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Submitted by:

HELAL Majda

SAOUDIA Wiem

Evaluation of Antimicrobial activity, Antioxidant activity, and Cytotoxicity of nanoparticles produced by green synthesis of silver nanoparticles from medicinal plants

By the jury composed of:

Dr. Masmoudi M	MCB	Univ Medea	President
Mr. Benhafri N	MCA	Univ Medea	Examiner
Mrs. Chaouati K	MCA	Univ Medea	Supervisor
Mrs. Hoceini A	MCA	Univ Medea	Co-Supervisor

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After a long study journey full of challenges, ordeals and fatigue, here I am today on the threshold of obtaining my master's degree.

I thank God, the most merciful, for giving me the ability to write and think, the strength to believe, the patience to follow the dream.

I dedicate this work,

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No dedication can express the love, esteem, devotion and respect that I have always had for you, my dear father. Thank you for your support, your encouragement, and the advice that followed me and allowed me to reach the end of the road. May Almighty God preserve you and grant your health, long life and happiness.

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Abstract

Green synthesis methods, utilizing plant extracts, offer a sustainable alternative to traditional methods for synthesizing silver nanoparticles (AgNPs), reducing harmful chemicals and ensuring effective nanoparticle production, attracting attention for biomedicine and environmental remediation. The thesis investigates the green synthesis of AgNPs using plant extracts from six species *Smyrnium olusatrum, Ferula communis*, *Teucrium polium, Peganum harmala, Atriplex halimus* and *Equisetum arvense* hypothesizing that these extracts will successfully mediate the process and exhibit significant antimicrobial and antioxidant and cytotoxicity properties, based on their bioactive compounds. AgNPs were synthesized using silver nitrate reduction using plant extracts. UV-visible and FTIR spectroscopy confirmed the biosynthesis, while well diffusion assays assessed antimicrobial properties. The DPPH free radical scavenging assay quantified antioxidant capacity. The study provides insights into AgNPs' structural properties. The nanoparticles confirming their role in reducing silver ions and stabilizing nanoparticles showed antimicrobial and antioxidant activity against bacterial and fungal pathogens, indicating potential applications in nanomedicine, agriculture, and environmental protection. The study highlights the potential of green synthesis methods in nanotechnology, suggesting their bioactivity could be used to develop antimicrobial agents and therapeutic applications. Future research should focus on optimizing synthesis conditions and scaling up production for industrial applications.

Keywords: green synthesis- AgNPs- antimicrobial activity- antioxidant activity- cytotoxicity- UV-visible- FTIR- DPPH

Résumé

Les méthodes de synthèse verte, utilisant des extraits de plantes, offrent une alternative durable aux méthodes traditionnelles de synthèse des nanoparticules d'argent (AgNPs), réduisant les produits chimiques nocifs et garantissant une production efficace de nanoparticules, attirant l'attention dans les domaines de la biomédecine et de la dépollution environnementale. La thèse étudie la synthèse verte des AgNPs à partir d'extraits de plantes de six espèces : *Smyrnium olusatrum, Ferula communis*, *Teucrium polium, Peganum harmala, Atriplex halimus* et *Equisetum arvense,* en postulant que ces extraits réussiront à médiatiser le processus et à présenter des propriétés antimicrobiennes, antioxydantes et cytotoxiques significatives, basées sur leurs composés bioactifs. Les AgNPs ont été synthétisées par réduction du nitrate d'argent à l'aide d'extraits de plantes. La spectroscopie UV-visible et FTIR a confirmé la biosynthèse, tandis que des essais de diffusion en milieu solide ont évalué les propriétés antimicrobiennes. L'essai de piégeage des radicaux libres DPPH a quantifié la capacité antioxydante. L'étude fournit des informations sur les propriétés structurelles des AgNPs. Les nanoparticules, confirmant leur rôle dans la réduction des ions argent et la stabilisation des nanoparticules, ont montré une activité antimicrobienne et antioxydante contre des agents pathogènes bactériens et fongiques, indiquant des applications potentielles en nanomédecine, agriculture et protection de l'environnement. L'étude souligne le potentiel des méthodes de synthèse verte en nanotechnologie, suggérant que leur bioactivité pourrait être utilisée pour développer des agents antimicrobiens et des applications thérapeutiques. Les recherches futures devraient se concentrer sur l'optimisation des conditions de synthèse et l'augmentation de la production pour des applications industrielles.

Mots clés : synthèse verte- AgNPs- activité antimicrobienne- activité antioxydante- cytotoxicité- UV-visible- FTIR- DPPH

الملخص

. تُعد طرق التخليق الأخضر، التي تعتمد على المستخلصات النباتية، بديلاً مستدامًا للطرق التقليدية في تخليق جسيمات الفضة النانوية (AgNPs)، حيث تقلل من استخدام المواد الكيميائية الضارة وتضمن إنتاجًا فعالًا للجسيمات النانوية، مما يجذب الانتباه في مجال الطب الحيوي ومعالجة البيئة. يناقش هذا البحث التخليق الأخضر لجسيمات الفضة النانوية باستخدام مستخلصات نباتية من ستة أنواع:,*Smyrnium olusatrum, Ferula communis* و ,*Teucrium polium, Peganum harmala* و *Atriplex halimus* و *Equisetum arvense*مفترضًا أن هذه المستخلصات ستنجح في تسهيل العملية وستظهر خصائص مضادة للميكروبات ومضادة للأكسدة وسامة للخلايا، بناءً على المركبات النشطة بيولوجيًا التي تحتويها. تم تخليق جسيمات الفضة النانوية باستخدام نترات الفضة مع المستخلصات النباتية. أكدت مطيافية الأشعة فوق البنفسجية وFTIR حدوث التخليق الحيوي، بينما تم تقييم الخصائص المضادة للميكروبات باستخدام اختبار نشر الأقراص. كما تم قياس قدرة الجسيمات النانوية على تحييد الجذور الحرة باستخدام اختبار DPPH. يوفر البحث رؤى حول الخصائص الهيكلية للجسيمات النانوية، حيث أكدت الجسيمات دورها في تقليل أيونات الفضة واستقرار الجسيمات النانوية. أظهرت الجسيمات النانوية نشاطًا مضادًا للميكروبات ومضادًا للأكسدة ضد مسببات الأمراض البكتيرية والفطرية، مما يشير إلى إمكانية استخدامها في الطب النانوي والزراعة وحماية البيئة. يبرز البحث إمكانيات طرق التخليق الأخضر في تقنية النانو، مقترحًا أن النشاط الحيوي لهذه الجسيمات يمكن استخدامه لتطوير عوامل مضادة للميكروبات وتطبيقات علاجية. وينبغي أن تركز الأبحاث المستقبلية على تحسين شروط التخليق وتوسيع الإنتاج لاستخدامات صناعية.

الكلمات المفتاحية: التوليف الأخضر- الجسيمات النانوية النانوية الفضية- النشاط المضاد للميكروبات- النشاط المضاد -للأكسدة- السمية الخلوية

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- ***A. halimus****: Atriplex halimus.*
- **AgNO3:** Silver nitrate.
- **AgNPs:** Silver Nanoparticles.
- **ATCC:** American Type Culture Collection.
- **DMSO:** Dimethyl Sulfoxide.
- **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl.
- ***E. arvense:*** *Equisetum arvense.*
- ***F. communis:*** *Ferula communis.*
- **FTIR:** Fourier-Transform Infrared.
- **LED:** Light Emitting Diodes.
- **NPs :** Nanoparticules.
- ***P. harmala:*** *Peganum harmala.*
- ***S. olusatrum:*** *Smyrnium olusatrum.*
- **SEM:** Scanning Electron Microscopy.
- **SPR:** Surface Plasmon Resonance.
- ***T. polium:*** *Teucrium polium.*
- **TSA:** Tryptic Soy Agar.
- **UV–Vis:** Ultra Violet Visible
- **XRD:** X-ray Diffraction.
- **ZOI**: Zone of Inhibition.
- **EO:** Essential Oil

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Introduction

Introduction

In recent years, the exploration of green synthesis methods for nanoparticle production has gained significant traction in the fields of nanotechnology, medicine, and environmental science. This burgeoning interest stems from the inherent advantages of green synthesis, which offers a sustainable and eco-friendly alternative to conventional chemical methods while harnessing the bioactive properties of natural resources. Among the various types of nanoparticles synthesized through green routes, silver nanoparticles (AgNPs) have emerged as particularly promising candidates due to their unique physicochemical properties and versatile applications.

The utilization of plant extracts in nanoparticle synthesis not only provides a renewable and cost-effective approach but also capitalizes on the rich phytochemical composition of medicinal plants, which possess inherent therapeutic properties.

This thesis endeavors to investigate the antimicrobial activity, antioxidant activity, and cytotoxicity of nanoparticles produced through green synthesis methods using the aerial parts of *S. olusatrum, F. communis, T. polium, P. harmala, A. halimus,* and *E. arvense* plant extracts.

This work is divided into three chapters:

- **The first chapter** is a bibliographical study of nanoparticles, including their synthesis, characterization, and applications. It also includes the botanical presentation and chemical composition of the species studied: *Smyrnium olusatrum, Ferula communis, Teucrium polium, Peganum harmala, Atriplex halimus,* and *Equisetum arvense,* along with their therapeutic value in traditional medicine.
- **The second chapter** covers the materials and methods used to synthesize silver nanoparticles, the characterization of these nanoparticles using UV-Visible spectroscopy and Fourier Transform Infrared (FTIR) spectroscopy, and the evaluation of their biological activity.
- **The third chapter** discusses and presents the results obtained during the course of this work. The dissertation ends with a conclusion.

Finally, a general conclusion summarizes the main results obtained during the course of this thesis, as well as the prospects for continuing this study.

Chapiter I: Literature Review

I.1.1 Nanoscience

In recent years, a small word with great potential has quickly made its way into the world's consciousness; the word is nano. In recent decades, research into nanomaterials has advanced dramatically. The prefix nano- means one billionth; for example, a nanometer is one billionth of a meter **(Poonia et al., 2022).**

Nanoscience is the study of structures ranging from 1 nm to 100 nm in size. It is concerned with materials and frameworks whose structure segments exhibit novel and significantly enhanced chemical, biological, and physical properties, phenomena, and succession due to their nanoscale size **(Pal & Zaheer, 2022).**

The nanoworld plays an important role between a solid and an atom, from the massive particle or the little strong item to the solid connection, surface, and volume. the nano planet has been around for a long time, and it is now up to scientific professionals to investigate particle qualities and configuration **(Poonia et al., 2022).**

I.1.2 Nanotechnology

Although the scientific community is interested in nanoscience, nanotechnology dominates discussions and definitions. As such, it is a broad phrase that demonstrates the heights of man's insatiable desire for knowledge with practical applications **(Nasrollahzadeh et al., 2019).**

Nanotechnology can be described as the science and engineering involved in the design, synthesis, characterization and application of materials and devices whose smallest functional organization in at least one dimension is on the nanoscale scale **(Shrestha, 2012).**

This rapid advancement in nanoscience demonstrates that nano-scale production will soon be integrated into practically every field of science and technology. Nanotechnology has uses in a variety of industries, including agriculture, food, cosmetics, medicine, healthcare, automotive, oil and gas, chemical, and mechanical **(Malik et al., 2023).**

I.1.3 Nanomaterials:

Materials containing fundamental structural elements, fibers, grains, particles, or other constituent parts smaller than 100 nm in at least one dimension are known as nanomaterials **(Zhang & Webster.,2009).** Nanomaterials are not just another stage of miniaturization; rather, they represent a completely new field that sits between the size of bulk materials and that of atomic and quantum processes. Rather than acting like conventional bulk materials, some material properties at the nanomaterial level are influenced by the rules of atomic physics

(Pokropivny et al.,2007). NMs are useful in a variety of domains, including chemical engineering, biology, electronics, medicine and pharmacology because of their unique and adaptable physicochemical characteristics **(Sanzari et al,2019).**

I.1.4 Nanoparticles

The term "nanoparticles" refers to particulated nanostructures of varying shapes that have at least one dimension on the "nano" scale, which is less than 100 nanometers. Shapes may be spherical, filamentous, tubular, or irregular. They can be made from a variety of materials **(Rada, 2014).**

Nanoparticles are effective instruments for imaging, diagnosis, and therapy, owing to their characteristics. They have the same magnitude as antibodies, membrane receptors, nucleic acids, and proteins. They also have biomimetic properties that, along with miniaturization, allow them to transform their applications (**Miziak et al., 2020).**

The green nanoparticles are eco-friendly, energy-efficient, reduce waste, and reduce greenhouse gas emissions. These nanoparticles have several advantages because of their unique size and shape properties. Green synthesis of nanoparticles removes harmful chemicals and pollutants from the environment and does not disturb the ecosystem while also conserving natural resources **(Rasal et al., 2023).**

I.1.4.1 Classification of Nanoparticles

Nanoparticles (NPs) are classed according to their form, size, and physical/chemical properties. They are mainly classified into organic, inorganic and carbon-based NPs.

1) Organic Nanoparticles

Organic nanoparticles can be defined as solid particles present in the diameter range of 10 nm to 1 m, mainly composed of organic compounds (lipids or polymers). Liposomes, dendrimers, carbon nanomaterials, and polymeric micelles are examples of organic nanoparticles **(Khan & Sharma, 2022)**.

Because organic nanoparticles have these features, they are an ideal option for pharmaceutical transportation. Additionally, they are commonly utilized in the process of transporting target drugs to their targeted areas **(Alshammari et al., 2023)**.

Organic nanoparticles are environmentally friendly, biodegradable, non-toxic, cost-effective, and ideal for biomedical applications **(Kumari & Sarkar, 2021).**

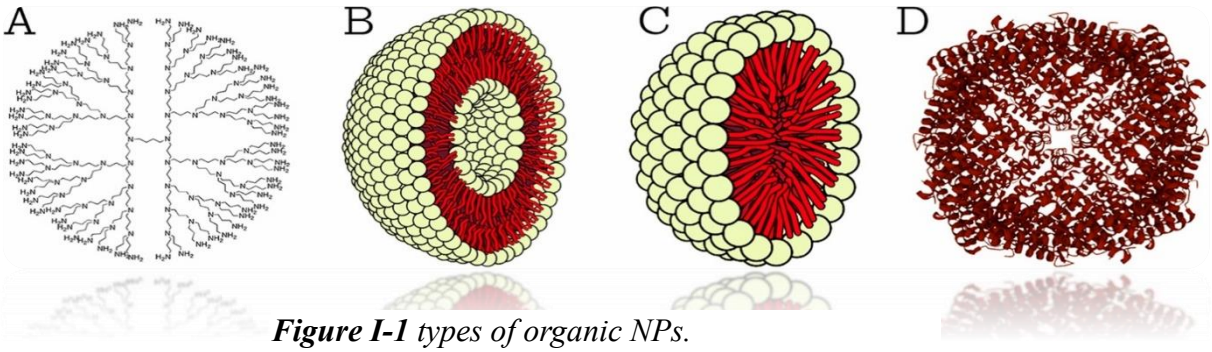


Figure I-1 *types of organic NPs.*

A Dendrimers; **B** liposomes; **C** micelles; and **D** ferritin **(Joudeh & Linke, 2022).**

2) Inorganic Nanoparticles

Inorganic nanoparticles, which lack carbon, are hydrophilic, non-toxic, and biocompatible with living systems. They are also more stable than organic nanoparticles **(Alshammari et al., 2023).**

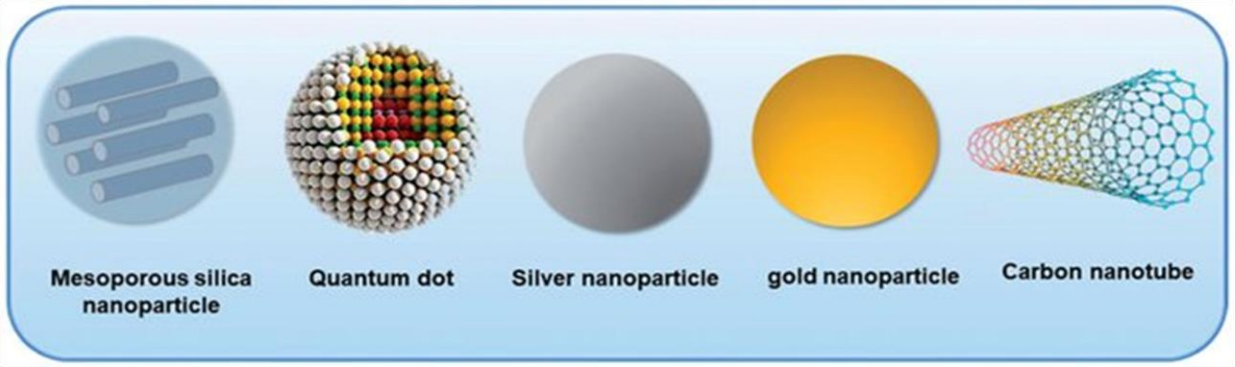


Figure I-2 *Different types of inorganic nanoparticles.*

(Li et al., 2019).

a) Metal Based Nanoparticles

Metal-based nanoparticles can be synthesized using destructive or constructive processes to achieve nanometric sizes **(Ealias & Saravanakumar, 2017).** These nanoparticles have uses in research fields, detection and imaging of biomolecules, and environmental and bioanalytical applications **(J et al., 2023).**

Metal-based nanoparticles typically range in size from 10 to 100 nm. They exist in numerous shapes, including spherical and cylindrical. These materials show particular characteristics, including a high surface area-to-volume ratio, pore size, surface charge and density, crystalline and amorphous morphologies, high reactivity, and sensitivity to environmental elements like air, moisture, heat, and sunlight **(Kumari & Sarkar, 2021).** Metallic nanoparticles have unique optoelectronic capabilities due to surface Plasmon. Their unique features make them suitable for a variety of research areas **(Khan & Sharma, 2022).**

Nanoparticles can be synthesized from almost any metal. Metals often utilized in nanoparticle synthesis include aluminum (Al), cadmium (Cd), cobalt (Co), copper (Cu), gold (Au), iron (Fe), lead (Pb), silver (Ag), and zinc (Zn) **(Ealias & Saravanakumar, 2017).**

b) Metal Oxides Based Nanoparticles

Metal oxide nanoparticles, also called metal oxide nanomaterials, consist of positive metallic ions and negative oxygen ions **(Mekuye & Abera, 2023).** Metal oxide nanoparticles are utilized in the creation of fuel cells, piezoelectric devices, and catalysts. **(Khan & Sharma, 2022).** Commonly synthesized materials include aluminum oxide (Al2O3), cerium oxide (CeO2), iron oxide (Fe2O3), magnetite (Fe3O4), silicon dioxide (SiO2), titanium oxide (TiO2), and zinc oxide (ZnO). These nanoparticles possess unique properties when compared to their metal counterparts **(Ealias & Saravanakumar, 2017).**

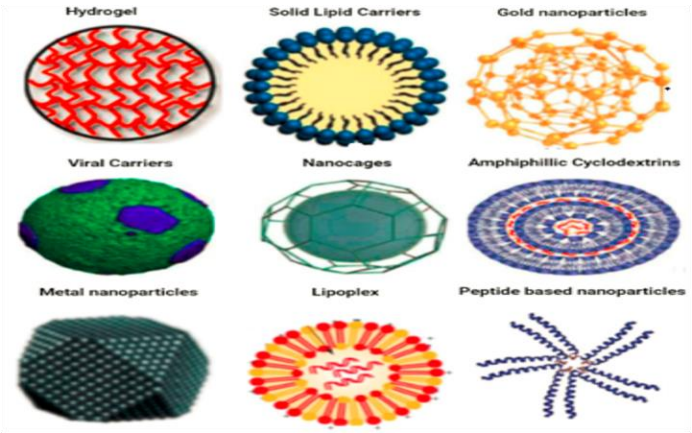


Figure I-3 *Metal nanomaterials*

(Alshammari et al., 2023).

