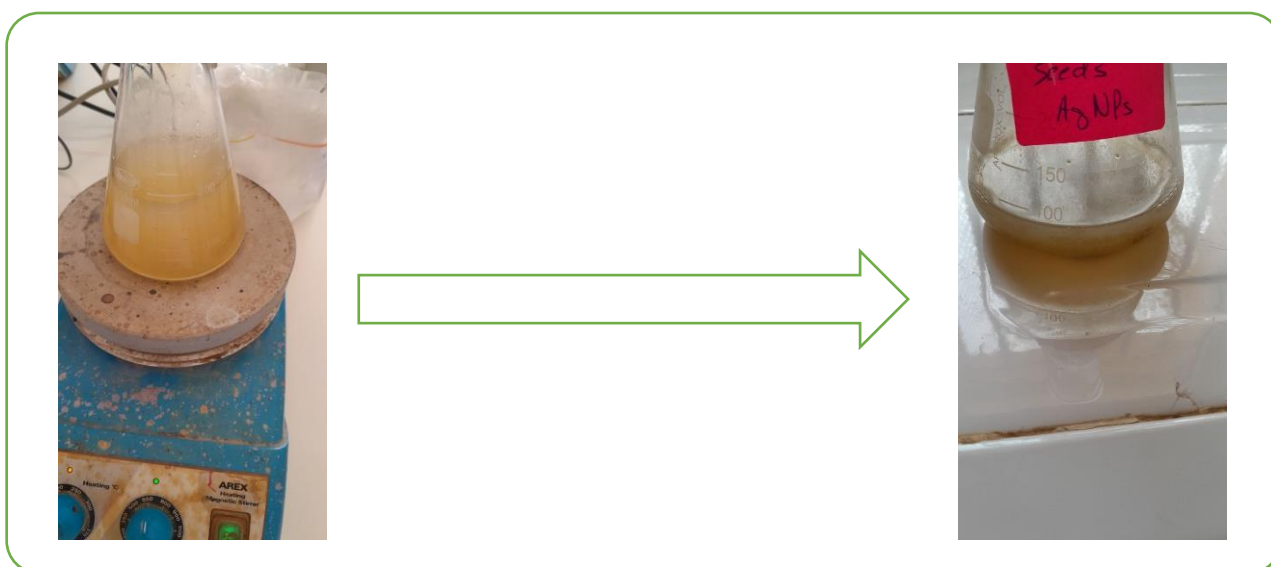


FIGURE II.4. Photo showing the color change during preparation for: Ag NPs seeds Ex

ZnO NPs leaves Ex:

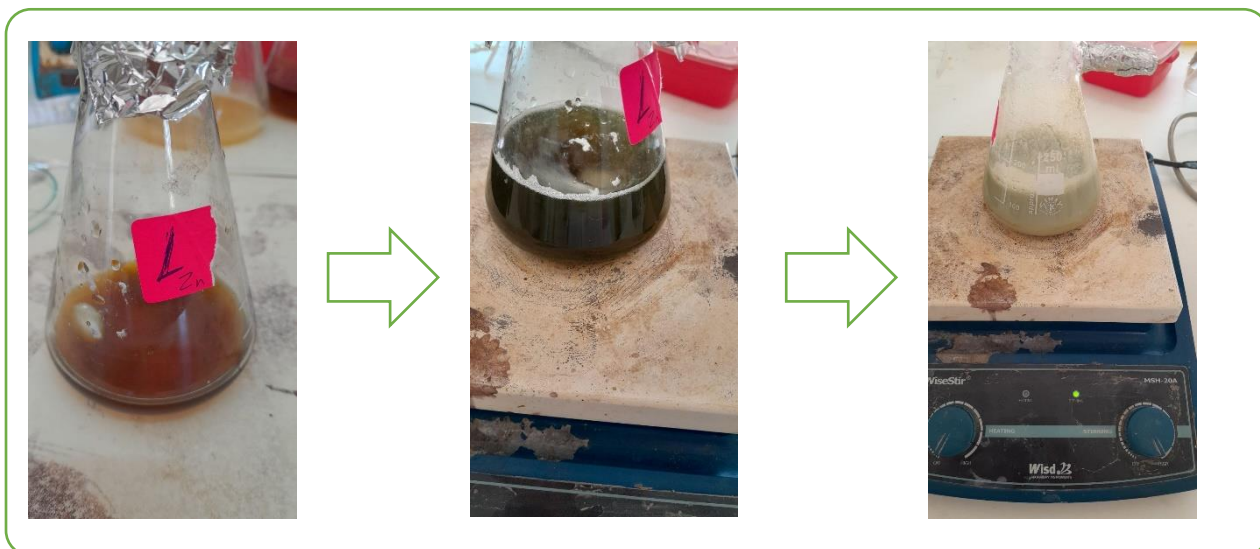


FIGURE II.5. Photo showing the color change during preparation for: ZnO NPs leaves Ex

ZnO NPs seeds ex:

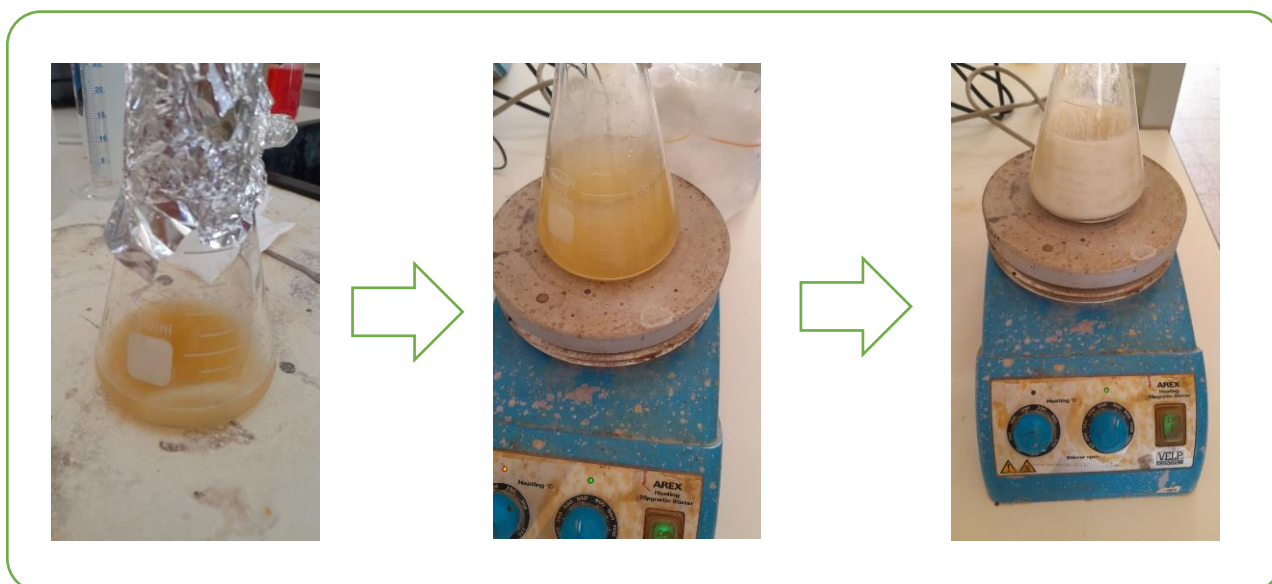


FIGURE II.6. Photo showing the color change during preparation for: ZnO NPs Leaves Ex

Ag/ZnO NPs leaves ex:

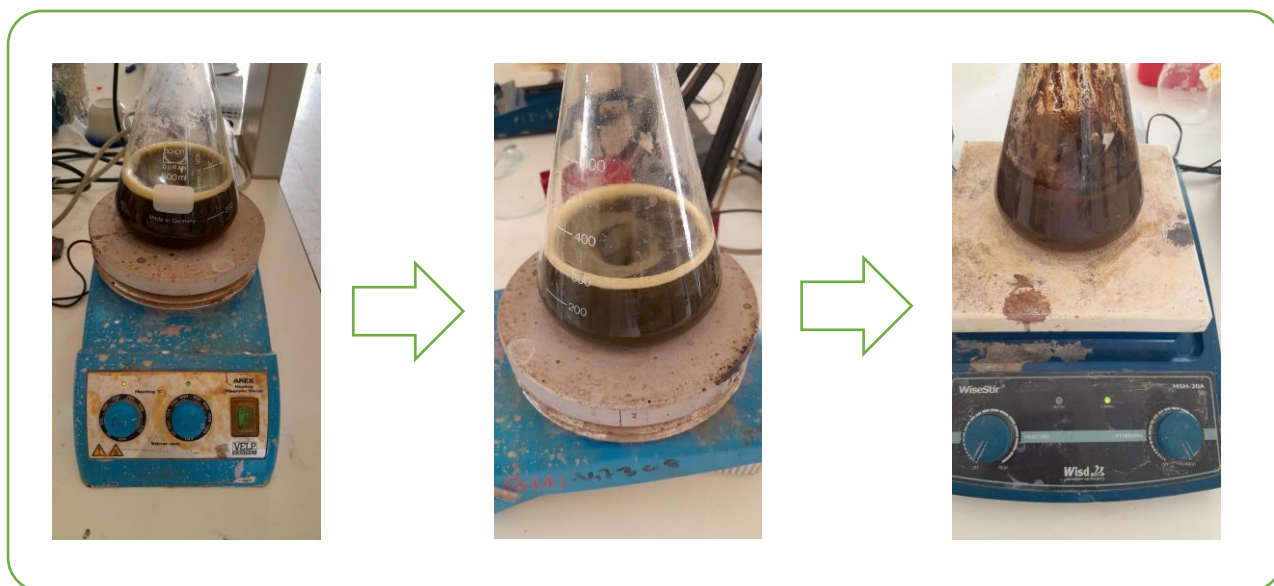


FIGURE II.7. Photo showing the color change during preparation for: Ag/ZnO NPs Leaves Ex