3) Carbon Based Nanoparticles

Carbon-based nanoparticles are highly fashionable in the fields of nanoscience, material science, engineering, and technology **(Khan & Sharma, 2022).**

This class includes NPs generated completely from carbon. Examples of this class include fullerenes, carbon black NPs, and carbon quantum dots. Fullerenes are carbon molecules with a closed, symmetrical form. C60 fullerenes feature 60 carbon atoms grouped in a soccer ball shape; however, additional varieties, including C70 and C540 fullerenes, have also been identified. Carbon black nanoparticles are clusters of tightly bonded spherical particles, similar to grapes. Carbon quantum dots are discrete, quasi-spherical carbon nanoparticles with diameters under 10 nm. Carbon-based NPs combine the distinct features of sp2-hybridized carbon bonds with unique physicochemical properties at the nanoscale **(Joudeh & Linke, 2022).**

Because of their unique physical, chemical, and mechanical properties, these materials are not only used in their pristine form but also in nanocomposites for many commercial applications **(J et al., 2023),** such as fillers, efficient gas adsorbents for environmental remediation, and as support medium for different inorganic and organic catalysts **(Khan et al., 2019).**

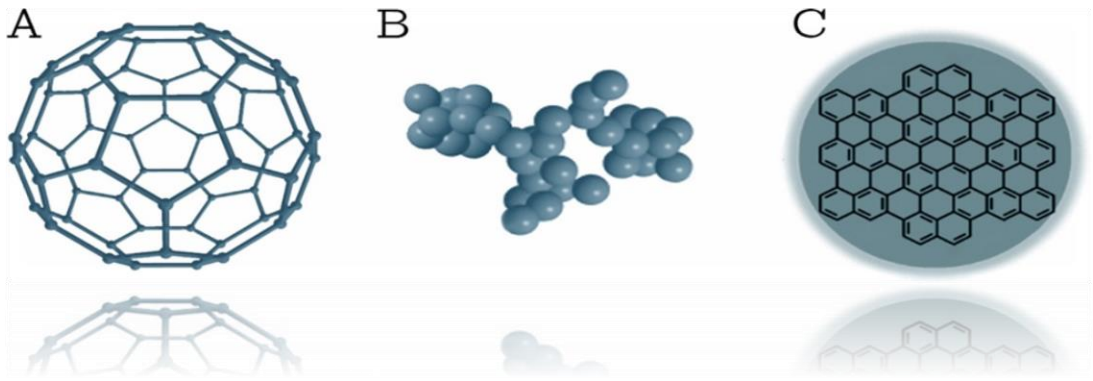


Figure I-4 *Different types of carbon-based NPs.*

A C_{60} fullerene; **B** carbon black NPs; and **C** carbon quantum dots **(Joudeh & Linke, 2022).**

I.1.4.2 Properties of Nanoparticles

a) Physical

The physical properties of nanoparticles include color, light penetration, absorption, reflection, and UV absorption in solution or coating. Mechanical qualities including elasticity, ductility, tensile strength, and flexibility are crucial for their use. Other features such as

hydrophilicity, hydrophobicity, suspension, diffusion, and settling have become common in modern products. Nanoparticles' magnetic and electrical properties, including conductivity, semi-conductivity, and resistance, make them suitable for use in electronics and renewable energy applications that require thermal conductivity **(Ealias & Saravanakumar, 2017).**

b) Chemical

Nanoparticles' chemical properties, including reactivity with targets, stability, susceptibility to moisture, oxygen, heat, and light, define their applicability. Nanoparticles possess antibacterial, antifungal, disinfectant, and toxicological characteristics, making them suitable for biomedical and environmental applications. The corrosive, anti-corrosive, oxidation, reduction, and flammability properties of nanoparticles determine their appropriate application **(Ealias & Saravanakumar, 2017).**

I.1.4.3 Silver Nanoparticles:

Silver, which has the atomic number 47 and the symbol Ag, is a shining, highly ductile, and malleable element that is marginally tougher than gold **(Keat et al.,2015).** Silver is an inorganic antibacterial agent that is harmless and nontoxic, and it can eradicate approximately 650 different types of microbes that cause diseases **(Ibrahim., 2015).** Scientists have been interested in silver among metal nanoparticles because of its broad utility in the creation of novel nanoscale technologies in the fields of electronics, material sciences, and medicine **(Salar et al.,2015).**

silver nanoparticles are valuable materials that have been the subject of much research. **(Ibrahim., 2015).** silver nanoparticles work best against bacteria, viruses, and other eukaryotic microorganisms at low concentrations and without causing any negative side effects. They are also benign to humans. Thus, in the fields of nanotechnology and nanomedicine, silver nanoparticles are crucial **(Salar et al.,2015).**

Physical and chemical methods are mostly used to create silver nanoparticles **(Phongtongpasuk et al.,2016).** Different forms of silver nanoparticles have uses in optics, biological detection, and drug delivery, as well as size- and shape-sensitive surface plasmon resonance bands **(Mansouri & Ghader.,2009).**

I.1.4.3.1 Synthesis Of Silver Nanoparticles:

Different techniques categorized as top-down or bottom-up procedures are applied in the synthesis of metallic nanoparticles. Top-down methods create stable AgNPs by synthesizing nanoparticles from solid or aerosolized metallic silver all the way down to the nanoscale. Physical techniques including sputtering, laser ablation, and ball milling fall under this category. Conversely, bottom-up approaches involve stabilizing and nano structuring silver atoms using various techniques to create nanoparticles. Chemical and biological processes are utilized in bottom-up ways to create nanoparticles **(Bruna et al.,2021)**.

a) Synthesis Of AgNPs Using Physical Methods:

Large amounts of nanoparticles can be obtained by physical processes, which can also provide highly pure nanoparticles depending on the approach utilized **(Bruna et al.,2021)**. The physical method of creating metallic nanoparticles usually involves evaporation-condensation, which can be done in a tube furnace at room pressure **(Tran & Le ,2018).**

AgNPs with a nearly narrow size distribution are often produced using the physical synthesis of AgNPs, which makes use of physical energy. AgNPs samples can be produced in large quantities using the physical technique in a single operation. Additionally, this is the most practical way to make AgNPs powder. Primary costs for equipment investment should be taken into account, nevertheless **(Natsuki et al,2015).**

b) Synthesis of AgNPs using Chemical Methods:

Chemical reduction using both inorganic and organic reducing agents is the most widely used method for producing silver nanoparticles **(Abbasi,2016).** Three primary ingredients are often used in this process: metal precursors, reducing agents, and stabilizing/capping agents. In essence, there are two steps involved in the reduction of silver salts: nucleation and subsequent growth **(Zhang et al.,2016).**

Furthermore, it is shown that these steps have a significant influence on the size and shape of the produced AgNPs **(Natsuki et al.,2015).**as a result, they are seen as scalable and easy to execute. However, by mandating the use of these agents, hazardous or polluting waste may be produced as poisonous reagents or solvents are frequently added **(Bruna et al.,2021)**.

c) **Synthesis of AgNPs using Biological Methods (Green Synthesis):**

Many researchers have argued that, when compared to chemical and physical synthesis approaches, the green synthesis approach represents the right decision **(Dhaka et al., 2023).** Green synthesis is a biocompatible and 'eco-friendly' technique that is typically carried out with

the aid of microorganisms, yeast, plant extracts, capping agents, or stabilizers **(Ahmad et al., 2019).**

- **Green syntheses of silver nanoparticles using plant extracts:**

The production of nanoparticles has emerged as one of the most exciting scientific areas of the last several years, and interest in employing plant extracts to make NPs has increased **(Amor et al.,2023).** Due to its quick, safe, non-pathogenic, and affordable procedure; it also offers a one-step method for the biosynthetic processes and contains significant phytochemicals that are utilized as reducing agents in the AgNPs synthesis **(Abdelghany et al.,2018).**

Due to their abundance and accessibility, plant components such leaves, fruits, stems, roots, seeds, and flowers are employed in the synthesis of AgNPs **(Tariq et al.,2022).**

Plant extract contains a variety of active biomolecules that are essential to the biological reduction and stability of silver ions, including proteins, phenolic acids, sugars, terpenoids, alkaloids, and polyphenols **(Akintelu et al.,2020).**

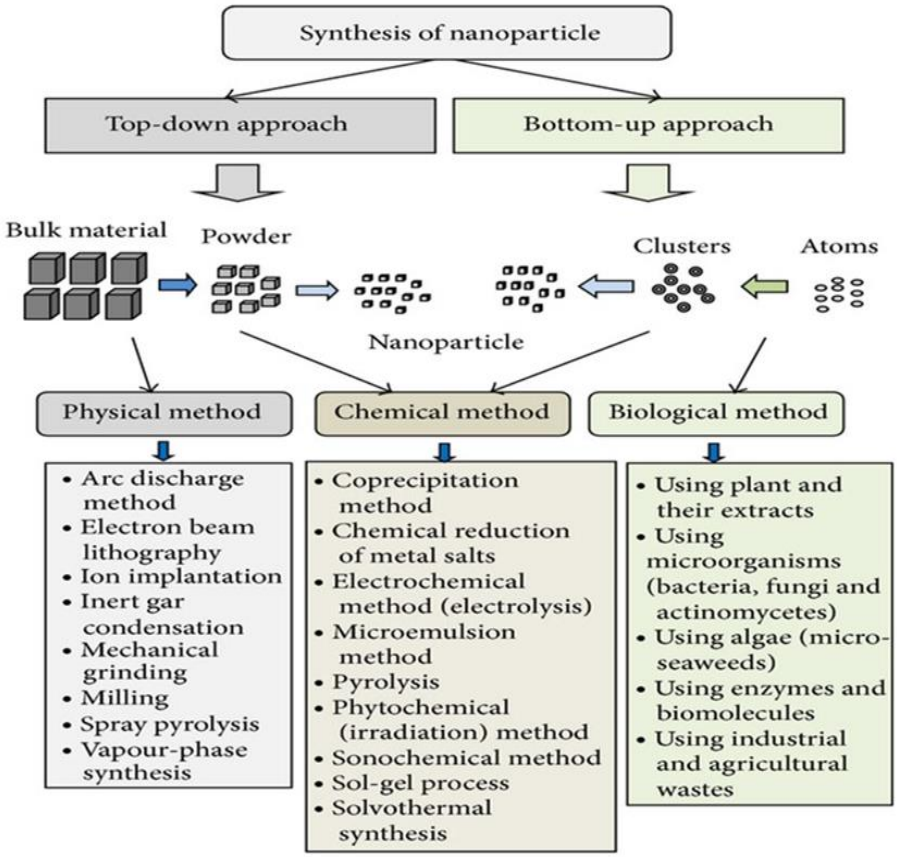


Figure I-5 *Different methods for synthesizing nanoparticles.*

(Patra & Baek.,2014).

I.1.4.3.2 Characterizations of silver nanoparticles

To describe the nature, size, shape, distribution, stability or aggregation state, morphology, elemental content, and dispersity of nanoparticles, a variety of analytical and spectroscopic techniques are employed **(Dawadi et al., 2021).** Some of these tools are:

I.1.4.3.2.1 UV–Visible Spectroscopy:

A quick, simple, useful, careful, and focused way to characterize various NP kinds is UV–vis spectroscopy. It works especially well for the initial characterization of produced nanoparticles. The UV–Visible spectrum can be used to comprehend how changes in a parameter, such as temperature, concentration, medium pH, etc., affect particle size. **(Akhter et al., 2024).** UV-Visible spectroscopy is utilized to quantify the plasmon absorbance that gives rise to the distinctive colors seen during AgNPs production **(Akintelu et al.,2020).**

The conducting electrons in metal nanoparticles' outermost orbitals collectively oscillate in resonance with specific wavelengths when they contact with an electromagnetic field; this phenomenon is known as surface plasmon resonance (SPR) **(Vanlalveni et al., 2021).** Surface plasmon resonance (SPR) allows AgNPs to exhibit strong absorption in the visible spectrum, with a maximum absorption in the 400–500 nm range **(Bamal et al., 2021).**

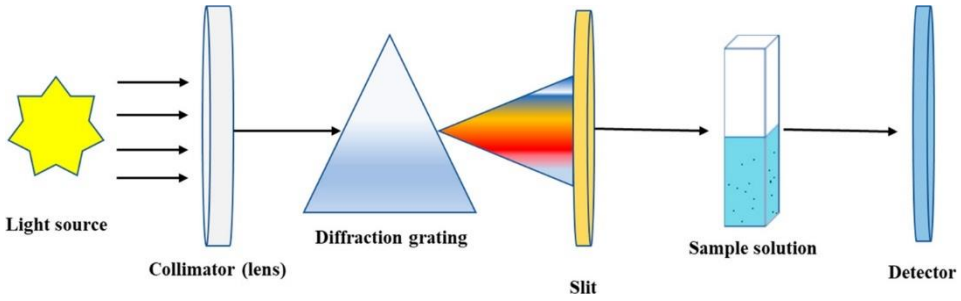


Figure I-6 *Schematic diagram of UV−visible spectrophotometer.*

(Sharma et al.,2022).

I.1.4.3.2.2 Fourier-Transform Infrared (FTIR) Spectroscopic Technique:

FTIR spectroscopy is a practical, inexpensive, simple, and non-intrusive way to determine the role of biological molecules in the synthesis for stabilizing green generated AgNPs **(Aldakheel et al., 2023)** and reducing Ag+ ions. **(Akhter et al., 2024).**

When IR radiation is sent through a sample during FTIR analysis, some of the radiation is absorbed by the sample and some of it goes through it. The resulting spectrum demonstrates

transmittance and absorbance by leaving a molecular fingerprint on the sample that serves as a representation of its identity **(Bamal et al., 2021).**

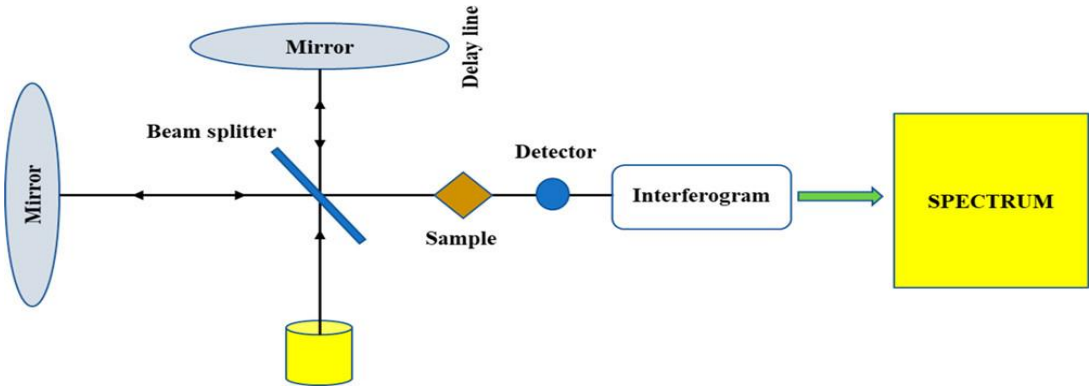


Figure I-7 *Schematic diagram of FTIR.*

(Sharma et al., 2022).