Ag/ZnO NPs Seeds ex

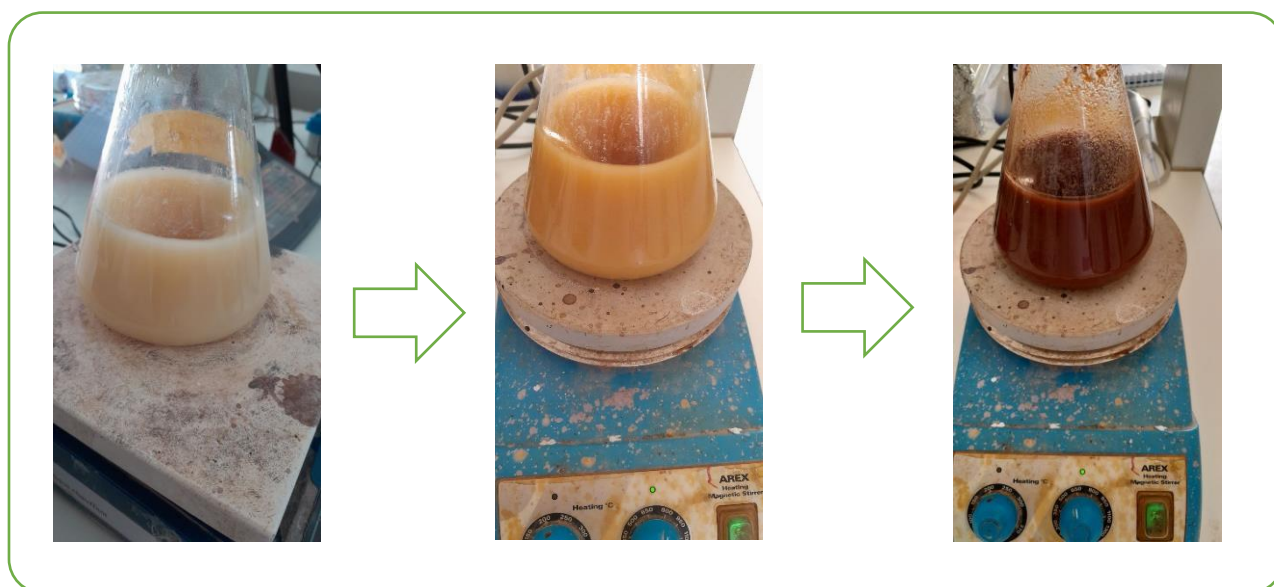


FIGURE II.8. Photo showing the color change during preparation for: Ag/ZnO NPs Seeds Ex

II.2.2.UV-Vis:

ZnO NPs Leaves Ex :

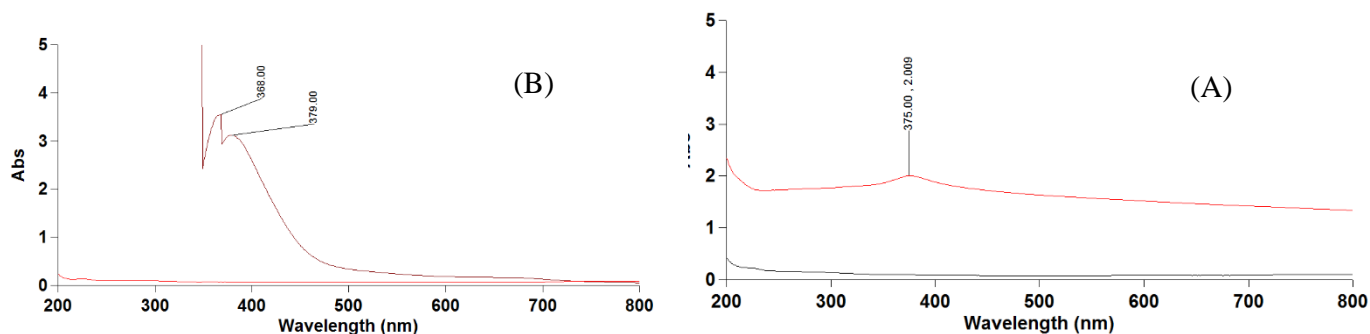


FIGURE II.9: UV-vis spectra of Zn NPs Leaves (A) & Leaves extract (B)

Interpretation:

The figure shows the UV-Vis absorption spectrum of ZnO nanoparticles (ZnO NPs) for the leaves, where the absorption intensity peaks at a wavelength of 375 nm. Theoretically, ZnO NPs possess an absorption peak within the range of 200-400 nm[88]. By comparing this with the spectrum of the extract, we observe a difference in wavelength, where the absorption peak of the extract reaches 368 nm.

ZnO NPs Seeds Ex:

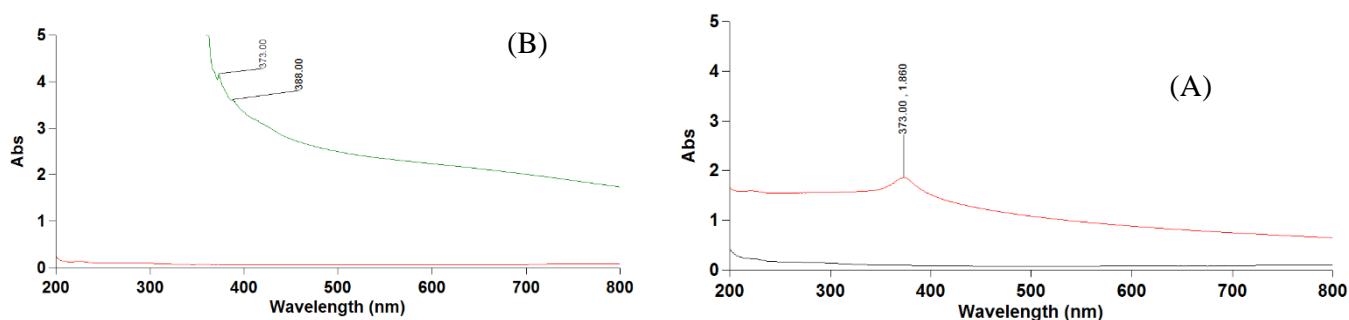


FIGURE II.10 : UV-vis spectra of ZnO NPs Seeds (A) & seeds extract (B)

Interpretation:

The figure shows the UV-Vis absorption spectrum of zinc oxide nanoparticles (ZnO NPs) for pumpkin seeds, where the absorption peak appears at a wavelength of 373 nm. Zinc oxide nanoparticles have an absorption peak in the range of 200-400 nm[88]. Comparing this with the absorption spectrum of the extract, we observe a difference in wavelength, where the absorption peak of the extract reaches 373 nm.

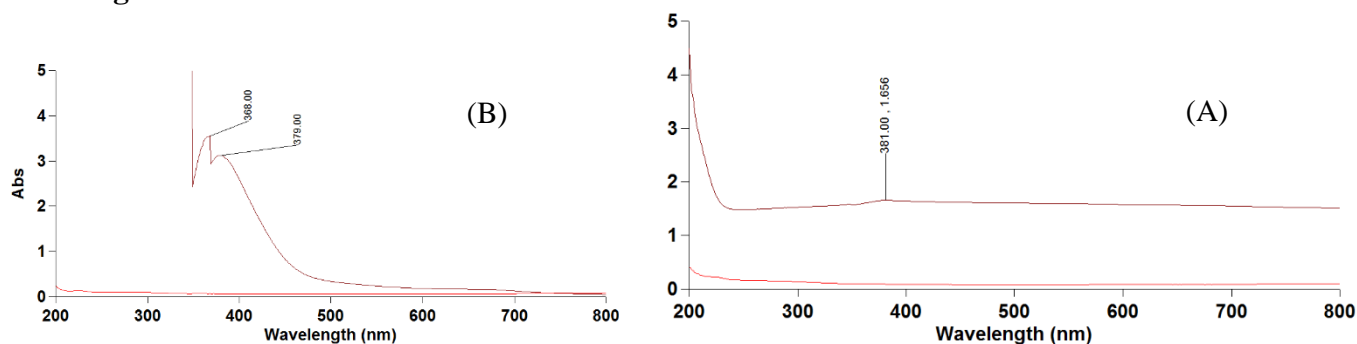
Ag/ZnO NPs Leaves Ex:

FIGURE II.11 : UV-vis spectra of Ag /ZnO NPs Leaves (A) & Leaves extract (B)

Interpretation

The figure shows the UV-Vis absorption spectrum of the nanoparticles of the silver and zinc oxide mixture (Ag/ZnO NPs) for the leaf extract, where the absorption intensity peaks at a wavelength of 381 nm. Theoretically, Ag/ZnO NPs have an absorption peak in the range of 377-397 nm[89]. By comparing this with the spectrum of the extract, we observe a difference in wavelength, where the absorption peak of the extract reaches 368 nm.

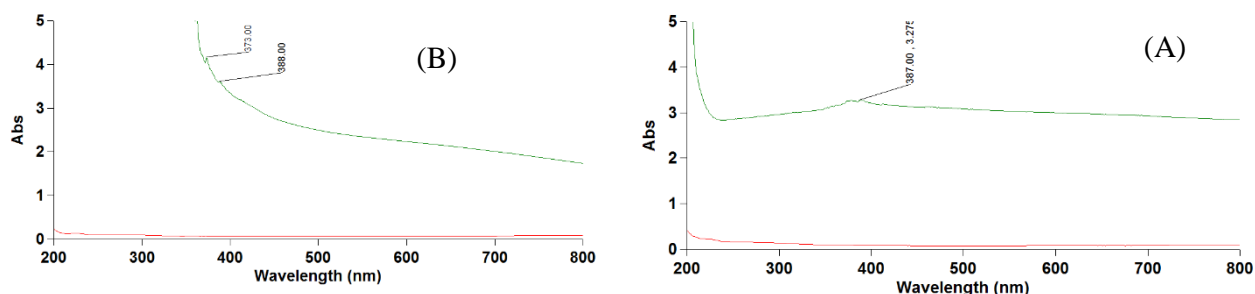
Ag/ZnO NPs Seeds Ex:

FIGURE II.12: UV-vis spectra of Ag /ZnO NPs seeds (A) & seeds extract(B)

Interpretation

The figure shows the UV-Vis absorption spectrum of the nanoparticles of the silver and zinc oxide mixture (Ag/ZnO NPs) for the pumpkin seed extract, where the absorption intensity peaks at a wavelength of 387 nm. By comparing this with the spectrum of the extract, where the wavelength reaches 373 nm, we observe an increase in the absorption peak.