I.1.4.3.2.3 X-ray diffraction (XRD):

Analysis of the atomic structures of materials is frequently done using X-ray diffraction (XRD). Determining the qualitative and quantitative characteristics of materials is a useful application of this technology. XRD analyses were utilized in the context of nanomaterials to determine and validate the dimensions and composition of crystalline materials **(Aldakheel et al., 2023).**

An X-ray beam is sent onto the crystal during an XRD study, and the atoms scatter the beam, creating diffraction patterns. According to Bragg's law, the interference of dispersed X-rays can be utilized to determine a variety of properties of the crystal or polycrystalline material. By determining the particles' oxidation state over time, XRD verifies the NPs' crystalline nature. Typically, XRD results are expressed in Angstroms (1 Å = 0.1 nm) **(Bamal et al., 2021).**

I.1.4.3.2.4 Scanning Electron Microscope (SEM):

The surface appearance, size, aggregation, and distribution of nanoparticles are characterized by scanning electron microscopy (SEM), which uses electron beams as imaging probes It is used to measure the size of nanoparticles and produces high-resolution images at the nanoscale scale, highlighting the function of biomatrix or AgNPs encapsulation **(Dawadi et al., 2021).** Synthesized AgNPs are positioned above a stub intended for SEM examination. Typically, the stub is composed of tiny copper cylinders with a diameter of 1 mm. The stub has a single layer of carbon. The stub is fastened to a holder when the synthesized AgNPs is

deposited on the carbon substance. Up to seven samples may be taken by the holder **(Akintelu et al.,2020).**

Application of Nanoparticles:

Table I-1 *the applications of nanoparticles in various fields.*

(Hano & Abbasi, 2021), (Reddy et al., 2020), (Ijaz et al., 2020).

Field	Application
Medicine	(antimicrobial, antiparasitic, antiproliferative, pro-apoptotic, pro- or anti-oxidative depending on the context, anti-inflammatory activities, etc.)
Environment	(destroy organic pollutants in air, purify ground water by removing carbon tetrachloride, breakdown oil in the biodegradable compounds, etc.)
Materials and Manufacturing	(provide the barrier to gases (or) humidity, protect textiles, wood, plastic from exposure to uv rays, kill bacteria that produce bad odor to clothes, etc.)
Energy and Electronics	(the light emitting diodes (LED) of modern displays, production of energy from splitting of electrochemical water and photo electrochemical (PEC), Generate renewable resources, energy storage, etc.)
Cosmetics	(sunscreen, anti-aging, hair growth, bioactive compounds delivery, nanoemulsion, etc.)
Food	(processing, storage, and packaging processes), in bioengineering (biocatalysts, photocatalysts, biosensors, etc.)
Agriculture	(precision farming with controlled release of agrochemicals, target-specific delivery of biomolecules, more efficient nutrients absorption, detection and control of plant diseases, etc.)

I.2 Presentation of the plants studied

I.2.1 Smyrnium olusatrum L

I.2.1.1 Botanical Description

Smyrnium olusatrum L, known as Alexanders or wild Celery **(Saida et al.,2020)** and in French as ‘‘Maceron’’ **(Maggi et al.,2012).** The germination of seeds occurs intermittently from October to March, with significant bursts of germination occurring in late October and January once again. It peaks in May/June and peaks in October **(Agro & Shattock. 1999).**

A stout, glabrous herb with a celery scent, semelparous, and facultative biennial. It has a thickened taproot (50–60 cm) with finer lateral roots near the surface. It features one or more solid stems, 50–150 cm tall, which become hollow at fruiting; upper branches are often opposite, with deeper furrows on the upper stems. The dark green, glossy leaves are 2–3-pinnate or -ternate, with lobes 25–80 mm, ovate to rhombic, serrate or lobed, and all stalked. Stem leaves have expanded sheathing petioles. Flowers are approximately 1.5 mm, yellow to yellow-green, actinomorphic, and shortly pedicellate. Sepals are small with a stylopodium formed from elongated style bases. The fruit is a black schizocarp, 7–8 mm thick, comprising two single-seeded, broadly ovoid, laterally compressed mericarps. Each mericarp has three sharp ridges and numerous oil-exuding veins **(Randall,2003).**



Figure I-8 *Morphological Features of Smyrnium olusatrum*

Leaves **[1]**, *Flowers* **[2],** *and Fruits* **[3].**

I.2.1.2 Botanical classification

The taxonomy of *Smyrnium olusatrum* according to **Kew Science - Plants of the World Online'.**

Table I-2 *Scientific classification of Smyrnium olusatrum.*

Smyrnium olusatrum L	
Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Subclass	Magnoliidae
Order	Apiales
Family	*Apiaceae*
Genus	*Smyrnium*
Species	*Olusatrum*

I.2.1.3 Geographical distribution:

The species' distribution in Europe spans from nations bordering the Mediterranean to Scotland, the region's northernmost point **(Tsasi et al.,2016**). in Algeria, Common around country houses of Algiers **(Munby,1847).** The plant grows in various habitats, including hedgerows, hedged banks, sea cliffs, quarries, and railway embankments, ranging from sea level to an altitude of 800 meters **(Maggi et al., 2012)**.

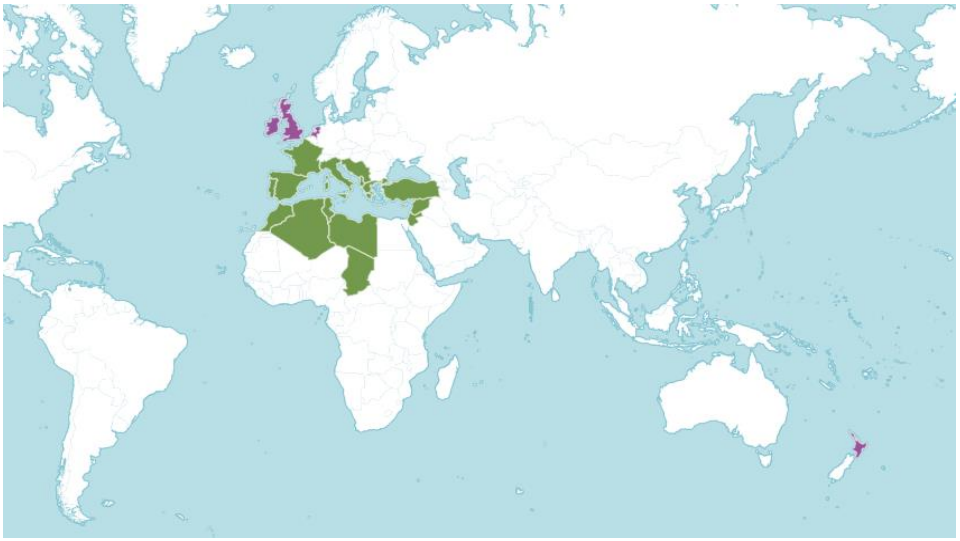


Figure I-9 *Distribution of Pistacia Smyrnium olusatrum in the world.* **[4]**

I.2.1.4 Phytochemical composition

A rich mixture of bioactive chemicals can be seen in *Smyrnium olusatrum* phytochemical profile. Among them are essential oils **(Kavallieratos et al.,2004).** The leaf, flower, and fruit of *S. olusatrum* were found to have a significant percentage of Furano sesquiterpenes in their essential oil composition **(Messaoud et al.,2013).** Furthermore, a number of flavonoids, acetylenic derivatives, and sesquiterpene lactones have been identified from *S. olusatrum* **(Tsasi et al.,2016**). From the Algerian species *Smyrnium olusatrum L*. and the genus Smyrnium (Apiaceae), four flavonoids 1–4 are reported for the first time **(Bencheraiet et al.,2011).** Fruits provide 51.1% carbohydrates, 10.4% crude fiber, 7.1% fat, and 15.4% protein **(Spinozzi et al.,2024).**

I.2.1.5 Traditional and medicinal uses

Traditional medicine uses the leaves of *Smyrnium olusatrum* as an antiscorbutic, the fruits as an antiasthma-tic and stomachic, and the roots for its laxative, diuretic, fragrant, and appetite-stimulating qualities **(Messaoud et al.,2013).** The herb was employed in Isle of Man veterinary treatment to cure mouth sores caused by scurvy in cattle. Seafarers in Scotland used it to stop scurvy and clear their blood **(Maggi et al., 2012)**. Numerous studies have demonstrated the beneficial effects of *S. olusatrum* as an insecticidal and antileishmanial agent. Recently, the isofuranodiene that was isolated from the EO of *S. olusatrum* demonstrated potential action as an acaricide, an antifungal, and a mosquito larvicidal agent against *Culex quinquefasciatus*. Isofuranodiene, which was recently discovered from *S. olusatrum*, has the ability to inhibit both Trypanosoma brucei and DHFR from *Escherichia coli*. According to one study, in rats with liver injury induced by a chemical, isofuranodiene was found to have hepatoprotective properties. **(Saida et al.,2020)**. Essential oils, particularly Isofuranodiene, showed cytotoxic effects on human tumor cell lines, with radical scavenging activity and antioxidant properties **(Quassinti et al.,2013).**

I.2.2 Ferula communis L

I.2.2.1 Botanical Description

Ferula communis L. (giant fennel) is a perennial wild plant in the Apiaceae family that grows naturally on the wasteland of the Mediterranean and Central Asia areas. It blooms in March and April and becomes dormant in early summer in dry climate conditions **(Distefano et al., 2024).**

It has deep tap roots, clumps of leaves that emerge in the winter and early spring, and a tall flowering stem (up to 3 meters) with numerous yellow flowers gathered in umbels. *Ferula communis* produces a large number of seeds, which vary in size and absolute weight depending on where the plant grows. They typically measure 3 to 6 mm in length, have an elongated and cylindrical shape, and come in a variety of colors, but most usually appear dark brown or black. *Ferula* seeds may be dormant, with a hard seed coat that must be scarified or stratified to improve germination rates **(Distefano et al., 2024).**



Figure I-10 *Morphological Features of F. communis*

a. *habitat* ***b.*** *whole plant* ***c.*** *umbels* ***d.*** *flowers* **(Maslo, 2020).**

I.2.2.2 Botanical classification

Scientific classification of *Ferula communis L* **[5].**

Table I-3 *Scientific classification of Ferula communis*

Ferula communis L	
Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Subclass	Magnoliidae
Order	Apiales
Family	Apiaceae
Genus	*Ferula*
Species	*Communis*

I.2.2.3 Geographical Distribution

F. communis is native to southern and central Europe, the Mediterranean Sea region, the Middle East, the Canary Islands, the Balkan Peninsula, the Apennine Peninsula, the Iberian Peninsula, and North Africa. The plant is common throughout Europe, ranging from Portugal to Greece, Spain, France, Italy, Croatia, Montenegro, Albania, and Cyprus. The plant was introduced in Great Britain **(Maslo, 2020).**

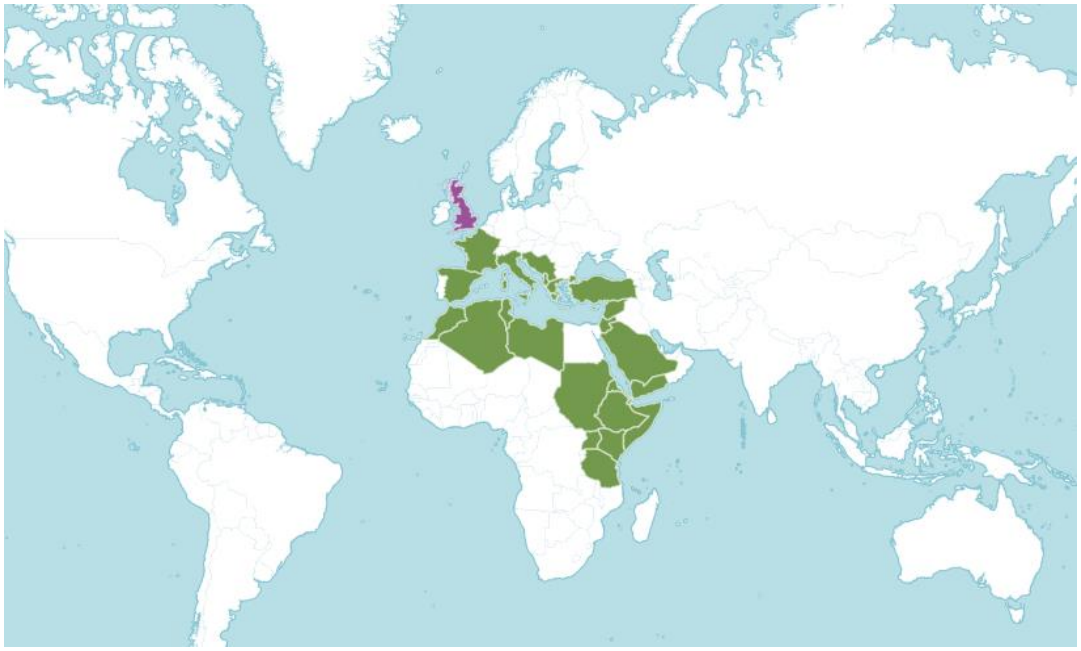


Figure I-11 *Map presenting the distribution pattern of Ferula spp. throughout the globe*

I.2.2.4 Phytochemical Composition

Despite its name, the plant is not a true fennel but rather a member of the other genus, Foeniculum. Ferulic acid, a phenolic component isolated from giant fennel, is named after the plant's Latin name.

Two chemotypes of *F. communis L*. have been identified, each with distinct biological effects. The poisonous chemotype is primarily composed of prenyl coumarins such as ferulenol and related substances that cause ferulosis (a deadly haemorrhagic condition primarily affecting goats, sheep, cattle, and horses) and its toxicity. The alternative chemotype is non-poisonous and has daucane esters. These two chemotypes are genetically distinct and occur in different geographical locations. Non-poisonous plants are typically utilized as hormonal plants due to their phytoestrogen concentration, whereas poisonous plants can induce intoxication and death. **(Akaberi et al., 2015).** More recently, it has been discovered that the difference between toxic and nonpoisonous *F. communis* chemotypes is related to the presence of various chemical makers such as acetylated ferulenol-oxy-ferulenol and the different enzymatic behavior of polyphenol oxidases **(Maggi et al., 2015).** In general, polyphenols exhibit strong antioxidant properties against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases. Furthermore, it has been shown that the hexane fraction of roots exhibits significant antioxidant-anticholinesterase activity **(Rahali et al., 2021).**

I.2.2.5 Traditional and medicinal uses

F. communisis is commonly used in traditional medicine to treat skin problems, rheumatism, helminthic infections, joint pain, infertility, hysteria, and dysentery. This species additionally comes with an antispasmodic, vermifuge, aphrodisiac effect and can be used as a depilatory, emetic, diuretic and analgesic **(Neghliz & Benabdelkader, 2021).**

Several studies have shown that the Ferula genus contains anti-cancer, anti-diabetic, antibacterial, anti-ulcerative, and anti-inflammatory properties. These biological qualities are mostly due to the quantity of active phytochemicals, which include sesquiterpene derivatives, organic acid glycosides, steroidal esters, and sulfur-containing compounds **(Rahali et al., 2021)**

According to legend, in ancient Rome, assa-foetida was stored in jars with pine nuts and used to provide pleasant and distinct smells and odors to various delicacies such as vegetables, barbecued meats, meatballs, pickles, and other cooked dishes. *F. communis L*. (Narthex) was used by Prometheus of Greek mythology to set fire to the ground where this

species thrived. Despite its extreme toxicity to humans and animals, certain chemotypes of this plant have been used to treat skin diseases, diarrhea, and fever **(Mohammadhosseini et al., 2019).**

I.2.3 Teucrium polium

I.2.3.1 Botanical Description

Teucrium polium, commonly known as felty germander **(Bahramikia & Yazdanparast .2012)**, as golden germander **(Albadr et al.,2022)**, or Jaadah as it is known in Algeria **(Belmekki et al.,2013)**. comprises around 340 species, with 20 of them found in Algeria. make up the Teucrium genus in the Lamiaceae family **(Kabouche et al.,2007).**

T. polium is a fragrant, perennial herbaceous plant with a woody base. The plant's stalks are pubescent and spherical. The plant has an upright stem that can reach a maximum length of 10 to 35 centimeters. The plant has a maximum growth potential of 30–50 cm. Anatomically, the upper body appears fully branched. The plant has leaves, that measure 2 cm in length and 4 mm in width. According to certain reports, the leaves have a length of one to three centimeters. The margins in the higher portions are extended and crenate, while the lower regions have unbroken, folded borders. From April to August, it blooms in a variety of colors, from pink to yellow. According to several reports, the flower's color can range from pale cream to white in various tones. It is well known that bruised leaves emit a nice, fragrant scent. Essential oil is reported to be present in the plant's flowering branches and leaves. The fruits are lattice-topped, light- to dark-brown nutlets **(Tepe et al.,2021).**



Figure I-12 *Morphological Features of T. polium*

I.2.3.2 Botanical classification

The taxonomy of *Teucrium polium* according to **(Rahmouni et al.,2021).**

Table I-4 *Scientific classification of Teucrium polium*

Teucrium polium L	
Kingdom	Plantae
Phylum	Spermatophyta
Class	Dicotyledones
Order	Lamiale
Family	Lamiaceae
Genus	*Teucrium*
Species	*polium*