II.2.3.EDX/SEM :

ZnO NPs Seeds Ex:

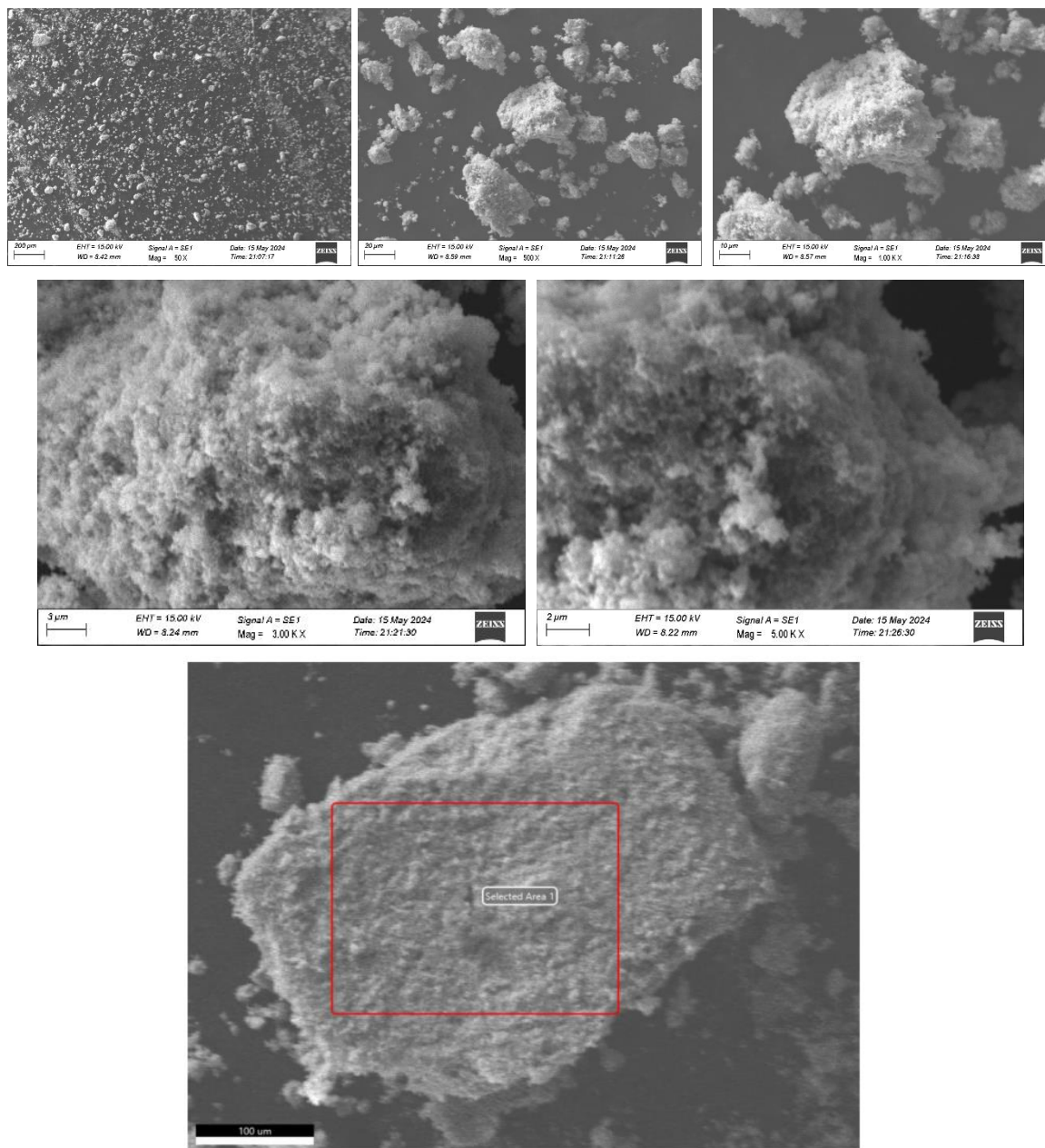


FIGURE II.13: images taken with a EDX/SEM of ZnO NPs seed extracts

Interpretation:

From this image, we observe that the morphological shape consists of aggregated particles, ranging in shape from circular to oval, with sizes varying between (100 μm to 10,000 nm).

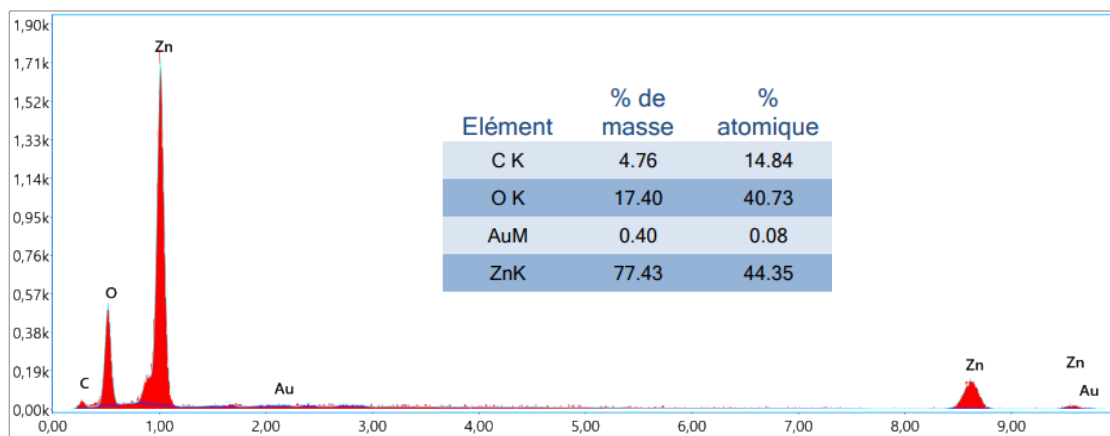


FIGURE II.14 : EDX of ZnO NPs seeds extract

The EDX analysis shows the presence of four elements - carbon (C), oxygen (O), gold (Au), and zinc (Zn) in the analyzed area.

Zinc is by far the major constituent, making up 77.43% by weight (44.35% atomic percentage). This high zinc concentration suggests the analyzed area likely contains a zinc-based material or zinc coating.

Oxygen is the next most abundant element at 17.40 wt% (40.73 at%). The presence of both zinc and oxygen indicates there are likely zinc oxide compounds in the sample .

ZnO NPs Leaves Ex :

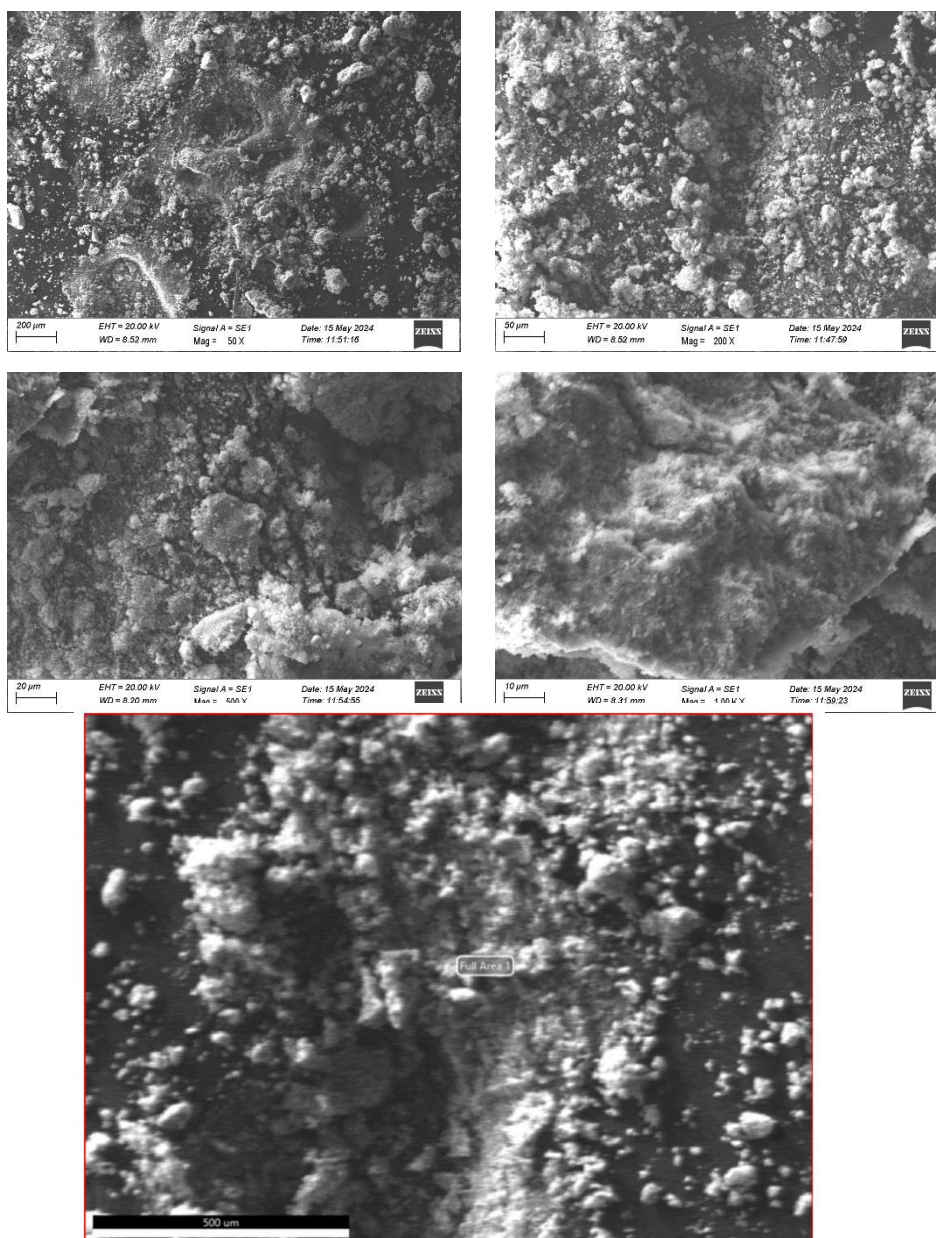


FIGURE II.15 : images taken with a EDX/SEM of ZnO NPs leaves extracts

Interpretation:

From this image, we notice that its morphological form is small, clustered granules close together, and their sizes range from 500 nm to 50000 nm .