I.2.3.3 Geographical distribution

The primary habitats of this plant are stony beaches, sand dunes, and arid regions. Geographically speaking, *T. polium* is found in Europe, North Africa, and certain regions of Asia. It is a pluri-regional plant **(Bardsiri et al.,2022).** but mostly in the area around the Mediterranean **(Bukhari et al.,2015).** Numerous species are dispersed throughout South America, the mountainous tropical Northeast, South Africa, and Australia **(Sadeghi et al.,2022).**12 subspecies of *Teucrium polium* were identified in North Algeria; they were common in the Tell Mountains **(Djabou et al.,2012).**



Figure I-13 *Distribution of Pistacia T. polium in the world.*

I.2.3.4 Phytochemical composition

Many chemicals, including terpenoids, flavonoids, and iridoids, have been identified in *T. polium* by phytochemical studies **(Bahramikia & Yazdanparast .2012).** Minerals that are essential to human health, such as calcium, potassium, iron, sodium, magnesium, manganese, and zinc, are abundant in *Teucrium polium L*. Furthermore, as demonstrated by in silico studies, it had encouraging antioxidant, antihyperglycemic, anti-Alzheimer, and anti-inflammatory properties, all of which could be linked to its phenolic components **(Benchikha et al.,2022).**

I.2.3.5 Traditional and medicinal uses

In traditional medicine, *T. polium* has been utilized. Diuretic, carminative, antibacterial, antifungal, antispasmodic, antirheumatic, and tonic qualities are all present in this plant. Additionally, it has been utilized as a hypoglycemic drug and has hypoglycemic effects in people with diabetes. When combined with standard blood sugar-controlling drugs, daily ingestion of this plant helps to maintain normal blood sugar levels. Moreover, *T. polium* contains lipid-lowering, antioxidant, and anti-inflammatory qualities. It also lowers blood pressure and excessive body weight **(Khazaei et al.,2018).** *T. polium* extracts enhance the cytotoxic and apoptotic effects of vinblastine, doxorubicin, and other anticancer medications against a variety of malignant cell lines **(Stankovic et al.,2012).**

I.2.4 Peganum harmala L

I.2.4.1 Botanical Description

Harmal *(Peganum harmala L., Zygophyllaceae*) is a glabrous perennial that grows spontaneously in semi-arid conditions, steppe regions, and sandy soils. It is native to the eastern Mediterranean region. It is a shrub that grows to be between 0.3 and 0.8 meters tall. It has short creeping roots, white blooms, and circular seed capsules that contain more than 50 seeds. The plant is called "Espand" in Iran, "Harmel" in North Africa, and "African rue," "Mexican rue," or "Turkish rue" in the United States **(Majid, 2018).** *P. harmala* extracts are significant for drug development since they have been shown to exhibit a wide range of pharmacological actions throughout the Middle East, particularly Iran and Egypt. *P. harmala* has long been used in traditional remedies to relieve pain and as an antiseptic **(Asgarpanah & Ramezanloo, 2012)**.

Peganum harmala L. is a glabrous perennial herbaceous plant with 5-13 stems that can grow to a height of 30-100 cm. The leaves are palmatised into 3-5 linear lobes that are 3-6 cm long and 1.5-3.0 mm broad **(Khan et al., 2017).**

- **Seeds:** are found in a curved sac or capsule, which contains 50-60 seeds. These are dark brown to blackish-brown in hue, triangular in shape, and about 2mm length with a matriculate surface. The endosperm is quite greasy.
- **Fruit:** is spherical and dry, with three chambers containing 50-60 seeds each.
- **Flower:** the color Mostly white, but can be yellowish or lemon white. The flowers have a diameter of around 2-3 cm, with veins visible in the petals. Nectar is produced in small amounts and is rich in hexose sugars **(Sharma et al., 2022).**



Figure I-14 *Morphological Features of P. harmala*

*(**A**) plant; (**B**) flower; (**C**) ripe fruits; (**D**) seeds* **(Zhu et al., 2022*).***

I.2.4.2 Botanical classification

Scientific classification of *P. harmala* **(Shahrajabian et al., 2021).**

Table I-5 *Scientific classification of P. harmala*

Peganum harmala L.	
Kingdom	Plantae
Division	Angiospermae
Class	Dicotyledonae
Family	Nitrariaceae
Order	Sapindales
Genus	*Peganum*
Species	*Harmala*

I.2.4.3 Geographical Distribution

This species is native to arid and semiarid environments and is widely dispersed in North Africa, the Middle East, Turkey, Pakistan, China, India, Iran, Iraq, Syria, Jordan, Kazakhstan, Mexico, South America, and many more countries **(Sharifi-Rad et al., 2021).**

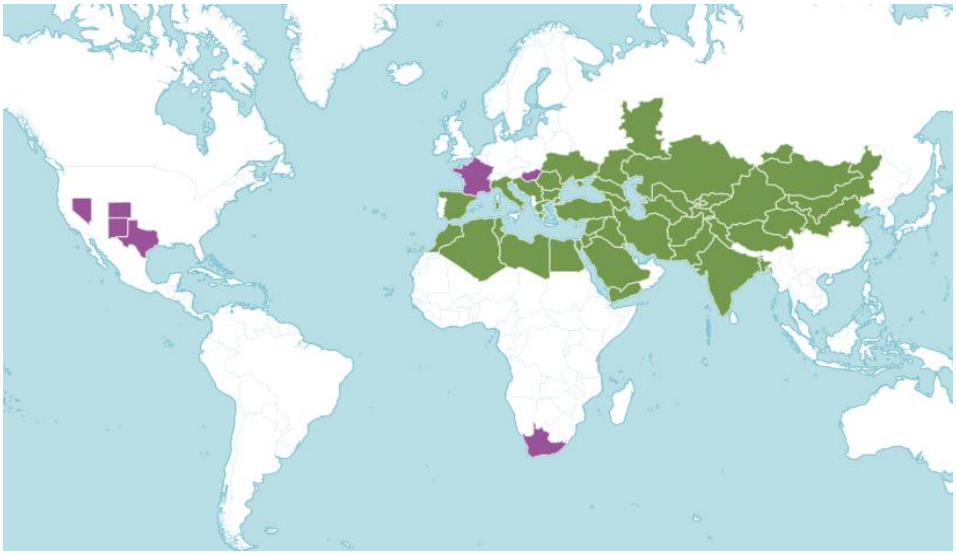


Figure I-15 *Map presenting the distribution pattern of Peganum spp. throughout the globe*

I.2.4.4 Phytochemical Composition

P. harmala's pharmacologically active components include numerous alkaloids found primarily in the seeds and roots. These include β carbolines such as harmine, harmaline (same with harmidine), harmalol and harman, and quinazoline derivatives: vasicine and vasicinone. Unripe seeds have a lower alkaline content than ripe ones **(Mahmoudian et al., 2002).** One study found that total harmala alkaloids comprised at least 5.9% of the dried weight. The plant's stems contain approximately 0.36% alkaloids, the leaves around 0.52%, and the roots up to 2.5%. Harmine and harmaline are reversible inhibitors of MAO A (RIMA) **(Pathan et al., 2012).**

I.2.4.5 Traditional and medicinal uses

Carboline alkaloids derived from different portions of the plant are used to treat a variety of diseases. *P. harmala* also has antiparasitic properties. Furthermore, the alkaloids discovered in *P. harmala* have pharmacological features such as anticancer and analgesic effects, vasorelaxant activity, antibacterial capabilities, and strong inhibitors of monoamine oxidase. The alkaloid extract of seeds from Peganum harmala is regarded to have anti-cancer activity, which could prove to be a potential anticancer treatment **(Khan et al., 2017).** The herb

is commonly used to treat epilepsy, Parkinson's disease, colds, malaria, fever, syphilis, rheumatism, scabies, skin problems, and cataracts **(Jan et al., 2021).**

Peganum harmala has been traditionally used for the treatment of diabetes in folklore medicine in some parts of the world. *Peganum harmala* seeds were reported to be burned after childbirth in Northwestern India and West Pakistan. The seeds are also burned during wedding ceremonies, and the plant has been used in traditional medicine for centuries to treat a variety of ailments. In Turkey and Iran, carpets are colored using a crimson dye produced from *Peganum* seeds. In traditional medicine, the species has been utilized to cure several nerve system ailments such as Parkinson's disease in psychiatric situations **(Khan et al., 2017).**

I.2.5 Atriplex halimus L

I.2.5.1 Botanical Description

Atriplex halimus L., commonly known as “Mediterranean” saltbush and "Lgtef," is a halophytic shrub belonging to the Amaranthaceae family. It is characterized by its tolerance to high salinity soils and its ability to exert allelopathic effects on other plants **(Elbouzidi et al.,2022).**

A. halimus is an upright perennial shrub that may grow up to 3 meters tall. It has branches growing from the base, and its grey-white bark is colored. The leaves on this shrub are 10–30 mm long and 5–20 mm broad. The leaves have a short petiole that attenuates them at the base, and their morphology varies greatly, ranging from lanceolate to deltaid-orbicular. Despite being evergreen, *A. halimus* loses some leaves in the hot, dry summers that are typical of its range. halimus with shorter fruit-bearing branches, a more upright habit, and a smaller stature. *Halimu*s has twisted upper branches with leafy twigs that resemble scorpioid twigs. Its fruit valves are reniform with whole margins, and its leaves are roughly hastate, appearing greenish when young and silvery-grey when older **(Walker et al., 2014).**



Figure I-16 *Morphological Features of A. halimus*

I.2.5.2 Botanical classification

Scientific classification of *A. halimus*

Table I-6 *Scientific classification of A. halimus*

Atriplex halimus L	
Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Subclass	Magnoliidae
Order	Caryophyllales
Family	Amaranthaceae
Genus	*Atriplex*
Species	*halimus*

I.2.5.3 Geographical Distribution

Natural distribution areas for *A. halimus* include Macaronesia, the Mediterranean basin, the Middle East, western Asia, Portugal, Spain, Italy, Greece, Turkey, Cyprus, Syria, Lebanon, Jordan, Tunisia, Morocco, Algeria, Libya, Egypt, and Saudi Arabia. It thrives in regions that are classed as arid or semi-arid because they have high potential evapotranspiration and little

annual rainfall. It is more prevalent in sunny locations on neutral or alkaline (pH 7.0–11.0) soils, which are frequently saline **(Walker, & Lutts, 2014).**

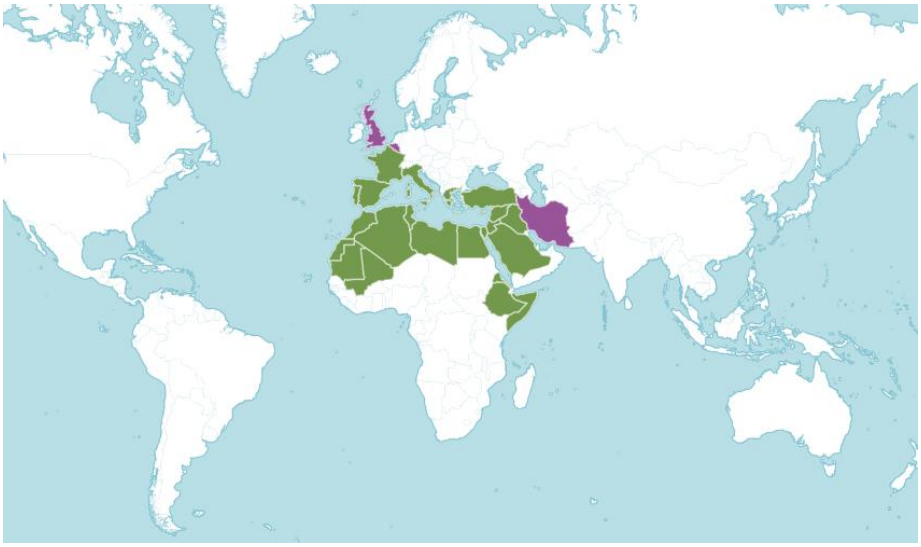


Figure I-17 *Distribution of Pistacia A. halimus in the world.*

I.2.5.4 Phytochemical Composition

The pharmacological activities of Atriplex species are linked to the existence of beneficial biochemicals like phenolics, and terpenoids. These plants are also rich in proteins, flavonoids, vitamins A and C, and amino acids, among other nutrients **(Ali et al.,2021).** According to its chemical composition, A. halimus includes up to 10% sodium chloride and secondary metabolites like tannins, flavonoids, saponins, alkaloids, and resins. It is distinguished by having low levels of crude fat, moderate levels of crude protein, and high levels of ash and crude fiber **(Benhammou et al.,2009).**

I.2.5.5 Traditional and medicinal uses

Due to the plant's high concentration of secondary metabolites such polyphenols, flavonoids, tannins, and saponins, its current uses are restricted to the pharmacology field. Its anti-oxidant, antidiabetic, and protoscolicidal properties make it useful **(Ennoury et al.,2023).** Recent research supports its potential by emphasizing its antibacterial, anticancer, antioxidant, and antidiabetic properties **(Roubi et al.,2023).** It is commonly used in Algerian therapy, mostly to treat several cyst forms, including "ovarian, uterine, and breast **cysts (Ounaissia et al.,2020).**

I.2.6 Equisetum arvense L

I.2.6.1 Botanical Description

Equisetum arvense L, also known as horsetail, is a bushy perennial herb. Because of their similarity to a horse's tail, E. arvense prefers acidic soil. **(Badole & Kotwal, 2014).**

In spring, a spore-bearing stem, like a thin asparagus shoot, rises 15-20cm; once shed, this is replaced by a pale green bush with erect hollow jointed stems with longitudinal furrows and sharply-toothed sheaths covering each joint; from the sheaths of the central stem emerge whorls of fine branches, each giving off finer whorls, the whole sometimes extending up to 60 cm in height, but usually less than 1, 2 **(Sandhu et al., 2010)**

- **Leaves** - Reduced to small scales, generally fused form sheaths around stems and branches; fertile stem sheaths have 8 - 12 large, pointed teeth; sterile stem sheaths are green, with 10 - 12 brownish or black teeth.
- **Spore Clusters** - In long-stalked, blunt-tipped cones at tip of fertile stems.
- **Other features** - Very varied; occasionally prostrate. Fertile stems are brown and appear earlier than sterile stems, do not become green, and die after spore release



Figure I-18 *Morphological Features of E. arvense*

A) *Sterile stems,* ***B)*** *Fertile stems* ***(Asgarpanah & Roohi, 2012)****.*

I.2.6.2 Botanical classification

Scientific classification of Equisetum arvense L. **(Badole & Kotwal, 2014).**

Table I-7 *Scientific classification of Equisetum arvense L*

Equisetum arvense L.	
Kingdom	Plantae
Superdivision	Pteridophytes
Division	Equisetophyta
Class	Sphenopsida
Subclass	Equisetidae
Family	Eqisetaceae
Order	Equisetales
Genus	*Equisetum*
Species	*arvense*

I.2.6.3 Geographical Distribution

E. arvense is an herbaceous perennial plant found in the northern hemisphere, specifically Europe, North America, and Asia. *E. arvense* is native to practically every country in the northern hemisphere, including India, Japan, Italy, Germany, France, the United States of America, China, Russia, Afghanistan, Greece, Pakistan, Ukraine, Colombia, Sweden, Portugal, Austria, Bulgaria, and Turkey. Additionally, it has been introduced in New South Wales, Bolivia, and Victoria **(Luanda et al., 2023).**

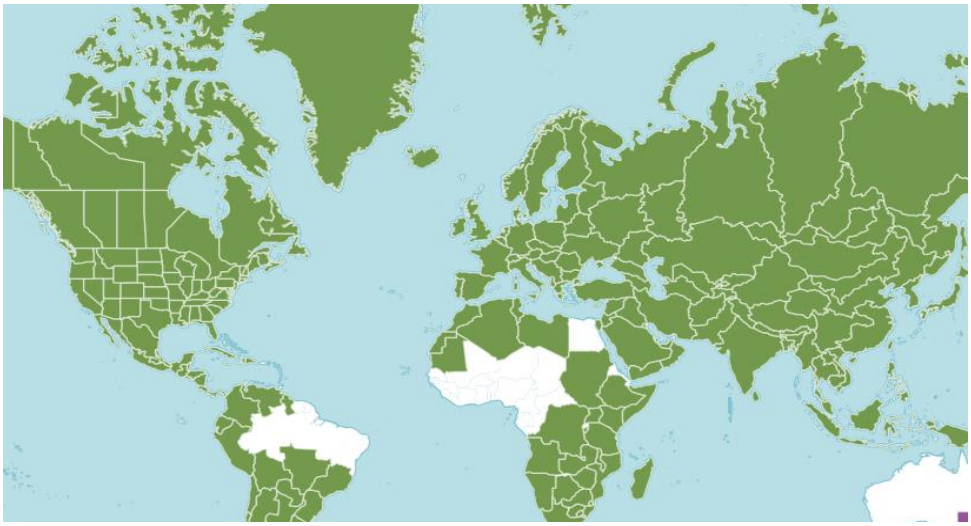


Figure I-19 *Map presenting the distribution pattern of Equisetum spp. throughout the globe*