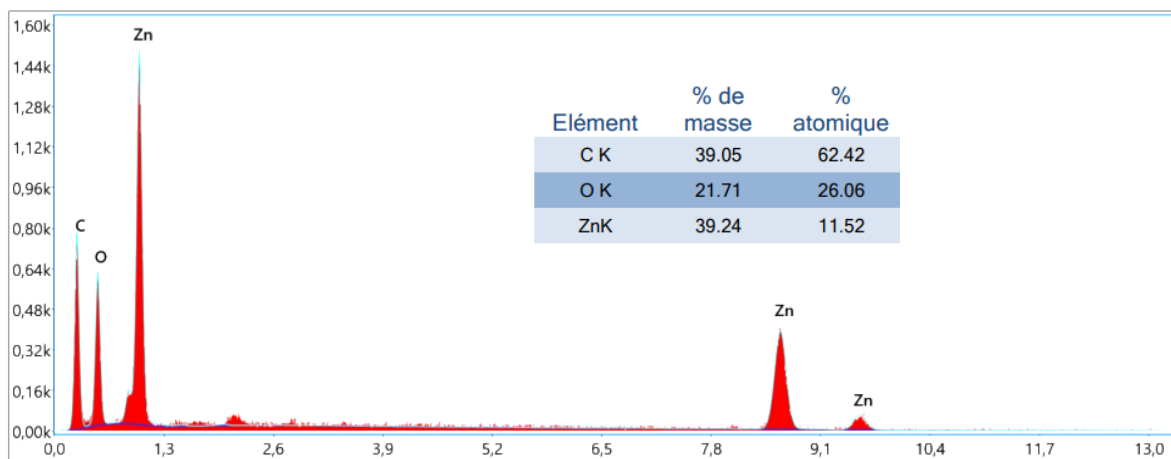


FIGURE II.16 : EDX of ZnO NPs leaves extract

Interpretation

The EDX analysis shows the sample contains three main elements - carbon (C), oxygen (O), and zinc (Zn).

Carbon is the most abundant at 39.05 wt% (62.42 at%). This high carbon content is likely due to the leaf material, which is made up of organic carbon compounds.

Oxygen is present at 21.71 wt% (26.06 at%), which along with the zinc indicates the presence of zinc oxide (ZnO) in the sample, as expected for ZnO nanoparticles.

Zinc makes up 39.24 wt% (11.52 at%) of the sample, which is a significant amount and confirms zinc oxide as a major constituent.

The relative zinc and oxygen percentages match the expected 1:1 ratio for ZnO quite well.

Ag/ZnO NPS Seeds Ex:

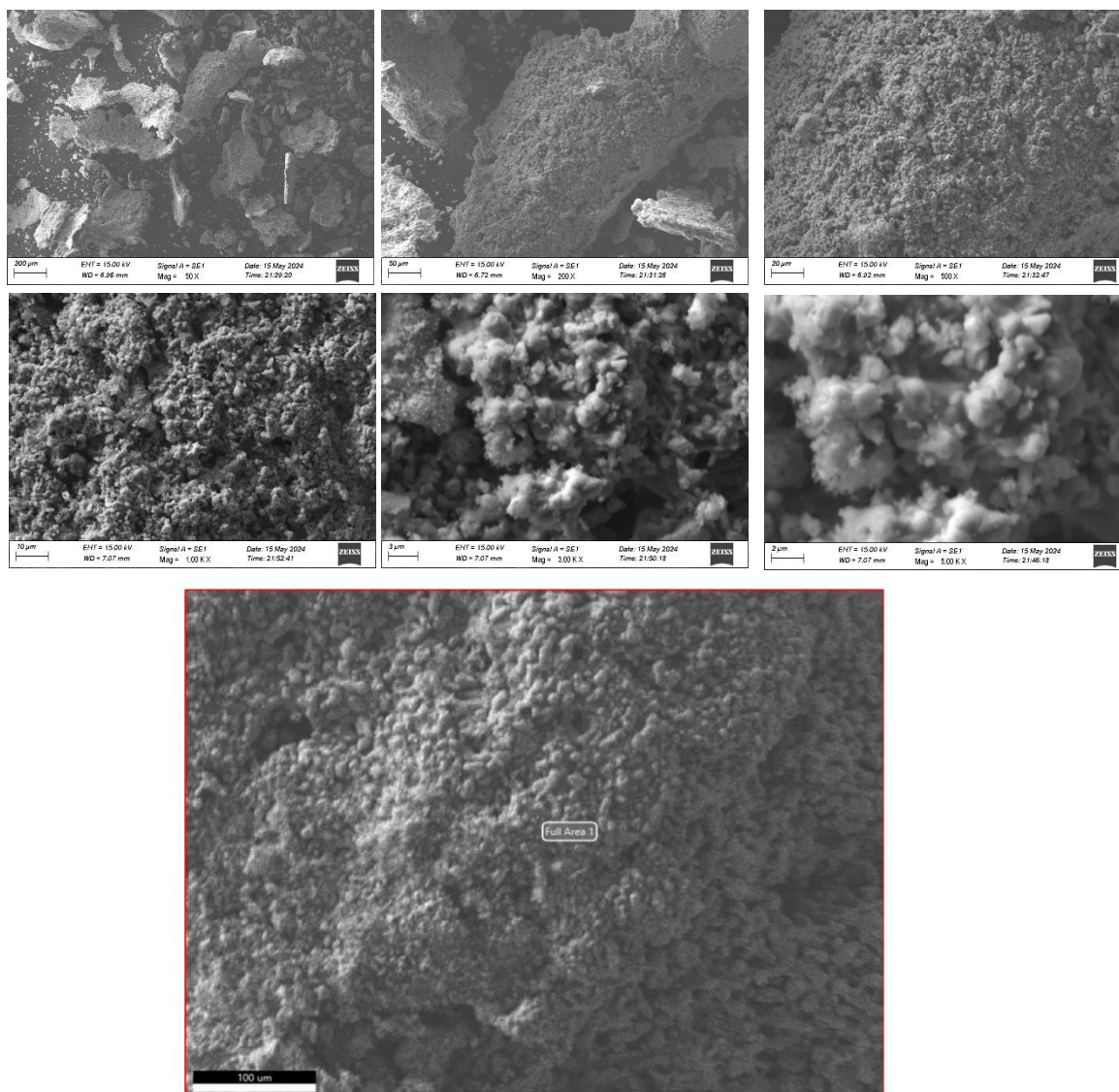


FIGURE II.17 : images taken with a EDX/SEM of Ag/ZnO NPs Seeds extracts

Interpretation :

From this image, we notice that its morphological form is small, clustered granules close together, and their sizes range from 100um to 10000 nm .

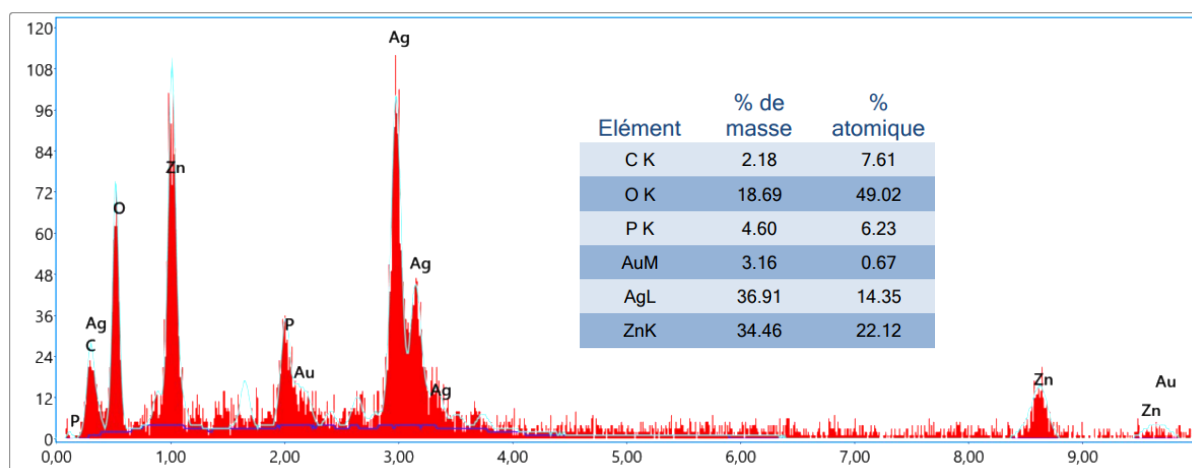


FIGURE II.18 : EDX of Ag/ZnO NPs Seeds extract

Interpretation :

The EDX data shows the presence of six main elements: carbon (C), oxygen (O), phosphorus (P), gold (Au), silver (Ag), and zinc (Zn).

Silver (Ag) and zinc (Zn) are the two most abundant elements by weight percentage at 36.91% and 34.46%, respectively. Their significant presence aligns with the intended composition of Ag and ZnO nanoparticles in the sample.