I.2.6.4 Phytochemical Composition

The plant's active compounds include minerals such as silicic acids and silicates, potassium, sulphur, manganese, magnesium; flavonoids such as quercetin glycosides; phenolic acids, alkaloids, equisetonin; phytosterols such as cholesterol, isofucosterol, and campesterol; and tannins. Equisetonin and flavone glycosides are thought to be the source of horsetail's diuretic effects. A silica compound helps the body retain calcium more efficiently, and it can even help rebuild bones and cartilage **(Amit et al., 2013).** Proteins, carbohydrates, amino acids, trace elements (sodium, potassium, strontium, calcium, magnesium, iron, phosphorus, copper, zinc, titanium, manganese, and silicon), as well as vitamins B1, B2, B6, C, E, and K, were all reported. These findings show that *E. arvense* can be used as a source of supplements **(Luanda et al., 2023).**

Current pharmacological applications of *E. arvense* have indicated antioxidant, anti-inflammatory, antidiabetic, antibacterial, antifungal, anticonvulsant, and anticancer properties, among others **(Asgarpanah & Roohi, 2012).**

I.2.6.5 Traditional and medicinal uses

Equisetum arvense was traditionally used for tuberculosis, as a catarrh in the kidney and bladder regions, as a hematostatic for profuse menstruation, nasal, pulmonary, and gastric hemorrhages, for brittle fingernails and hair loss, for rheumatic diseases, gout, poorly healing wounds and ulcers, swelling and fractures, and frostbite **(Al-Snafi, 2017).**

Horsetail can have harmful consequences when used over an extended period of time. Silicates cause stomach issues, especially when utilized over an extended period of time. Although alkaloids may not present in high concentrations, chronic usage can cause them to accumulate in the organism, facilitating premature births, neurological disorders, headaches, loss of appetite, swallowing problems, and so on. These intoxications force to a treatment that recovers the thiamine shortage, albeit in the case of the animals, they are no longer recoverable in many occasions **(Asgarpanah & Roohi, 2012).** *Equisetum arvense* has traditionally been used to treat prostate inflammation and hypertrophy, urine incontinence, and nocturnal enuresis in children. Nicholas Culpepper, a 19th-century English botanist, employed plant juices or decoctions to stop bleeding, as well as to cure skin ulcers, wounds, inflammation, kidney stones, and cystitis (**Carneiro et al., 2021).**

Chapiter II: Materials and methods

II.1 Objective

The objective of this study is to evaluate the antimicrobial activity, antioxidant activity, and cytotoxicity of nanoparticles synthesized through a green synthesis method using medicinal plants. Specifically, this research aims to:

- Synthesize silver nanoparticles (AgNPs) employing a green synthesis approach utilizing extracts from the aerial parts of *Smyrnium olusatrum, Ferula communis*, *Teucrium polium, Peganum harmala, Atriplex halimus* and *Equisetum arvense* plants extracts.
- Characterize the synthesized nanoparticles using UV-vis spectroscopy and Fourier transform infrared spectroscopy.
- Assess the antimicrobial efficacy of the synthesized nanoparticles against a panel of pathogenic microorganisms.
- Evaluate the antioxidant potential of the synthesized nanoparticles through relevant assays.
- Investigate the cytotoxic effects of the nanoparticles to determine their safety profile.

By achieving these objectives, this study aims to contribute to the understanding of the potential biomedical applications of green-synthesized nanoparticles derived from medicinal plants, emphasizing their antimicrobial efficacy, antioxidant properties, and cytotoxicity profile.

II.2 Time and place of study:

This study is being done in collaboration with the biology laboratory of Yahia Fares University's Faculty of Sciences, as well as the microbiology and physio-chemical unit SAIDAL_Medea, the internship ran from April through June 2024.

This chapter describes the different substances and materials utilized in the experiments as well as the experimental procedures and methodologies used in this work. The study's primary steps are depicted in the diagram below:

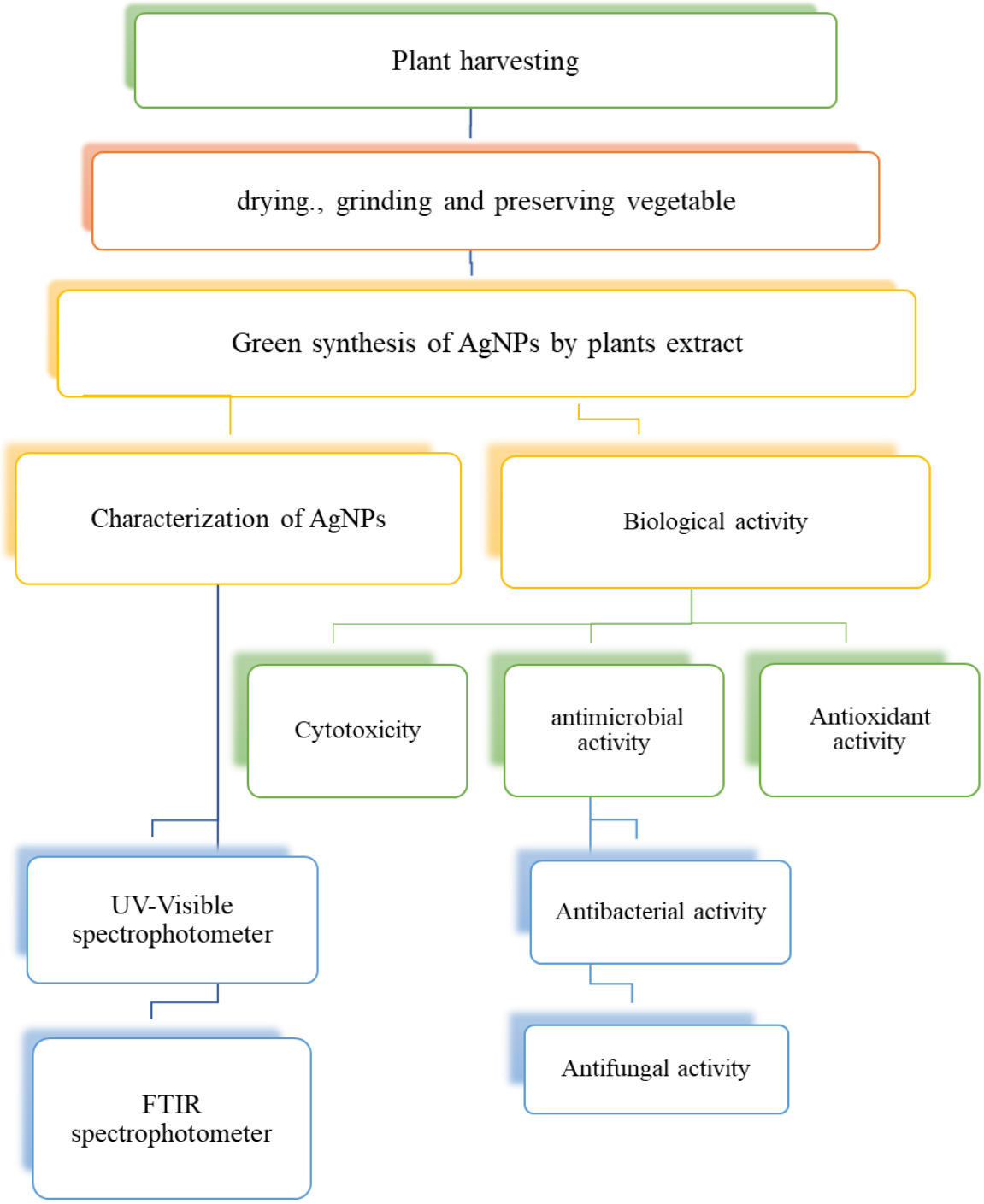


Diagram (1): The main steps of the study

II.3 Material:

II.3.1 Plant Material:

The aerial parts of *Smyrnium olusatrum*, *Ferula communis* **(Figure II-1)**, *Teucrium polium* **(Figure II-2)**, *Peganum harmala* **(Figure II-3)**, *Atriplex halimus* and *Equisetum arvense* **(Figure II-4)** were collected in February 2024 from different areas of Medea province in northern Algeria.



Figure II-1 Photographs of (A) *Smyrnium olusatrum*, (B) *Ferula communis* (Original).



Figure II-2 *Photographs of Teucrium polium*

(Original)



Figure II-3 *Photographs of Peganum harmala*

(Original)



Figure II-4 *Photographs of (A)Atriplex halimus, (B) Equisetum arvense.*

(Wild Flowers Provence)

II.3.2 Biological Materials

II.3.2.1 Microbial Strains

Four bacterial strains and two fungal strains were utilized in this study. These strains were provided by the SAIDAL laboratory in Medea. They were selected based on their high pathogenicity and multi-resistance to antibiotics.

Table II-1 *The bacterial reference strains used.*

bacterial strains	Gram	Supplier
Escherichia coli	negative	ATCC 8739
Pseudomonas aeruginosa	negative	ATCC 9027
Bacillus subtilis	positive	ATCC 6633
Staphylococcus aureus	positive	ATCC 6538

II.3.2.2 Fungal Strains:

Table II-2 *The reference fungal strains used*

fungal strains	Supplier
Candidats albicans	ATCC 10231
Aspergillus brasiliensis	ATCC 16404

II.3.2.3 Antibiotics and antifungals used:

- ✓ Amoxicillin
- ✓ Penicillin
- ✓ Chlortetracycline
- ✓ Lamidaz

II.3.2.4 Culture media used:

- ✓ Trypticase soy agar
- ✓ Sabouroud dextrose agar

II.3.3 Laboratory Materials

II.3.3.1 The Chemicals

- ✓ Silver nitrate AgNO3
- ✓ DPPH
- ✓ Ethanol
- ✓ Methanol
- ✓ Ultra-purified distilled water
- ✓ distilled water
- ✓ DMSO
- ✓ 0.9% NaCl (normal saline)

II.3.3.2 Apparatus and Instrument used

- Autoclave for sterilizing solutions and culture media.
- Digital Sensitive Balance.
- Centrifuge.
- UV–Vis spectrophotometer.
- FTIR spectrophotometer.
- Funnels.
- Incubator.
- Refrigerator.
- Hot plate with magnetic stirrer.
- Vortex.
- Filter paper.
- Antibiotic disk.
- Micro pipette.
- Bunsen Burner.
- Erlenmeyer Flasks, Beakers, Volumetric Flask.
- Test Tubes, Spatula, Syringe.
- Oven.

II.4 Methods:

II.4.1 Plant Harvesting:

The plant material used in this study was collected in February 2024 from different areas of Medea province in northern Algeria. The plants were chosen based on their profitability, availability, and medicinal properties.

The following map shows the location of the sampling site:

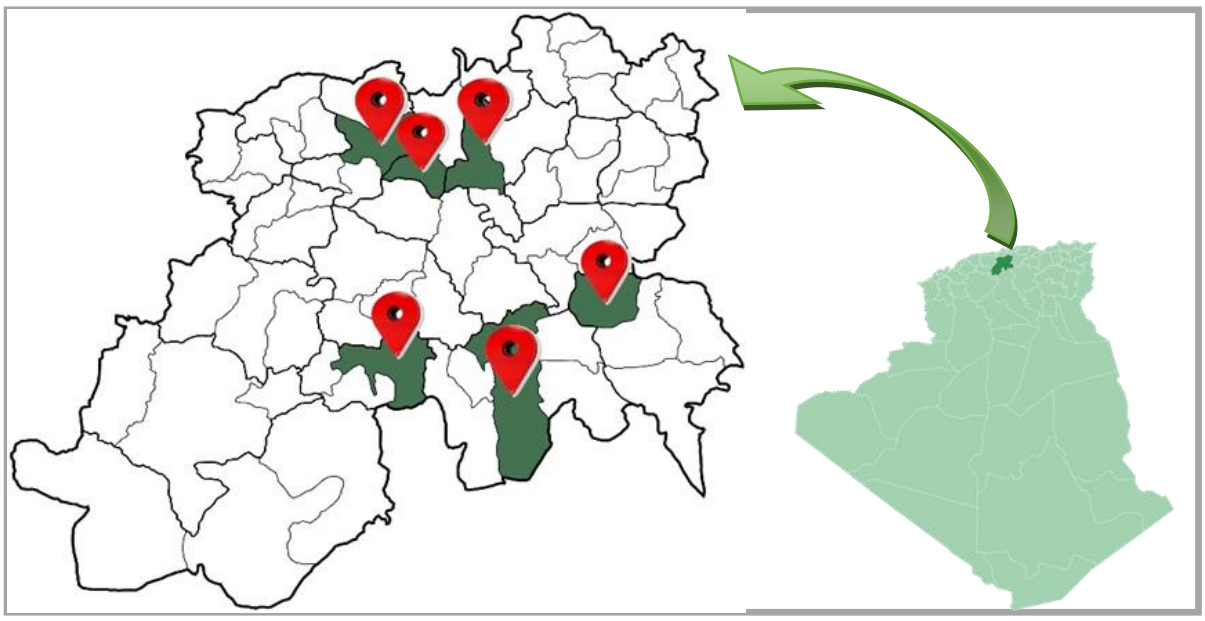


Figure II-5 Geographical location of harvesting site.

II.4.2 Method of drying, grinding, and preserving vegetable

II.4.2.1 Method of drying

The gathered plant materials were thoroughly washed with tap water, followed by distilled water. They were then allowed to dry in the shade at room temperature for two weeks.



Figure II-6 *The aerial parts of Teucrium polium during the drying process.*

(Original)

II.4.2.2 Method of grinding and preserving

After the plants had dried, the aerial parts were ground into a fine powder using an electric lab blender. The powder was stored in glass containers, which were securely sealed and covered with aluminum foil to protect them from light and moisture. These containers were kept in a dark place.

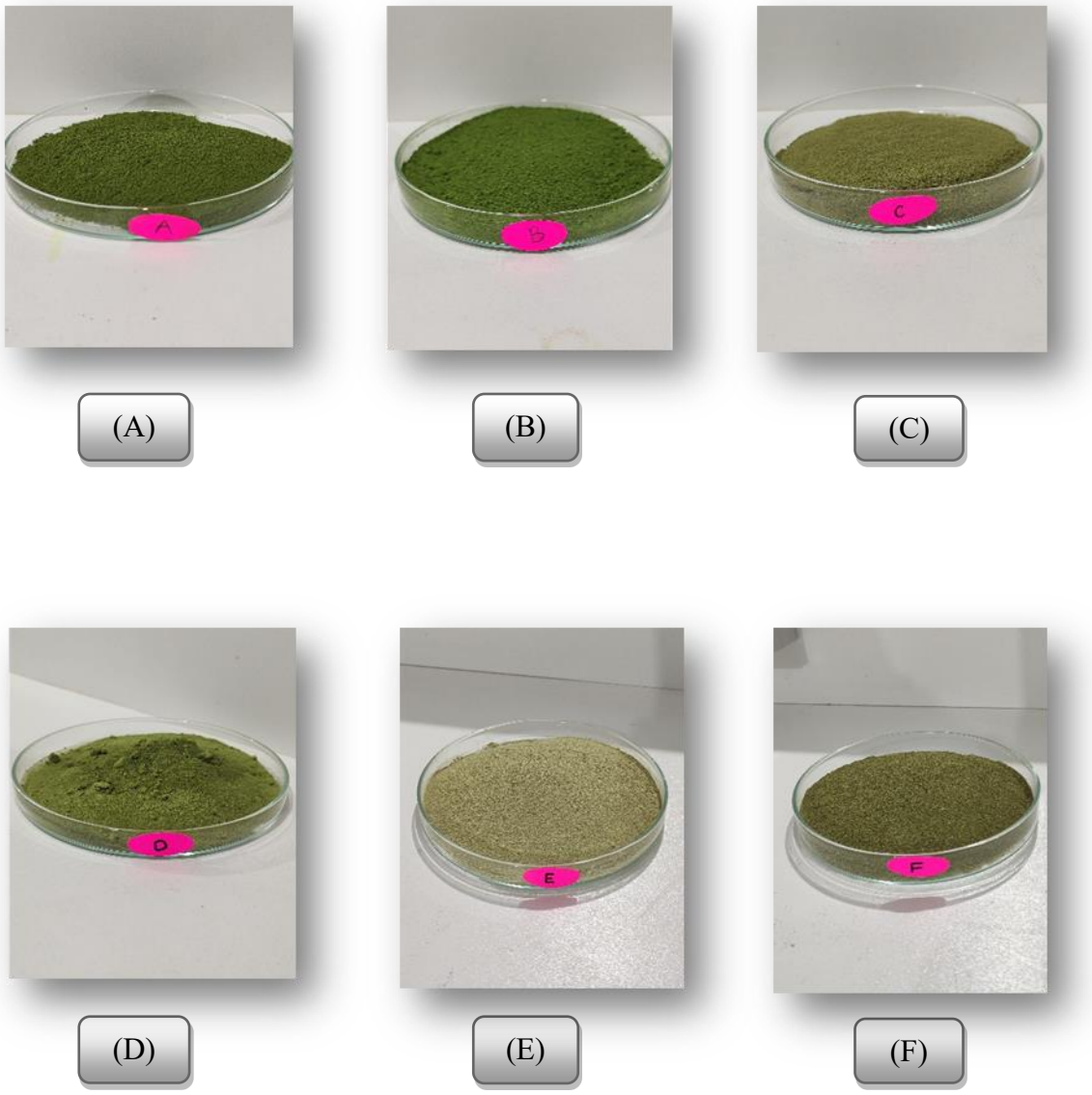


Figure II-7*: Powder of the upper parts of the six plants*

(A): S. olusatrum, (B): F. communis, (C): T. polium, (D): *P. harmala*, (E*): A. halimus, (F): E. arvense.* (Original).