Oxygen at 18.69 wt% further supports the presence of zinc oxide (ZnO), which requires oxygen along with zinc.

Ag/ZnO NPS Leaves Ex :

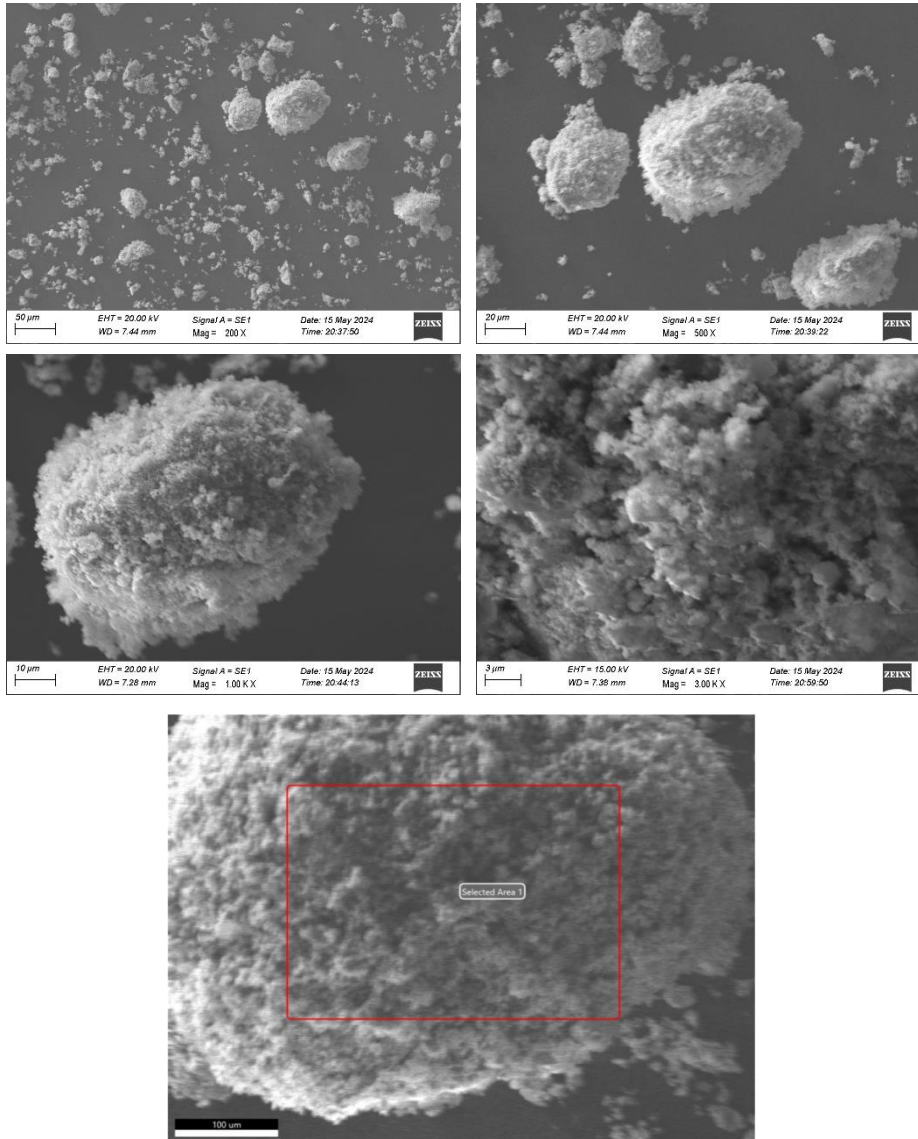


FIGURE II.19 : images taken with a EDX/SEM of Ag/ZnO NPs Leaves extracts

Interpretation :

From this image, we notice that its morphological form is small, clustered granules close together, and their sizes range from 100um to 10000 nm

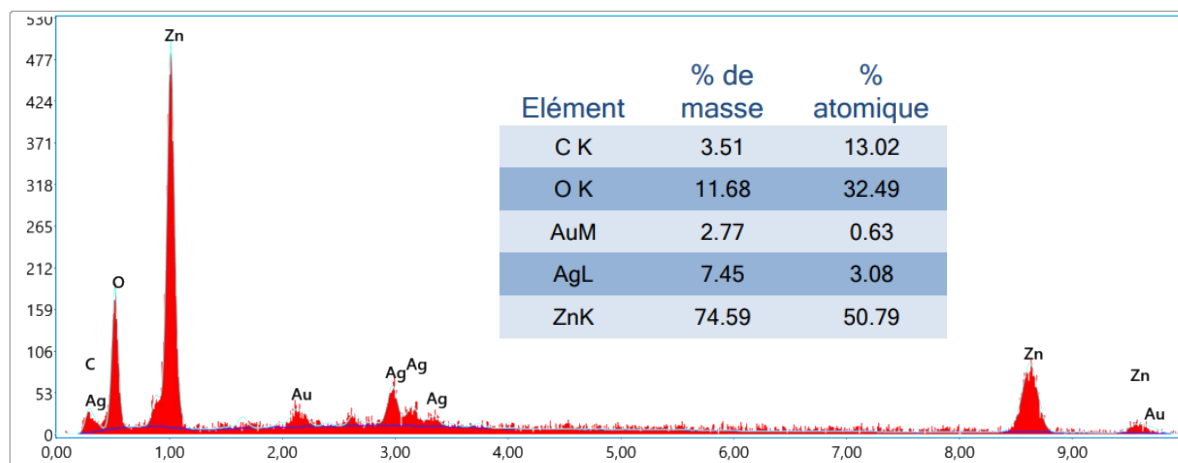


FIGURE II.20 : EDX of Ag/ZnO NPs leaves extract

Interpretation:

Based on the EDX results provided and the context that this is an analysis of a sample containing silver (Ag) and zinc oxide (ZnO) nanoparticles (NPs) on leaves, I can provide the following analysis:

The major element detected is zinc (Zn) at 74.59 wt% (50.79 at%), indicating a very high concentration of zinc in the analyzed area.

Oxygen is present at 11.68 wt% (32.49 at%), which combined with the high zinc content strongly suggests the presence of zinc oxide (ZnO) nanoparticles.

Silver (Ag) is detected at 7.45 wt% (3.08 at%), confirming the presence of silver nanoparticles as intended for the Ag/ZnO sample.

The high zinc and oxygen content relative to silver suggests that the ZnO nanoparticles are more abundant than the Ag nanoparticles in this analyzed area.

II.2.4.FTIR :

ZnO NPs Seeds Ex :

TABLE II.19 : Table showing peak and functional potential ZnO NPs Seeds

Peak At (cm ⁻¹)	Peak Height	Possible Assignment
1738.6713	92.5363	C=O stretching (carbonyl compounds)
1700.0426	91.5822	C=O stretching (carbonyl compounds)
1652.9049	92.1471	C=C stretching (alkenes)
1558.1025	89.8012	N-O stretching (nitro compounds) or C=C stretching (aromatic ring)
1515.7841	87.6966	C=C stretching (aromatic ring)
1493.2694	92.196	C-H bending (aromatics) or C=C stretching (aromatic ring)
1475.6493	92.7885	C-H bending (aromatics)
1458.6315	92.1548	C-H bending (alkanes)
1422.7136	93.1523	O-H bending (phenols) or C-H bending (alkanes)
1395.1538	93.0637	O-H bending (phenols) or C-H bending (alkanes)

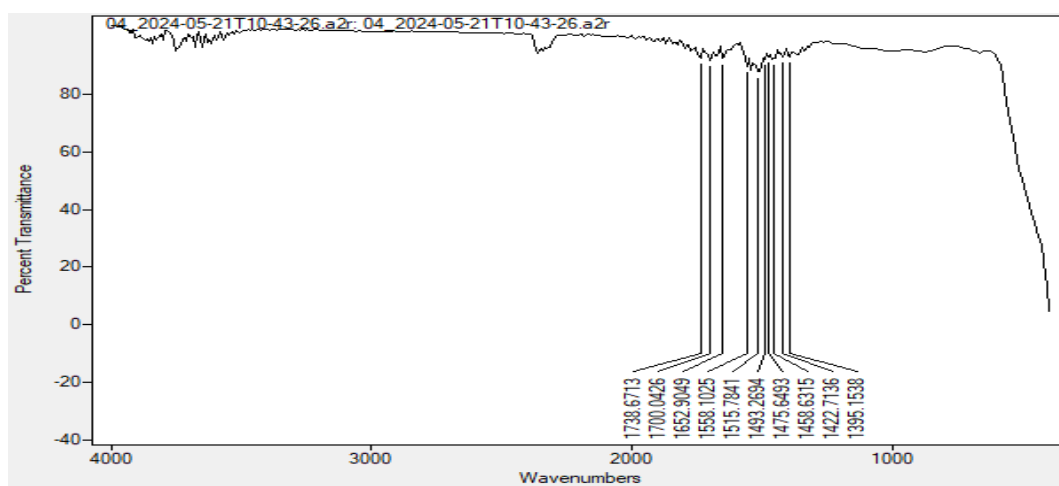


FIGURE II.21 : FTIR spectrom of ZnO NPs seeds extract

Interpretation:

The FTIR spectrum indicates the presence of various functional groups. The peaks at 1738.6713 cm⁻¹ and 1700.0426 cm⁻¹ suggest the presence of carbonyl compounds (C=O stretching). Peaks around 1500-1600 cm⁻¹ are indicative of aromatic rings and nitro groups, while those near 1400 cm⁻¹ correspond to phenolic O-H bending and alkane C-H bending

vibrations. The presence of C=C stretching vibrations around 1652.9049 cm^{-1} and 1515.7841 cm^{-1} suggests alkenes and aromatic rings.

ZnO NPs Leaves Ex :

TABLE II.3 : Table showing peak and functional potential ZnO NPs Leaves

Peak At (cm^{-1})	Peak Height	Possible Assignment
1699.892	90.4126	C=O stretching (carbonyl compounds)
1557.6507	86.692	N-O stretching (nitro compounds) or C=C stretching (aromatic ring)
1542.139	84.5667	N-O stretching (nitro compounds) or C=C stretching (aromatic ring)
1514.2781	82.9505	C=C stretching (aromatic ring)
1491.4622	85.7502	C-H bending (aromatics) or C=C stretching (aromatic ring)
1473.0891	84.5971	C-H bending (aromatics)
1399.4459	85.5855	O-H bending (phenols) or C-H bending (alkanes)
1368.7236	87.0009	C-H bending (alkanes, often due to methyl groups)
1343.2723	89.9869	C-N stretching (amines) or O-H bending (phenols)

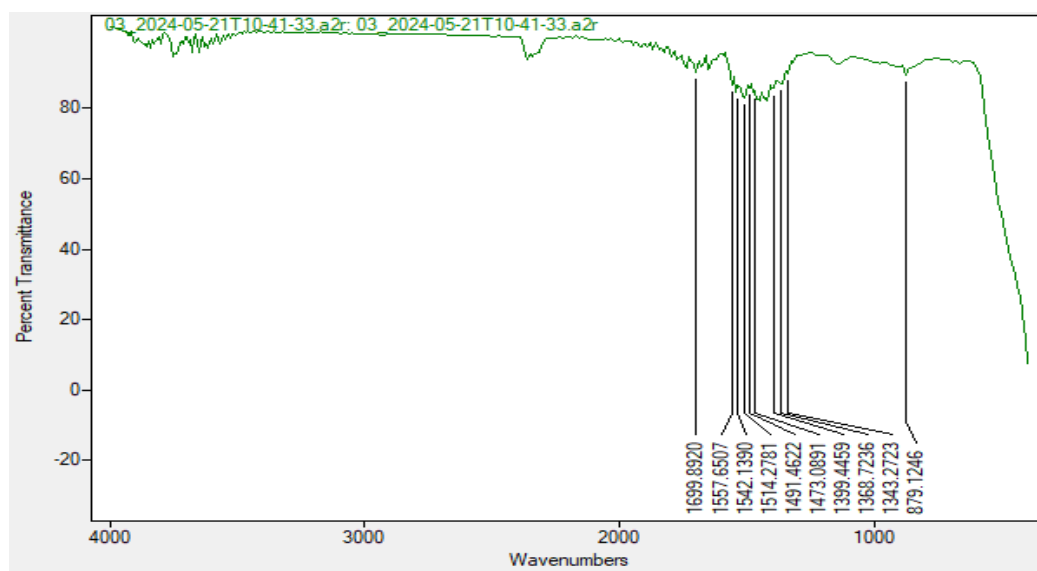


FIGURE II.22 : FTIR spectrum of ZnO NPs Leaves extract

Interpretation:

The FTIR spectrum indicates the presence of various functional groups. The peak at 1699.892 cm^{-1} suggests the presence of carbonyl compounds (C=O stretching). Peaks around $1500\text{-}1600\text{ cm}^{-1}$ are indicative of aromatic rings and nitro groups, while those near 1400 cm^{-1} correspond to phenolic O-H bending and alkane C-H bending vibrations. The presence of C-N stretching vibrations around 1343 cm^{-1} suggests amines, and peaks below 900 cm^{-1} indicate alkenes and aromatic ring.

Ag/ZnO NPs Leaves Ex :**TABLE II.4** : Table showing peak and functional potential Ag/ZnO NPs Leaves

Peak At (cm^{-1})	Peak Height	Possible Assignment
1557.8013	80.1492	N-O stretching (nitro compounds) or C=C stretching (aromatic ring)
1541.6872	77.9336	N-O stretching (nitro compounds) or C=C stretching (aromatic ring)
1512.6215	74.5869	C=C stretching (aromatic ring)
1489.0526	75.1965	C-H bending (aromatics) or C=C stretching (aromatic ring)
1402.7591	68.5832	O-H bending (phenols) or C-H bending (alkanes)
1344.4771	78.1786	C-N stretching (amines) or O-H bending (phenols)
1137.9299	78.7079	C-O stretching (alcohols, ethers, esters)
988.8364	82.4126	C-H bending (alkenes)
925.3587	81.9531	C-H bending (alkenes) or C-Cl stretching (halo compounds)
878.8234	75.3204	C-H bending (alkenes) or out-of-plane bending (aromatic compounds)

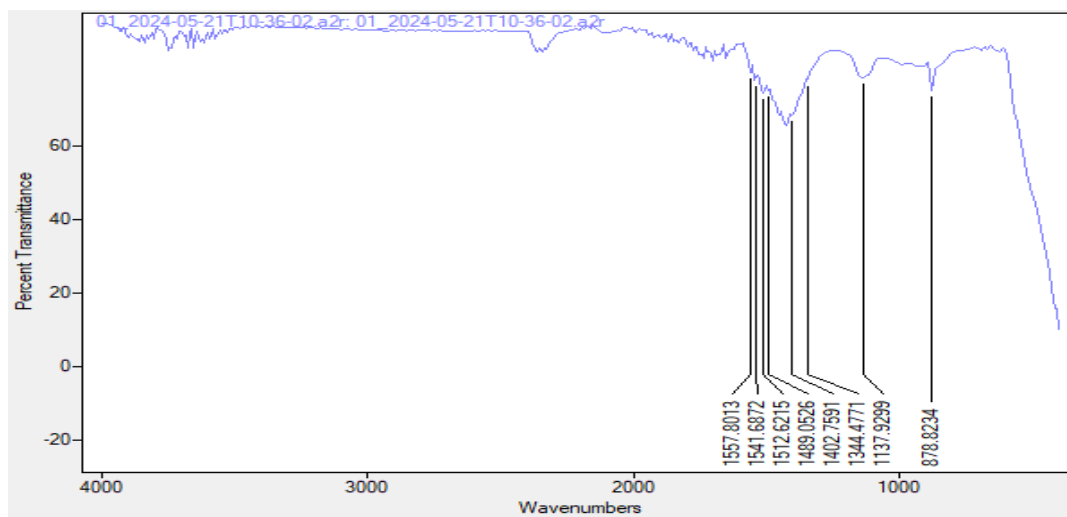


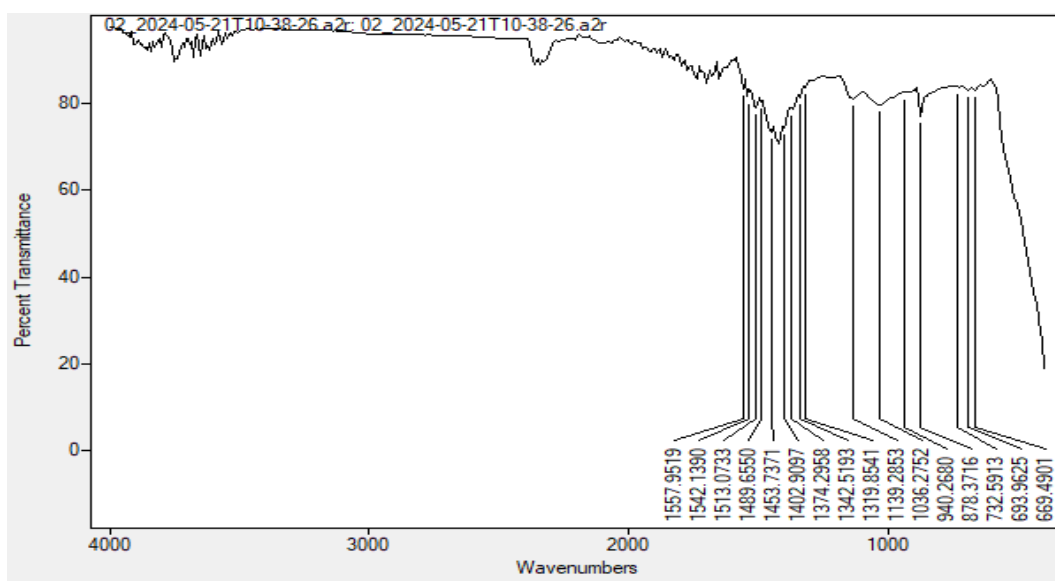
FIGURE II.23 : FTIR spectrum of Ag/ZnO NPs Leaves extract

Interpretation:

The FTIR spectrum indicates the presence of aromatic compounds, nitro groups, alkanes, alcohols/ethers/esters, and alkenes. Peaks around $1500\text{--}1600\text{ cm}^{-1}$ are indicative of aromatic rings and nitro groups, while those near 1374 cm^{-1} and 1454 cm^{-1} correspond to alkane bending vibrations. The presence of C-O stretching vibrations around 1036 cm^{-1} and 1139 cm^{-1} suggests alcohols, ethers, and esters. The peaks below 900 cm^{-1} indicate alkenes and aromatic ring.