II.5 Method for Determining Moisture Content:

a) Principle:

The method described by **(Pinelo et al.,2004)** was used to determine the moisture content of the plants. This involves measuring the mass loss by drying the plant material in an oven at 105°C.

The following formula is used to calculate the moisture content:

$$MC=\frac{(w-d)}{w}\times 100$$

Where:

MC: is the moisture content (%)

w: is the weight while wet (g)

d: is the weight while dry (g)

b) **Procedure:**

The humidity test protocol consisted of placing 3 samples of the six fresh plants in an oven at a temperature of 105°C until the weight stabilized.

II.5.1 Green Synthesis of Silver Nanoparticles from Aerial Part of Plants Powder:

II.5.1.1 Preparation of the plant extract:

Following the protocol of **(Manimegalai et al.,2020):**

- 50 g of plant powder of each plant type were weighed separately and transferred into 250 mL beakers containing 500 mL of ultra-purified distilled water. The mixture was boiled for 20 minutes at 60 °C.
- The extracts were then filtered through Whatman n°1 filter paper to remove particulate matter and obtain clear solutions.
- The filtrate was kept for further use at 4°C.

II.5.1.2 Green Synthesis of Silver Nanoparticles

The green synthesis of silver nanoparticles was conducted following a commonly used method with several adjustments tailored to the specific requirements of this study.

Using ultra-purified distilled water and a magnetic stirrer, a transparent aqueous solution of AgNO3 was prepared by swirling until all the nitrate had dissolved.

Following the method described by **(Rani & Prabhu.,2023)** with minor adjustments, the synthesis of AgNPs was performed as follows:

1. 10 mL of the aqueous plant extract was combined with 90 mL of a 3 mM silver nitrate solution using a magnetic stirrer at 600 rpm for 30 minutes to aid in the reduction of metal ions. The mixture was incubated at room temperature in the dark for 24 hours.
2. After centrifuging the reaction solution for 15 minutes at 4°C and 10,000 rpm, the resulting pellet was rinsed with deionized water and left to incubate for an additional 24 hours.
3. The pellets were then stored for further use in bioassay and characterization investigations.

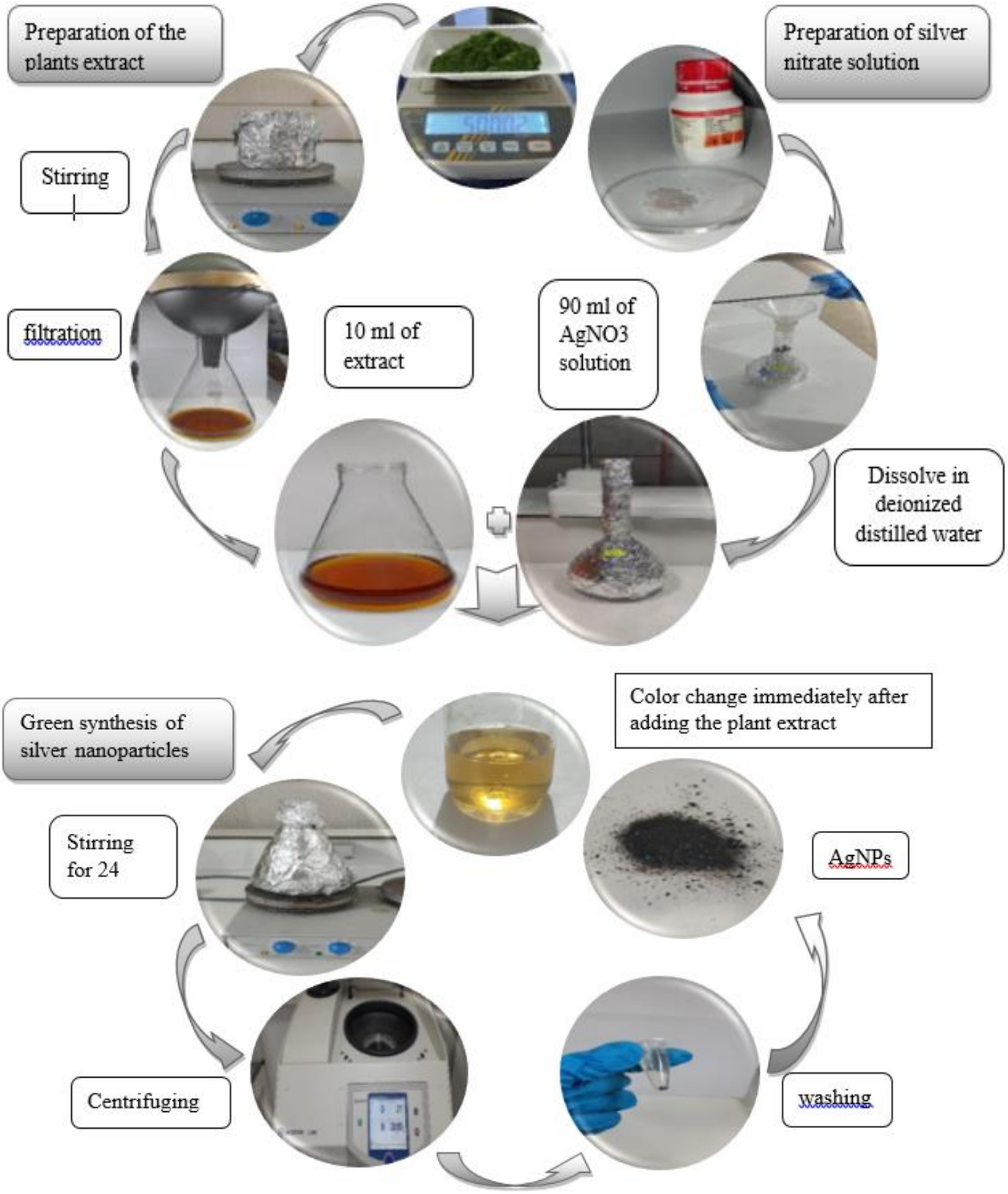


Figure II-8 *Schematic diagram illustrating the protocol for preparing silver nanoparticles using extracts from aerial plant parts.*

II.5.2 Characterization of silver nanoparticles:

There are several ways of characterizing nanoparticles. The first and most practical is the change in the color of the solution.

UV-vis spectroscopy and Fourier transform infrared spectroscopy were utilized to characterize the silver nanoparticles that were synthesized through biosynthesis.

II.5.2.1 UV-visible spectroscopy :

a) Principle:

UV-Visible spectroscopy is the simplest and essential method for identifying silver nanoparticles **(Ashkarran et al.,2013).** UV-Visible spectroscopy is a widely used technique for organic and inorganic substances. It is based on the transition of valence electrons from the ground state to the excited state by means of an electromagnetic wave **(Rocha et al.,2018).**

b) Procedure:

Spectrophotometric measurements were performed with a double-beam UV-visible spectrophotometer for the six extracts prepared (AgNPs and extract) using a quartz cell with a 10 mm optical path. the wavelength range between 300 and 800 nm, and using deionized water as the reference.



Figure II-9 *UV-Visible spectroscopy used*

II.5.2.2 Fourier-transform infrared spectroscopy (FTIR):

a) Principle:

Infrared is a widely used method for characterising and identifying compounds or their functional groups (chemical bonds) in a mixture of extracts. The bonds are identified using the corresponding wave number and the characteristic group is determined using a spectrum of an unknown compound, which is identified by comparison with the library of known compounds **(Khan et al.,2018)**

b) **Procedure:**

Fourier transform infrared spectroscopy (FTIR) analyses were carried out using a Fourier transform IR spectrometer in the range 500 to 4000 cm-1. This technique was used to identify the functional groups present in a sample and the bonds developed after the formation of silver nanoparticles.



Figure II-10 *Fourier-transform infrared spectroscopy (FTIR) used*

II.5.3 Biological Activity:

II.5.3.1 Antibacterial and antifungal studies:

a) The principle:

Zone of inhibition (ZOI) plates examine bacteria's sensitivity to antibiotics. Antibiotics are placed in agar plates and allowed to incubate. After incubation, growth inhibition or bacterial destruction is evaluated by examining the empty zone around the antibiotic (inhibition zone).

The zone inhibition test is a popular laboratory method due to its low cost, speed, suitability for water-soluble antimicrobials, and versatility in testing liquids, coated surfaces, and antimicrobial impregnated solids. However, the zone of inhibition plates test has certain limitations. The approach assesses bacterial mortality indirectly by evaluating the growth inhibition zone. This method is not a true quantitative method and cannot detect viruses. However, it can quantify the diameter of a zone **(Crisan et al., 2021).**

b) **Methodology:**

Escherichia coli (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027*), Bacillus subtilis* (ATCC 6633), and *Staphylococcus aureus* (ATCC 6538) were obtained from the Pharmaceutical Laboratory SAIDAL in Medea. Standard bacterial cultures were sub- cultured into newly prepared soy agar and incubated at 37 °C for 24 hours to produce fresh bacterial cultures **(Priya et al., 2023).**

The microbiology and physiochemical unit SAIDAL_Medea provide fungal cultures of *Candida albicans* (ATCC 10231) and *Aspergillus brasiliensis* (ATCC 16404). The fungus was sub-cultured for 72 hours to initiate the sporulation process, and the growing spores were tested for antifungal properties **(Priya et al., 2023)**

Preparation of the inoculum:

- **Preparation of the pre-culture:**

Antibacterial tests should be carried out on young cultures (18 to 24 hours) in the exponential growth phase. Strains are reactivated by inoculating the bacterial strain in a liquid medium. After incubation for 24 hours at 37°C, a second subculture is carried out in petri dishes containing Tryptic soy agar (TSA), then incubated at 37°C for 18 hours.

- **Preparation of the bacterial suspension:**

From young cultures on (TSA). 3 to 5 well-isolated, identical colonies are picked in 5 ml of sterile physiological water and vortexed for a few seconds. The suspension is standardized to 10^6 CFU/ml using a spectrophotometer set to a wavelength of 620 nm.

According to **Mac Farland**, an OD of between 0.08 and 0.1 corresponds to a concentration of 10^7 to 10^8 germ / ml; the inoculum suspension is diluted 1:10 in sterile distilled water to give a concentration of 10^6 germ / ml.

- **Preparing the discs:**

Discs made from 6mm Whitman paper are used. These discs should have a regular outline to provide easily measurable zones of inhibition. Sterilize the discs in an autoclave at 120°C for 20 minutes.

II.5.3.1.1 Assay for antibacterial activity by well diffusion:

The method described by **(Rais & Hasson, 2022)** was used with some modifications:

The antibacterial activity of green Ag-NPs was assessed using Agar Well Diffusion The turbidity of bacterial cultures was analyzed by comparing the McFarland turbidity standard (1.5X 10^8 cells.ml-1), a sterile swab was cast off to the bacteria suspension, and the inoculum was streaked on the Trypticase soy agar plate before being let to dry. Green Ag-NPs (100, 70, 50, and 25 µg.ml-1) were injected into 8mm corn borer wells, and allowed to scatter at ambient temperature for one hour before incubating at 37°C for 24 hours, as a positive control two well with 15 µL of streptomycin antibiotic and 15 µL of AgNO3 was utilized. To assess the organism's tolerance or sensitivity to green Ag-NPs, zones of inhibition were measured with a ruler or caliper and compared to previously determined zones.

II.5.3.1.2 Assay for antifungal activity by well diffusion:

The well diffusion experiment was used to investigate the antifungal activity of green produced AgNPs, following the methods of **(Shahbaz et al., 2023)** with minor modifications. The fungus spore suspension (1.5 x 108 CFU/mL) was widely spread on sterilized, solidified Sabouraud's Dextrose agar. Each agar plate included four 5.5 mm diameter wells formed with a sterile cork-borer. The wells were loaded with 15 µl of varied doses of AgNPs (150, 200, 250, and 300 µg/mL). The plates were incubated at 25°C for seven days and the zones of inhibition were determined. Two wells containing 5.0 mg of Lamidaz and 15 µL of AgNO3 as a positive control were used. The Petri plates were put into an incubator for one day at 37°C and 5 days at 28°C.

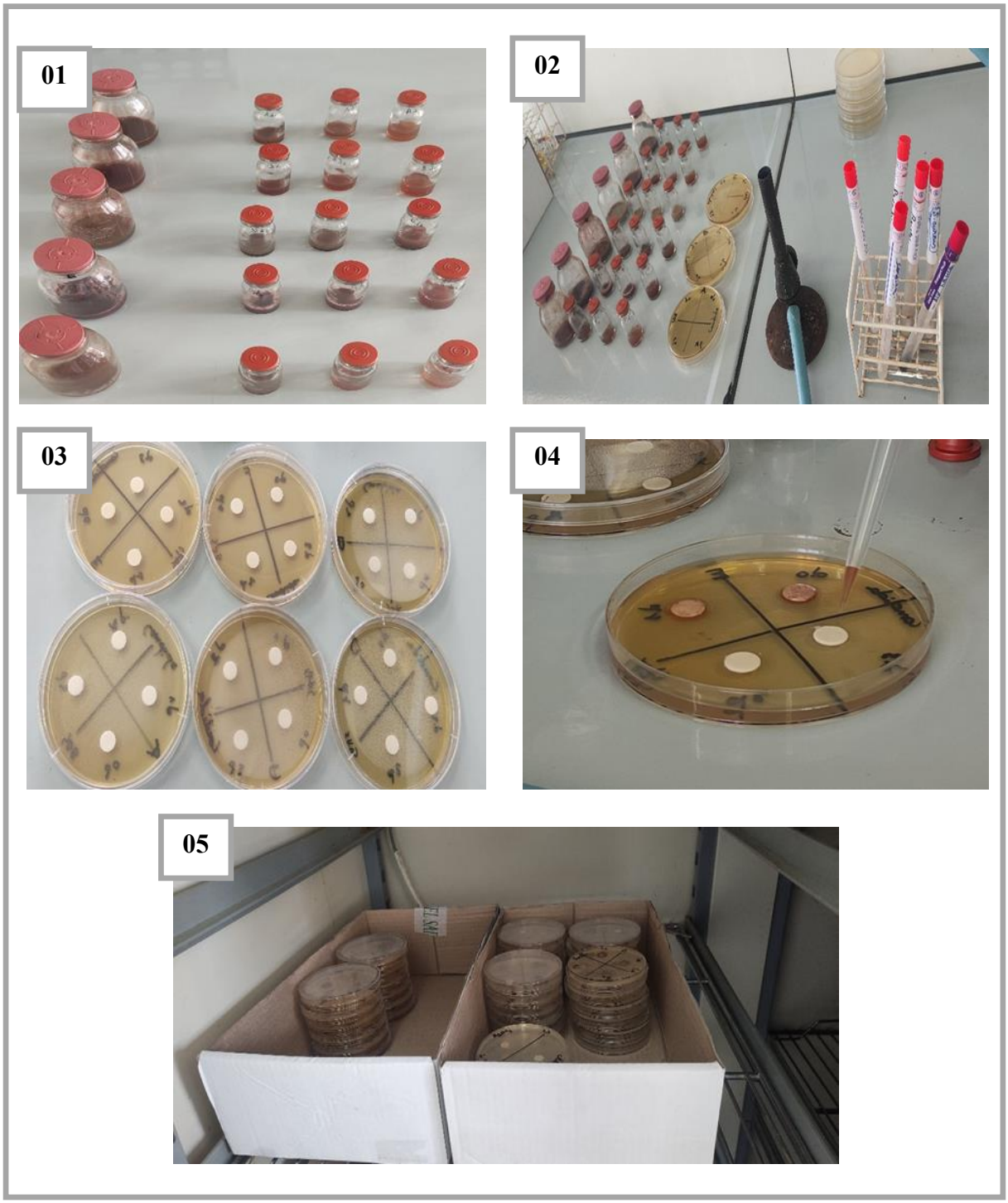


Figure II-11 *Key steps for antibacterial and antifungal studies*

01: Preparation of dilutions,**02:** Preparation of the pre-culture.**03:** streak plate method ,**04:** Disc deposition,**05:** incubation.

II.5.3.2 Antioxidant activity of silver nanoparticles:

Free radical scavenging ability on 2, 2-diphenyl-2- picrylhydrazyl (DPPH):

a) The principle:

The DPPH test is based on the ability of the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical to react with hydrogen donors. after the reaction it gives the reduced form DPPH (hydrazine form), which results in the color change from purple to pale yellow. The level of disappearance of purple color depends on the concentration of the antioxidant. It is a rapid, simple, and an inexpensive method for antioxidant capacity determination **(Bedlovičová et al., 2020).**

b) Protocol:

The antioxidant capabilities of AgNPs and ascorbic acid were assessed by measuring their ability to scavenge free radicals using the stable DPPH radical. Various concentrations (20, 40, 60, and 100 µg /ml) of AgNPs were combined with freshly prepared DPPH solution (1 mM in methanol) and thoroughly mixed before being left to incubate in darkness at room temperature for 30 minutes. The absorbance was then measured at 517 nm using a UV-Vis spectrophotometer. A control solution containing only DPPH (all reagents except the sample) and a blank solution of methanol were used for comparison **(Keshari et al., 2020)**. The percentage of inhibition, indicative of the free radical scavenging activity, was calculated using a specific formula:

$$I\,(\%) = \frac{(Pc\text{-}Ps)}{Pc} \times 100$$

Where:

I (%) of scavenging: is the inhibition percentage,

Pc: is the absorbance of the control reaction (with all reagents except the test compound),

Ps: is the absorption of AgNPs/ascorbic acid.