Ag/ZnO NPs Seeds Ex :**TABLE II.5 :** Table showing peak and functional potential Ag/ZnO NPs Seeds

Peak At (cm ⁻¹)	Peak Height	Possible Assignment
1557.9519	83.1371	N-O stretching (nitro compounds) or C=C stretching (aromatic ring)
1542.139	81.4416	N-O stretching (nitro compounds) or C=C stretching (aromatic ring)
1513.0733	78.8719	C=C stretching (aromatic ring)
1489.655	80.1619	C-H bending (aromatics) or C=C stretching (aromatic ring)
1453.7371	73.3001	C-H bending (alkanes, methylene groups)
1402.9097	74.3552	O-H bending (phenols) or C-H bending (alkanes)
1374.2958	78.7683	C-H bending (alkanes, often due to methyl groups)
1342.5193	81.3135	C-N stretching (amines) or O-H bending (phenols)
1139.2853	81.0343	C-O stretching (alcohols, ethers, esters)
1036.2752	79.6338	C-O stretching (alcohols, ethers, esters) or Si-O stretching (silicones)
940.268	82.4621	C-H bending (alkenes) or C-Cl stretching (halo compounds)
878.3716	76.9351	C-H bending (alkenes) or out-of-plane bending (aromatic compounds)
693.9625	82.8545	C-H bending (alkenes) or out-of-plane bending (aromatic compounds)
669.4901	82.8465	C-H bending (alkenes) or out-of-plane bending (aromatic compounds)

**FIGURE II.24 :** FTIR spectrom of Ag/ZnO NPs Leaves extract

Interpretation:

The FTIR spectrum indicates the presence of aromatic compounds, nitro groups, alkanes, alcohols/ethers/esters, and alkenes. Peaks around $1500\text{-}1600\text{ cm}^{-1}$ are indicative of aromatic rings and nitro groups, while those near 1374 cm^{-1} and 1454 cm^{-1} correspond to alkane bending vibrations. The presence of C-O stretching vibrations around 1036 cm^{-1} and 1139 cm^{-1} suggests alcohols, ethers, and esters. The peaks below 900 cm^{-1} indicate alkenes and aromatic ring

II.2.5.XRD :

The DRX results for the samples were processed using HighScore Plus software. After inspection and verification, we obtained the following results:

ZnO NPs leaves and seeds

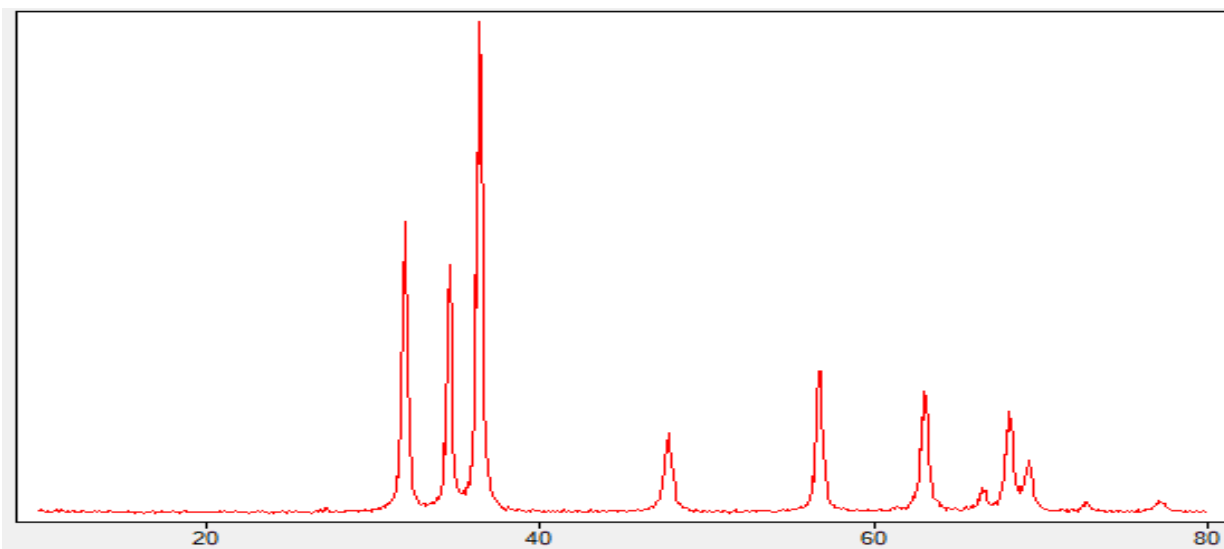


FIGURE II.25 : XRD spectrum of ZnO leaves and seeds Extracts

After analyzing the spectra of the two distinctive patterns for this sample, the corresponding crystalline structure was identified, matching the database table CSD, specifically from card number 01-079-0205:

- **Compound name:** Zinc oxide (ZnO)
- **Chemical formula:** ZnO
- **Crystal structure:** Hexagonal system

Ag/ZnO NPs seeds Ex:

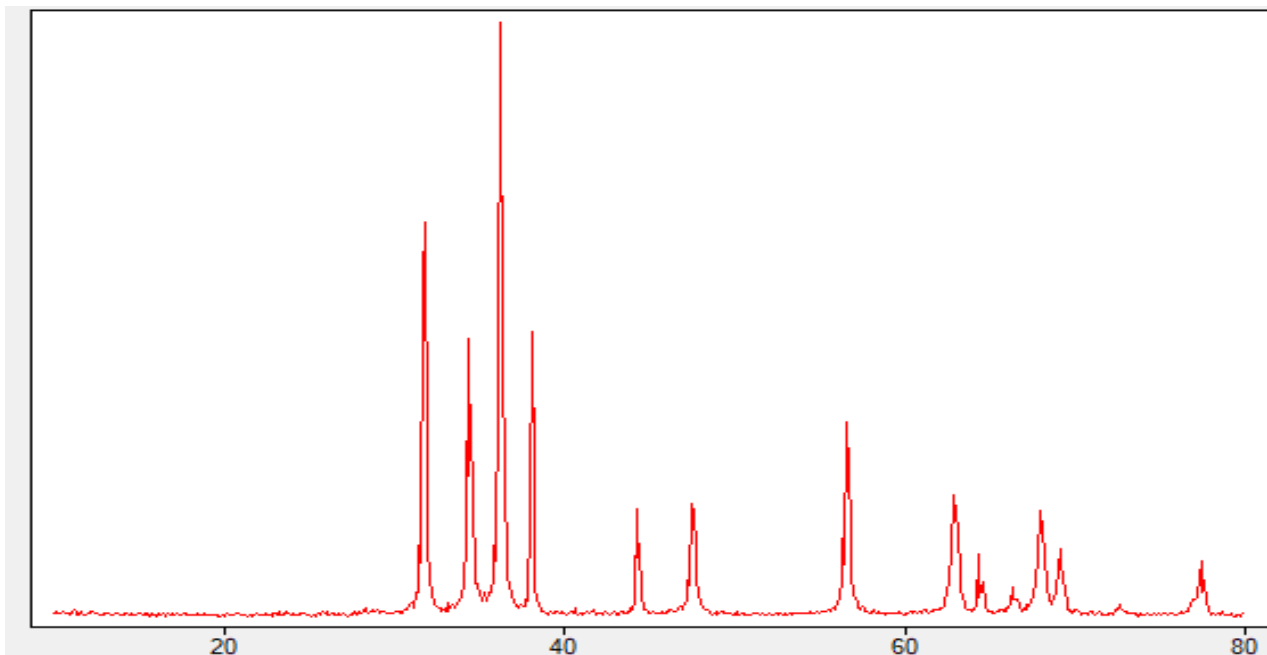


FIGURE II.26 : XRD spectrum of Ag/ZnO seeds Extracts

After analyzing the spectra of the two distinctive patterns for this sample, the corresponding crystalline structures were identified, matching the database table CSD:

- From card number 01-087-0717:
 - **Compound name:** Silver
 - **Chemical formula:** Ag
 - **Crystal structure:** Cubic system

- From card number 01-074-0534:
 - **Compound name:** Zinc oxide (Zincite, syn)
 - **Chemical formula:** ZnO
 - **Crystal structure:** Hexagonal system.

Ag/ZnO NPs Leaves Ex:

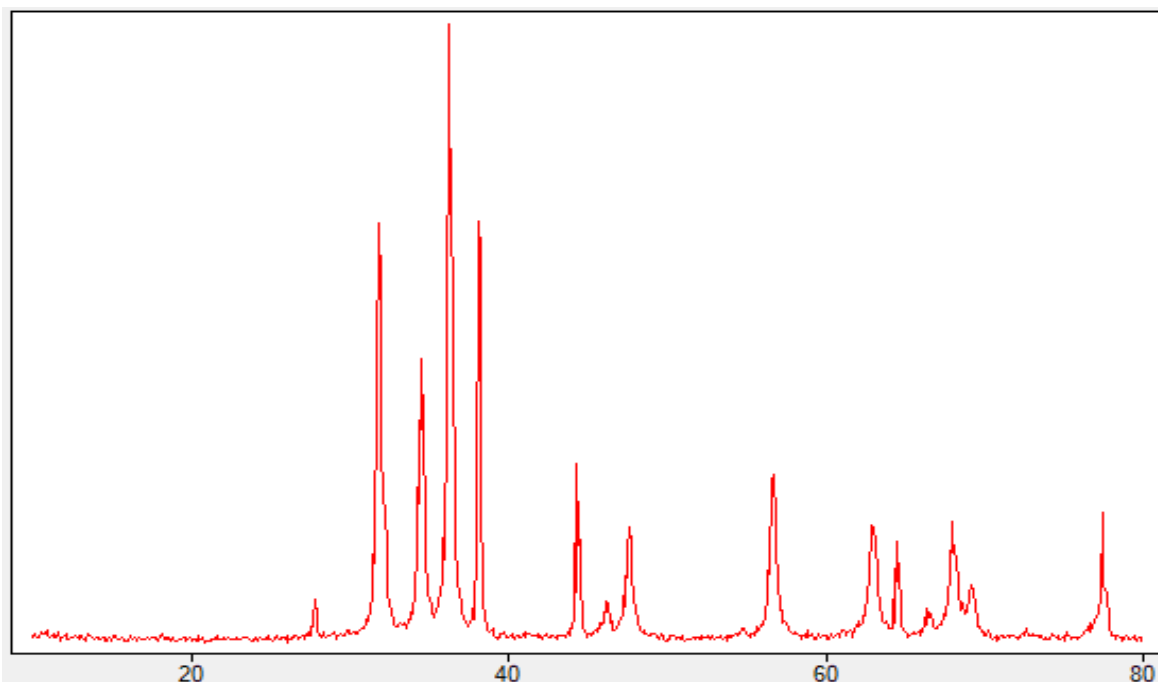


FIGURE II.27 : XRD spectrum of Ag/ZnO seeds Extracts

After analyzing the spectra of the two distinctive patterns for this sample, the corresponding crystalline structures were identified, matching the database table CSD:

- From card number 01-089-7102:
 - **Compound name:** Zinc oxide (Zincite, syn)
 - **Chemical formula:** ZnO
 - **Crystal structure:** Hexagonal system
- From card number 01-087-0718:
 - **Compound name:** Silver
 - **Chemical formula:** Ag
 - **Crystal structure:** Cubic system

***Chapter III. Application: Antimicrobial
Activity***

III.1. Biological Materials

We used a reference strain of *Candida albicans* ATCC 10231 and four bacterial strains, including two Gram-negative strains, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922, and two Gram-positive strains, *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 25973.

III.2. Used tools :

Laboratory Equipment
Mueller-Hinton culture media
Petri dishes
Refrigerator
Incubator set at 37°C
Autoclave Bunsen burner
Precision balance
Water bath
Sterile loop
Sterile swab
5% DMSO
Micropipette
Cones
Hemolysis tubes
Sterile Pasteur pipettes
McFarland Standard No. 0.5 (1.5×10^8 CFU/ml)
VITEK® DENSICHEK® McFarland
Sterile physiological saline solution (0.9% NaCl)
• Sterile distilled water

III.3. Agar Diffusion Method (Well Method)

The agar diffusion method, specifically the well method, is a fundamental technique used to evaluate the antimicrobial activity of a substance. To begin, Petri dishes are prepared with Sabouraud dextrose agar supplemented with 2% glucose for yeasts, and Mueller-Hinton agar for bacteria. These dishes are then aseptically inoculated with a suspension containing 1.5×10^8 CFU/mL, standardized using McFarland Standard No. 0.5. The suspension is prepared using a VITEK® DENSICHEK® device and a mixture of sterile physiological saline solution (0.9% NaCl) and sterile distilled water.

Once the agar surfaces have dried, wells are created at the center of each dish using the upper part of a Pasteur pipette. These wells are subsequently filled with 50 μ L of an aqueous solution of the test compounds (Ag/Zn NPs leaves, Ag/Zn NPs seeds, ZnO NPs leaves, ZnO NPs seeds) at concentrations of 80, 40, 20, and 10 mg/mL, prepared with 5% DMSO to ensure proper dissolution of the compounds.

The inoculated plates are incubated at 37°C for 48 hours for yeasts and 24 hours for bacteria. Antimicrobial activity is indicated by forming a clear zone of inhibition around the wells, where microbial growth has been prevented. The diameter of these inhibition zones is measured, and a product is considered to exhibit antimicrobial activity if the zone of inhibition is greater than 6 mm [90], [91].



FIGURE III.1: Pictures of laboratory tools in the protocol for studying the effectiveness against bacteria of the four compounds.

III.4. Results and discussions :

III.4.1. Ag/ZnO NPs leaves :

Table III.1. Results of antimicrobial tests: **Ag/ZnO NPs leaves**

Microbial inhibition Ag/ZnO NPs leaves					
Strains used	80mg/ml	40mg/ml	20mg/ml	10mg/ml	CIP
<i>Escherichia coli</i> ATCC 25922	10	12	10	9	33
<i>Pseudomonas aeruginosa</i> ATCC 27853	10	10	13	9	33
<i>Staphylococcus aureus</i> ATCC 25932	10	12	9	7	31
<i>Bacillus subtilis</i> ATCC 25973	10	10	10	13	33
Anti-Candida activity					
<i>Candida albicans</i> ATCC 10231	NI	NI	NI	NI	/

NI = No Inhibition, CIP = Ciprofloxacin (CIP) 5ug Discs.



FIGURE III.2 : photo of antimicrobial tests of **Ag/ZnO NPs leaves**.

III.4.2. Ag/ZnO NPs seeds :

TABLE III.2:Results of antimicrobial tests: **Ag/ZnO NPs seeds**

Microbial inhibition Ag/ZnO NPs leaves					
Strains used	80mg/ml	40mg/ml	20mg/ml	10mg/ml	CIP
<i>Escherichia coli</i> ATCC 25922	18	13	7	NI	33
<i>Pseudomonas aeruginosa</i> ATCC 27853	8	7	7	NI	33
<i>Staphylococcus aureus</i> ATCC 25932	8	7	7	NI	31
<i>Bacillus subtilis</i> ATCC 25973	NI	NI	NI	NI	33
Anti-Candida activity					
<i>Candida albicans</i> ATCC 10231	NI	NI	NI	NI	/

NI = No Inhibition, CIP = Ciprofloxacin (CIP) 5ug Discs.



FIGURE III.3 :. photo of antimicrobial tests of Ag/Zn NPs seeds.

III.4.3. ZnO NPs leaves :

TABLE III.3: Results of antimicrobial tests: ZnO NPs leaves

Strains used	Microbial inhibition ZnO NPs leaves				
	80mg/ml	40mg/ml	20mg/ml	10mg/ml	CIP
<i>Escherichia coli</i> ATCC 25922	7	NI	NI	NI	33
<i>Pseudomonas aeruginosa</i> ATCC 27853	NI	NI	NI	NI	33
<i>Staphylococcus aureus</i> ATCC 25932	10	9	NI	NI	31
<i>Bacillus subtilis</i> ATCC 25973	NI	NI	NI	NI	33
Anti-Candida activity					
<i>Candida albicans</i> ATCC 10231	NI	NI	NI	NI	/

NI = No Inhibition, CIP = Ciprofloxacin (CIP) 5ug Discs.



FIGURE III.4: photo of antimicrobial tests of ZnO NPs leaves.

III.4.4. ZnO NPs seeds:

Table III.4. Results of antimicrobial tests: ZnO NPs seeds

Strains used	Microbial inhibition ZnO NPs Seeds				
	80mg/ml	40mg/ml	20mg/ml	10mg/ml	CIP
<i>Escherichia coli</i> ATCC 25922	NI	NI	NI	NI	33
<i>Pseudomonas aeruginosa</i> ATCC 27853	NI	NI	NI	NI	33
<i>Staphylococcus aureus</i> ATCC 25932	7	NI	NI	NI	31
<i>Bacillus subtilis</i> ATCC 25973	NI	NI	NI	NI	33
Anti-Candida activity					
<i>Candida albicans</i> ATCC 10231	NI	NI	NI	NI	/

NI = No Inhibition, CIP = Ciprofloxacin (CIP) 5ug Discs.



FIGURE III.4: photo of antimicrobial tests of ZnO NPs seeds.

III.5. Conclusion:

Ag/ZnO Leaves

- Showed antimicrobial activity against all tested bacterial strains at various concentrations, but no activity against *Candida albicans*.

- Example: *E. coli* (10-12 mm compared to CIP's 33 mm), *P. aeruginosa* (9-13 mm compared to CIP's 33 mm), *S. aureus* (7-12 mm compared to CIP's 31 mm), *B. subtilis* (10-13 mm compared to CIP's 33 mm).

Ag/ZnO Seeds

- Effective against *E. coli*, less so against other bacteria, and ineffective against *B. subtilis* and *Candida albicans*.

- Example: *E. coli* (7-18 mm compared to CIP's 34 mm), *P. aeruginosa* (7-8 mm compared to CIP's 33 mm), *S. aureus* (7-8 mm compared to CIP's 34 mm), *B. subtilis* (NI compared to CIP's 33 mm).

ZnO Leaves

- Limited antimicrobial activity, mainly against *S. aureus*, ineffective against others including *Candida albicans*.

- Example: *E. coli* (7 mm at 80 mg/ml compared to CIP's 33 mm), *S. aureus* (9-10 mm compared to CIP's 33 mm), all others (NI compared to CIP's range of 26-33 mm).

ZnO Seeds

- Ineffective against most strains, with minimal activity against *S. aureus* and none against *Candida albicans*.

- Example: *S. aureus* (7 mm at 80 mg/ml compared to CIP's 34 mm), all others (NI compared to CIP's range of 26-33 mm).

Key Findings

Ag/ZnO leaves showed the broadest range of activity against the bacterial strains but were less effective than ciprofloxacin (CIP).

Ag/Zn seeds displayed selective activity, notably against *E. coli*, but still less effective compared to CIP.

ZnO leaves and seeds were less effective overall, with ZnO leaves showing some potential against *S. aureus*, but much lower than CIP.

- No tested compound was effective against *Candida albicans*

Ciprofloxacin (CIP) served as a positive control and was significantly more effective across all tested bacterial strains, highlighting the relatively lower efficacy of the test compounds.

Conclusion

The green synthesis of nanoparticles (NPs) is a modern field in preparing NPs using organic molecules as reducing agents. Recent studies aim to define strategies for the scalable production of NPs either from plants or using microorganisms that have multiple effects and can be used in agriculture, wastewater treatment, engineering, medicine, and the food industry [92].

Plants contain biomolecules and bioreducing agents such as enzymes, proteins, flavonoids, terpenoids, and cofactors that provide a versatile, economical, and eco-friendly method to fabricate metal NPs[92].

Pumpkin is an edible plant that can be included in the daily human diet and can improve human health by curing many diseases. The health benefits of pumpkin include anti-diabetic, anti-microbial, anti-carcinogenic, anti-ulcer, anti-viral, anti-tumor, anti-oxidant, and anti-inflammatory effects [93].

In this study, we focused on the green synthesis of nanocompounds (zinc oxide and silver) from pumpkin seeds and leaves by following a clean and environmentally friendly method. These compounds were characterized based on color change and UV-Vis, FTIR, EDX, and XRD spectroscopy, and their effectiveness against bacterial tissues was compared with some medications.

We also utilized the seed oil after extracting it to maximize its organic benefits before using the seeds in the green synthesis of nanomaterials. This way, we achieved both eco-friendly manufacturing and the full utilization of the organic mass of the used material.

In conclusion, through our research and work, we have demonstrated that it is possible to exploit the biomass from plants, bacteria, and viruses in the synthesis of nanomaterials, reducing the environmental damage caused by the use of toxic chemicals. These materials have various applications in several fields and hold promising future prospects.

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