The results were expressed as IC50 which is the concentration of the sample required to inhibit 50 % of DPPH concentration.

II.5.3.3 Estimation of the cytotoxic effects of silver nanoparticles (In-vitro cellular toxicity (blood hemolysis):

a) The principle:

Hemolysis is the process of rupturing red blood cells and releasing their contents into the surrounding fluid. It also goes by various names. It can happen both in vivo or in vitro. To create drugs, it's important to consider the toxicity of active molecules, and hemolytic activity can be a suitable starting point. Compounds with hemolytic activity are hazardous to healthy cells **(Kumar et al., 2011).**

b) **protocol**:

The hemolysis of blood was carried out as described by **(Bouma, 2002)** as below:

Silver nanoparticle solutions (50-60 µg/ml) were mixed with 0.2 ml of blood from healthy, non-smoking individuals. Mix gently for 5 seconds, then add 20 ml of normal saline to prevent hemolysis. Centrifuge at 3000 rpm for 10 minutes. To test for 100% hemolysis, 30 µl of DMSO was mixed with normal saline and blood in the same ratio as the positive control. The blood was then diluted with 100 times more distilled water than normal saline **(Hashim, 2018)**. Absorption was measured at 540 nm, and the percentage of hemolysis was calculated using the following equation:

$$Hemolysis\ \% = \frac{(A\ test\ solution - A\ normal\ saline)}{(A\ 100\%\ hemolysis - A\ normal\ saline)} \times 100\%$$

Chapiter III: Results and Discussion

III.1 Moisture Content:

The results of Moisture content in aerial parts of *S. olusatrum, F. communis, T. polium, P. harmala, A. halimus and E. arvense* at 105°C is showed in **Figure III-1**

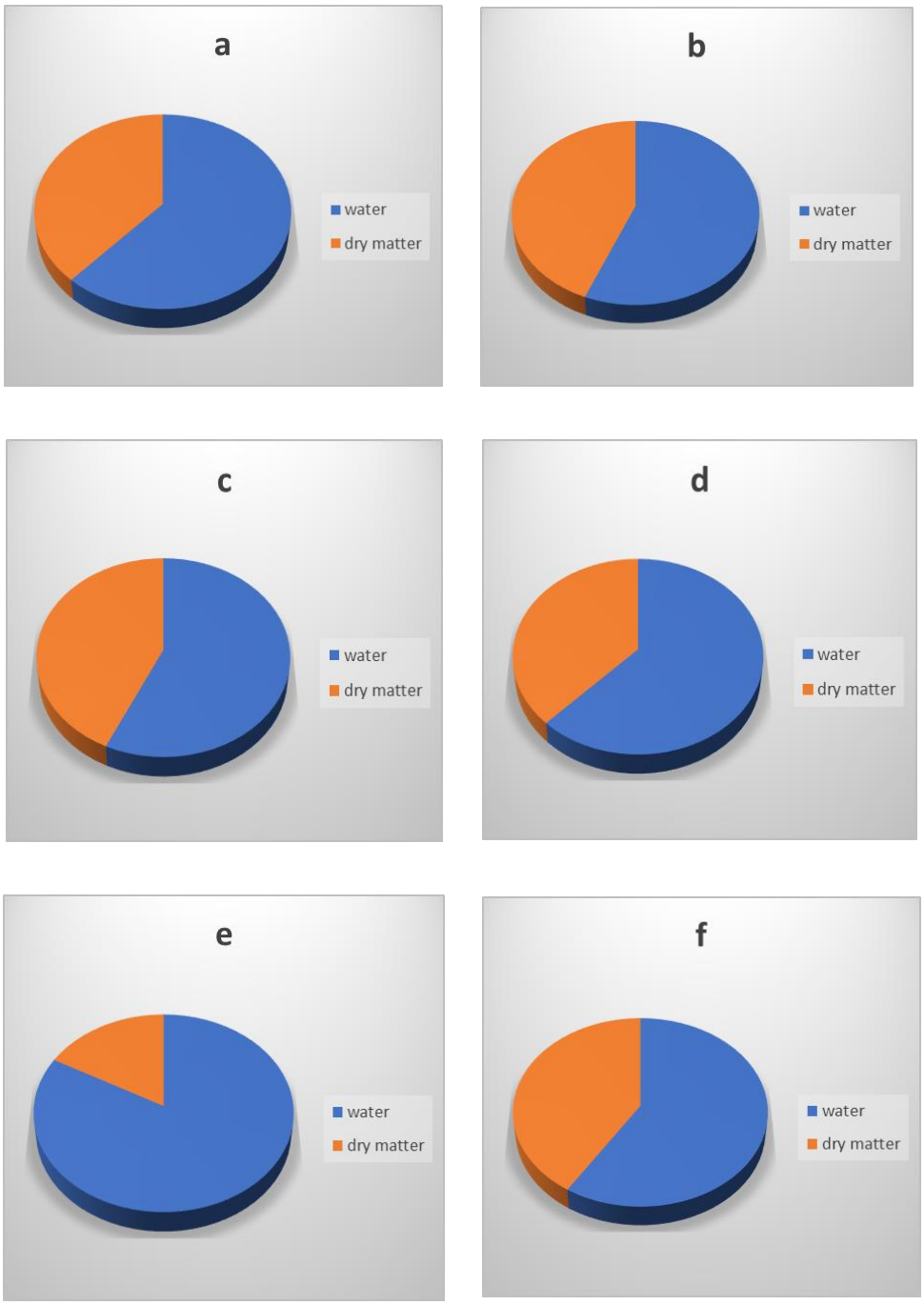


Figure III-1 *Graphical representation of the water content of the aerial part of (a) S. olusatrum; (b) F. communis; (c) T. polium; (d) P. harmala; (e) A. halimus and (f) E. arvense.*

The dry matter assessment is based on the determination of the water content of the to be analyzed that were harvested in February 2024. Drying three tests of each sample revealed Moisture content in aerial parts of S. *olusatrum, F. communis, T. polium, P. harmala, A. halimus and E. arvense*.

These results indicate that more than ¾ of the weight of fresh plants is water, but it is the aerial part of *A. halimus* that revealed a greater content. This small variation is explained by the different climatic conditions that characterize the different regions. Due to the retardation of enzymatic reactions, drying is usually applied in the processing of herbs to preserve their bioactivity and to expand the shelf life **(Georgé et al., 2011).**

III.2 Synthesis Of Silver Nanoparticles:

III.2.1 Characterization of silver nanoparticles:

III.2.1.1 Visual inspection:

The color change in the mixture was observed visually; the addition of extracts from the aerial parts of *S. olusatrum*, F. *communis*, T. *polium*, *P. harmala*, *A. halimus* and *E. arvense* **(Figure III-2).**

The results of this experience showed that silver nanoparticles cannot be synthesized in darkness in case of preparing in cold extract while boiled aqueous extract can be synthesized in darkness after 24 hr. caused the colorless solution of AgNO3 to shift from colorless to light yellowish and then to reddish-brown, indicating the synthesis and description of silver nanoparticles **(Figure III-2).**

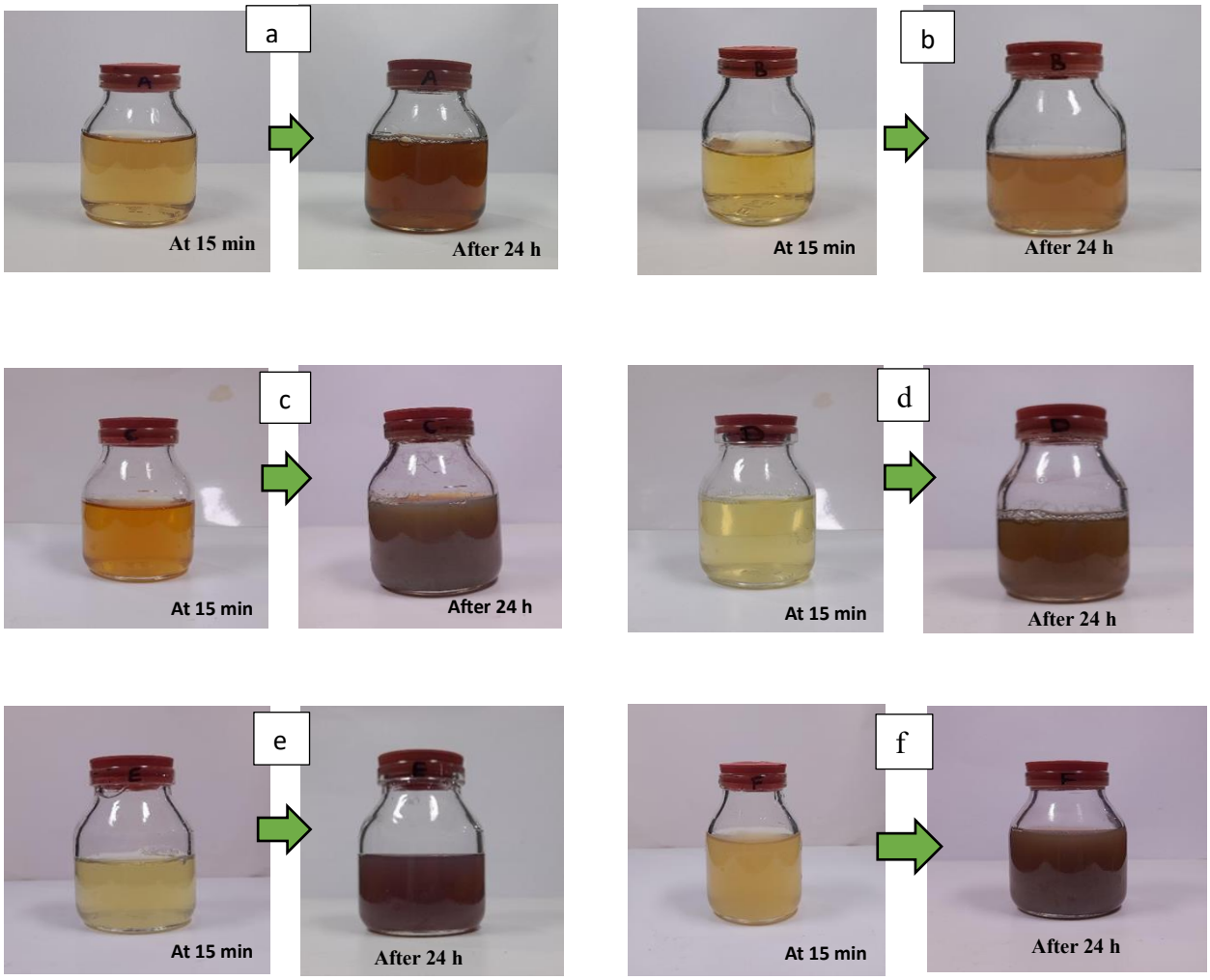


Figure III-2 *Color change of reaction solution containing*

the aerial parts of plant (a)and plant (b); plant (c); plant (d); plant (e) and plant (f). extract samples with AgNO3 at 15min and after 24 h

III.2.1.2 UV-Visible spectroscopy

Silver nanoparticles were successfully synthesized using botanical extracts from the aerial parts of various plants, namely *Smyrnium olusatrum*, *Ferula communis*, *Teucrium polium*, *Peganum harmala*, *Atriplex halimus*, and *Equisetum arvense*. The synthesis involved the reduction of silver nitrate to form silver nanoparticles, which were characterized using UV-visible spectroscopy within the wavelength range of 300 to 800 nm. The UV-visible spectra of these biosynthesized silver nanoparticles exhibited distinctive absorption peaks, indicating the formation of nanoparticles.

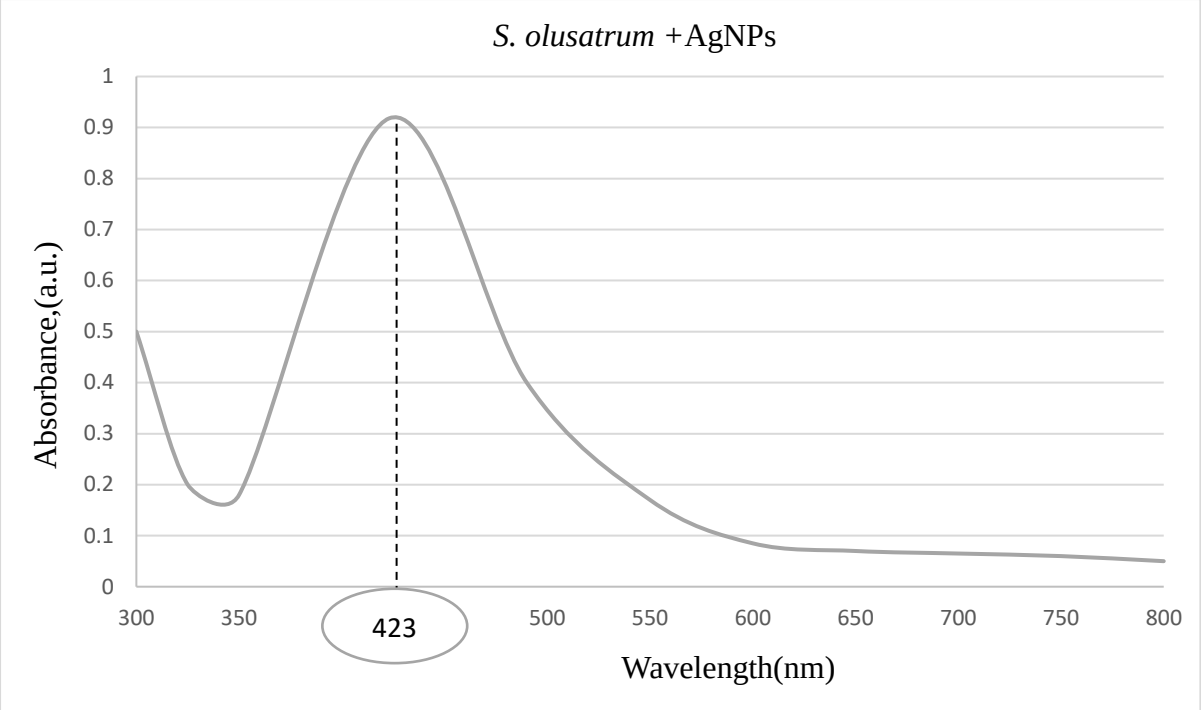


Figure III-3 *UV-visible spectrum of biosynthesized silver nanoparticle using S.olusatrum*

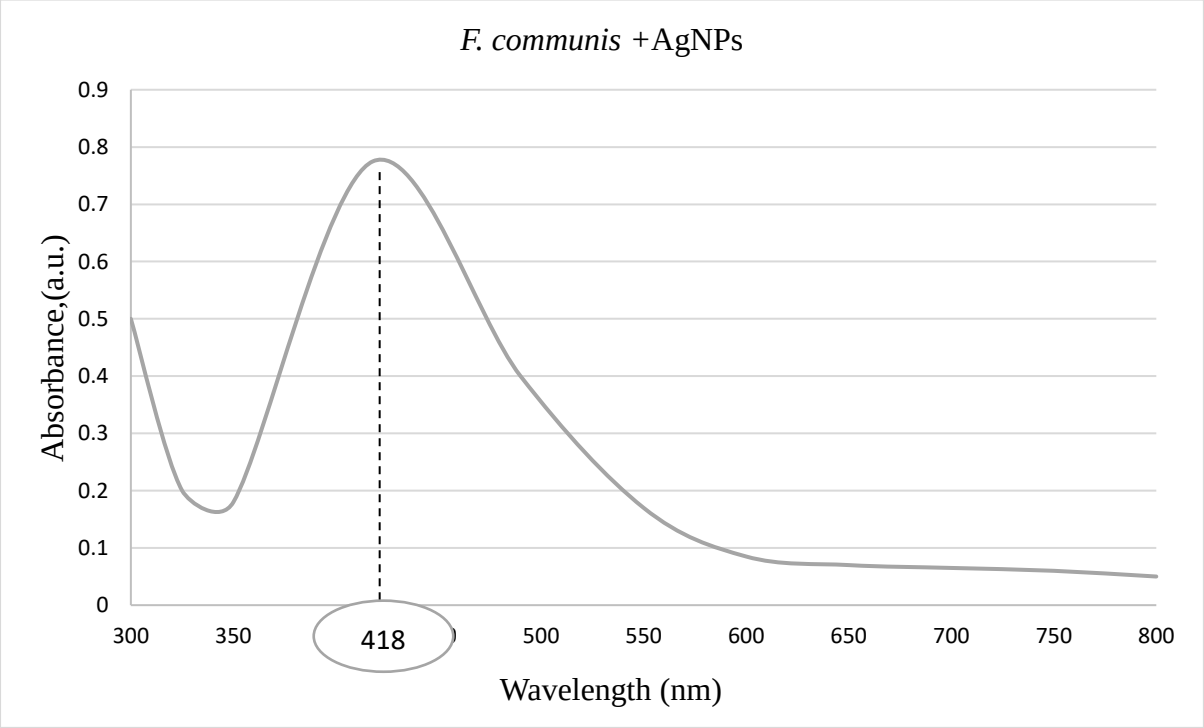


Figure III-4 *UV-visible spectrum of biosynthesized silver nanoparticle using F. communis*

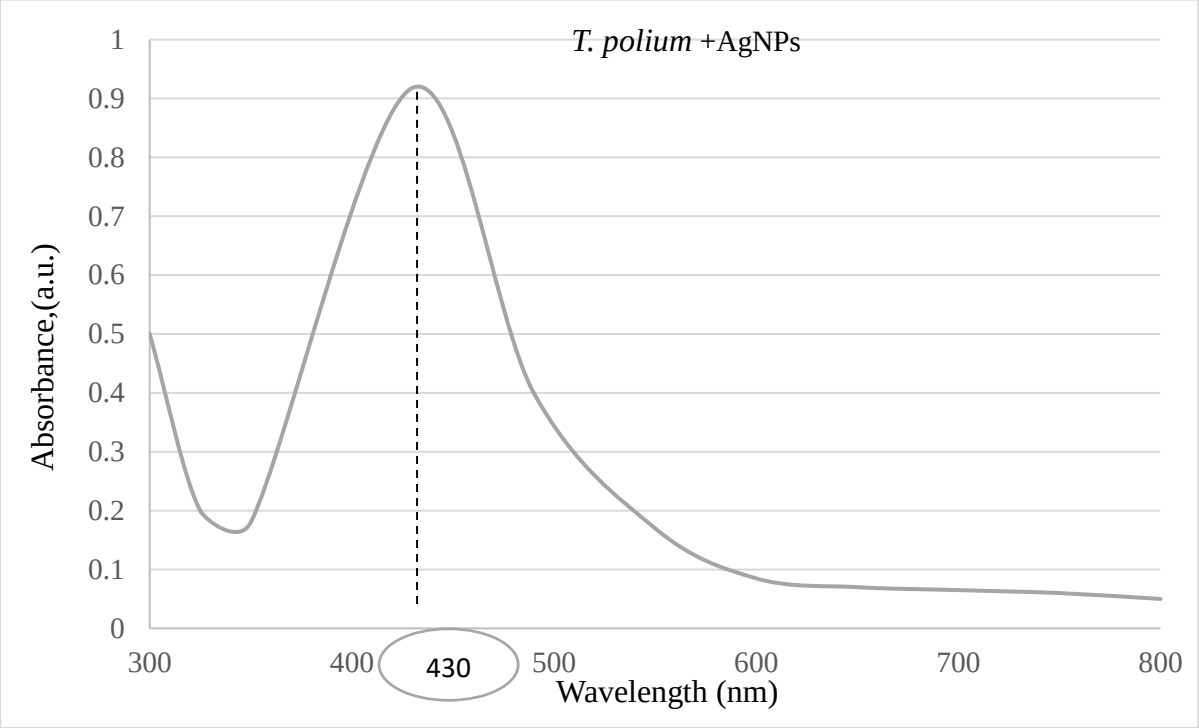


Figure III-5 UV-visible spectrum of biosynthesized silver nanoparticle using *T. polium*

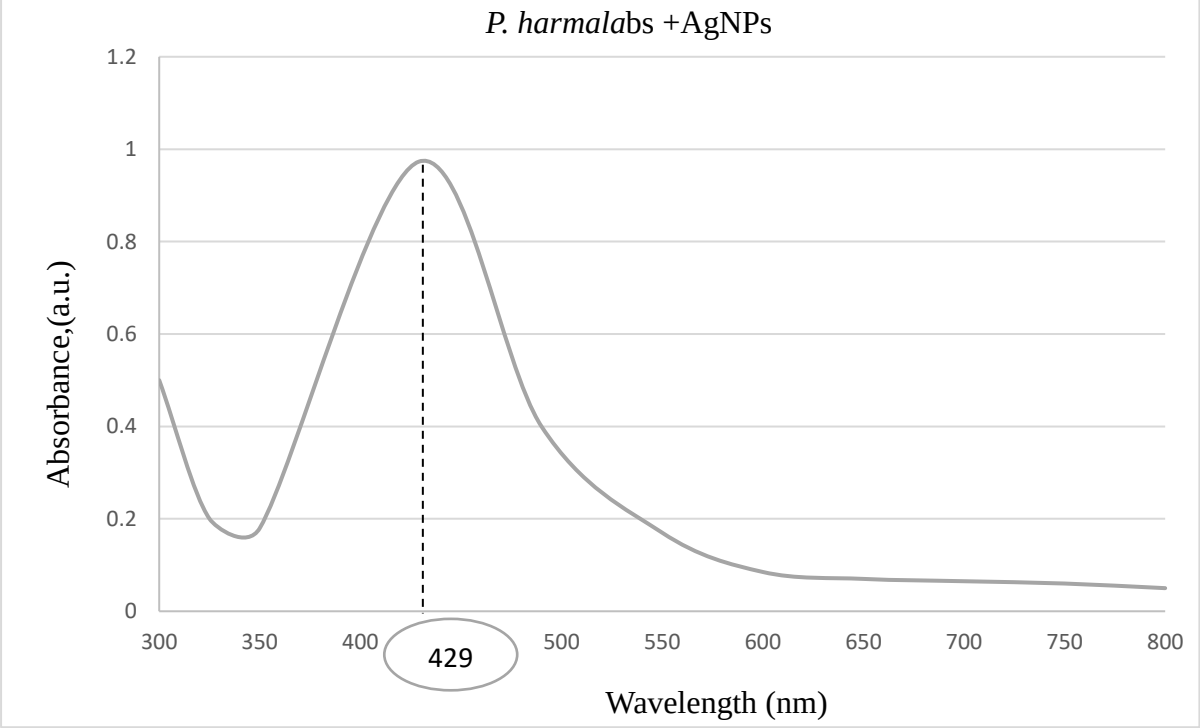


Figure III-6 *UV-visible spectrum of biosynthesized silver nanoparticle using P. harmala*

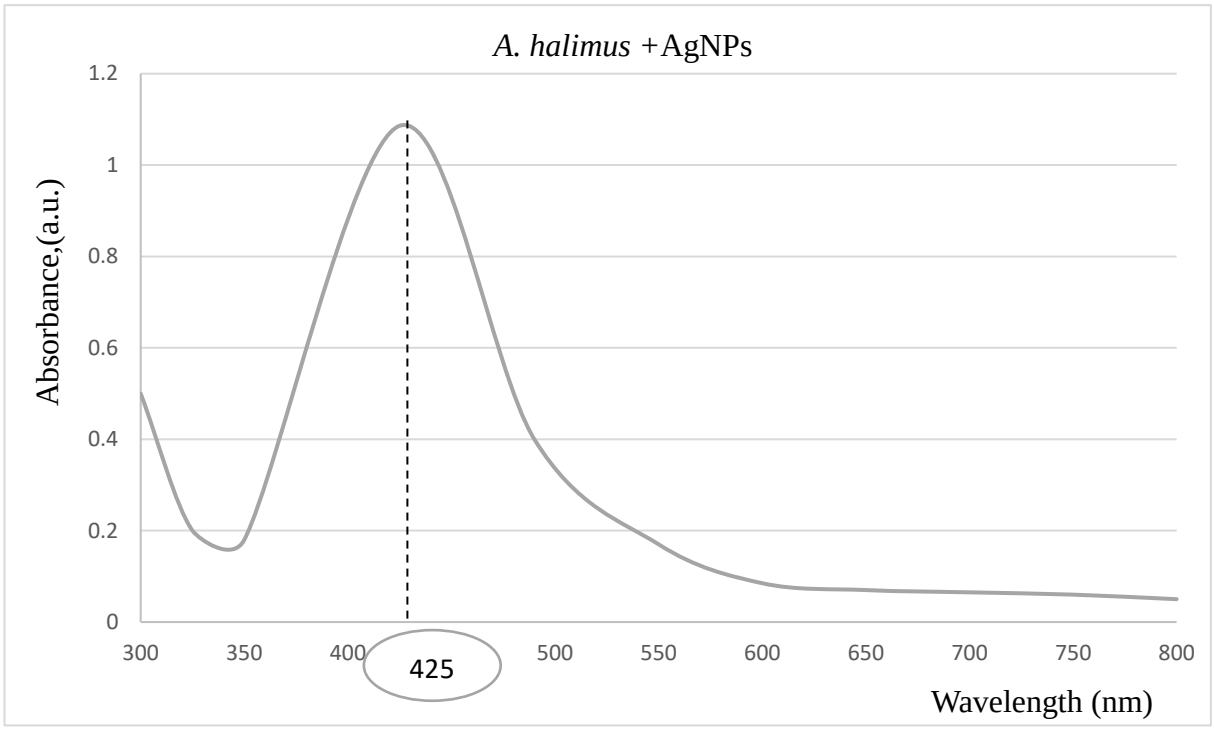


Figure III-7 *UV-visible spectrum of biosynthesized silver nanoparticle using A. halimus*

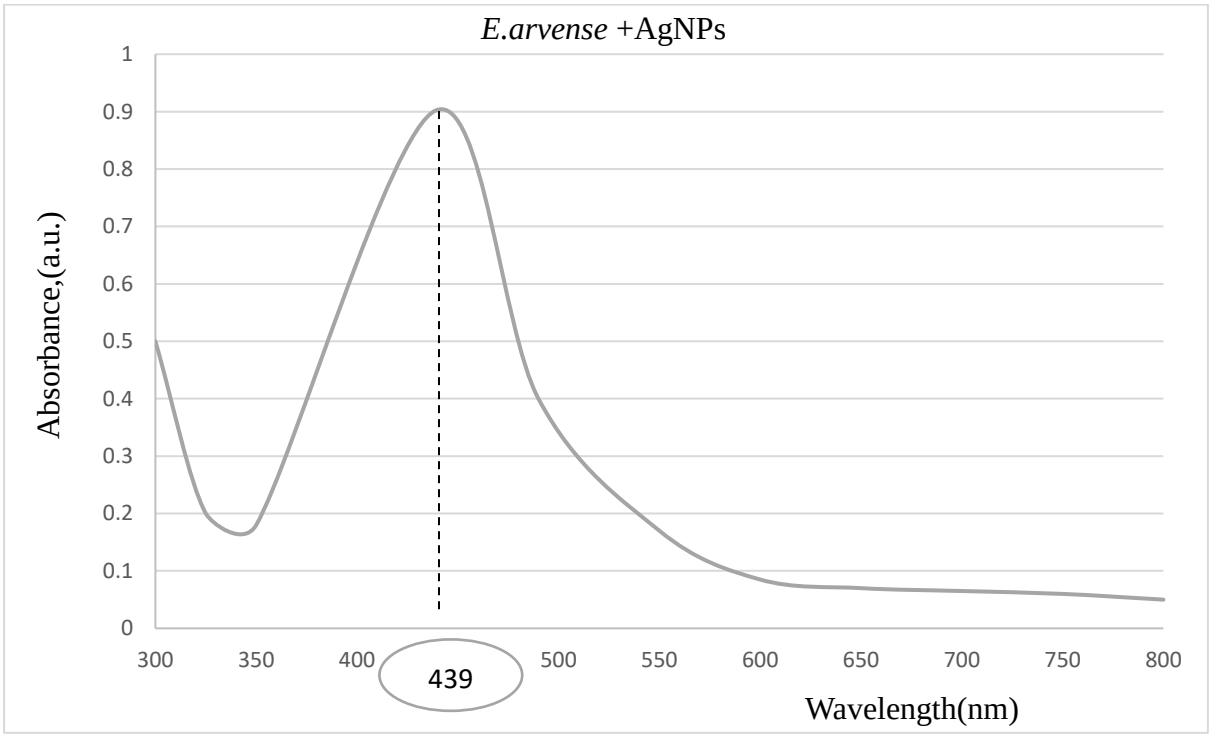


Figure III-8 *UV-visible spectrum of biosynthesized silver nanoparticle using E. arvense.*

The specific absorption peaks for the synthesized AgNPs are summarized as follows:

- *S. olusatrum*: 423 nm
- *F. communis*: 418 nm
- *T. polium*: 430 nm
- *P. harmala*: 429 nm
- *halimus*: 425 nm
- *E. arvense*: 439 nm

The UV-visible spectroscopy results clearly demonstrate the successful biosynthesis of silver nanoparticles using various plant extracts. The absorption peaks between 400 and 500 nm in all samples confirm the formation of silver nanoparticles, as these peaks are characteristic of the surface plasmon resonance (SPR) of silver nanoparticles. The variations in the observed peaks reflect differences in the nanoparticle size, shape, and the stabilizing agents present in the plant extracts. The appearance of an absorption peak at a wavelength of 400–500 nm is an indication that AgNPs have formed **(Umadevi et al.,2012; Chuchita,2018).**

The higher absorption intensity. shows that the number of AgNPs formed in the solution increases with increasing reaction time **(Umadevi et al.,2012; Chuchita,2018).** The absorption peak formed. also looks wider, indicating that there is aggregation. Silver nanoparticles can be stable if there is strong Coulomb repulsion between particles **(Desai et al.,2012).**

III.2.1.3 FTIR Analysis of Silver Nanoparticles Synthesized Using Various Plant Extracts

FTIR (Fourier Transform Infrared Spectroscopy) was utilized to investigate the chemical composition and identify the functional groups present in the synthesized silver nanoparticles (AgNPs) using the aerial parts of *Smyrnium olusatrum*, *Ferula communis*, *Teucrium polium*, *Peganum harmala*, *Atriplex halimus* and *Equisetum arvense* plant extracts. The spectra obtained provide insights into the interactions between the nanoparticles and the biomolecules from the plant extracts used for synthesis.

The six distinct plant extracts produced AgNPs' FTIR spectra revealed multiple distinctive peaks that suggested the existence of distinct functional groups. For every extract, key peaks were found at various wavenumbers that demonstrated the variations and convergences of the functional groups involved.

The different FTIR spectra showed that each plant extract contributed different biomolecules to the nanoparticle production. The different spectra show the different chemical environments that each plant extract creates, which can affect the characteristics of the nanoparticles that are generated. This variability is important because it implies that, depending on the functional groups present, the type of plant extract used can be specifically customized to attain particular nanoparticle characteristics, such stability, size, and reactivity.

An extensive visual representation of the FTIR spectra for each plant extract is shown in Figures 0-1 through 06 of the documents. These graphs highlight how various plant extracts affect the creation of nanoparticles by showing how peak positions and intensities vary throughout wavenumber ranges. A more comprehensive comprehension of the chemical interactions involved in the synthesis process is made possible by the identification of important peaks and the functional groups that correspond with them.

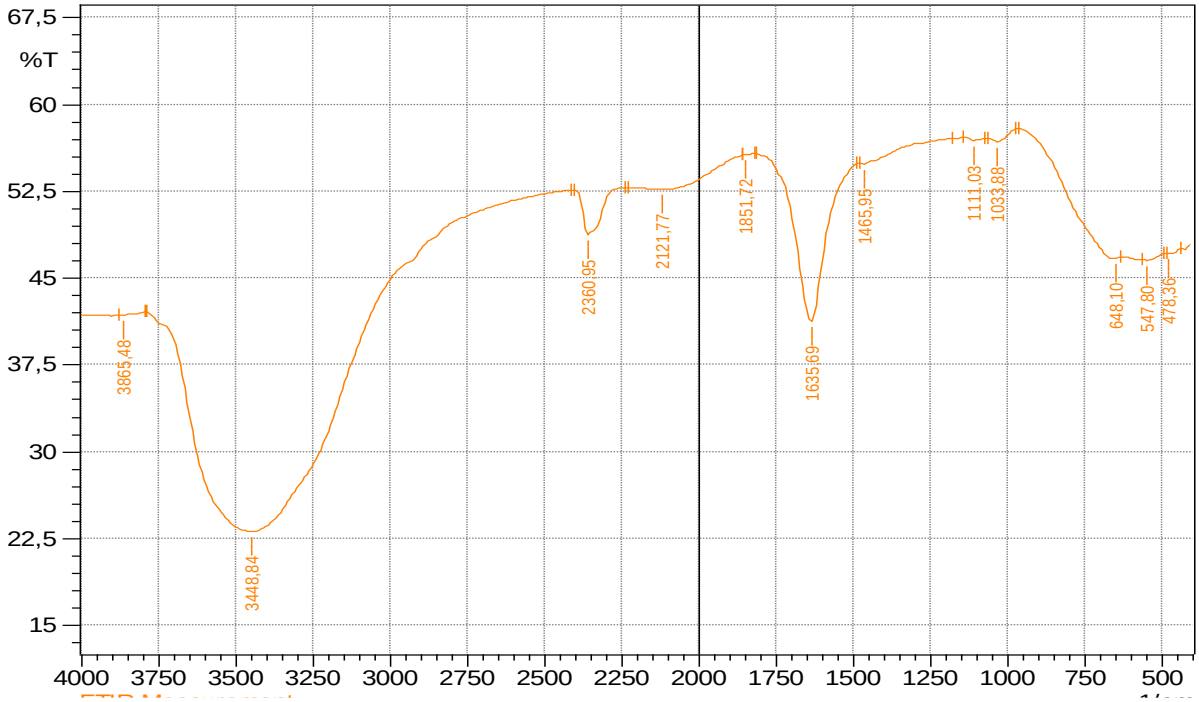


Figure III-9 *FTIR spectrum of silver nanoparticles synthesized by aqueous aerial parts of S. olusatrum extract.*

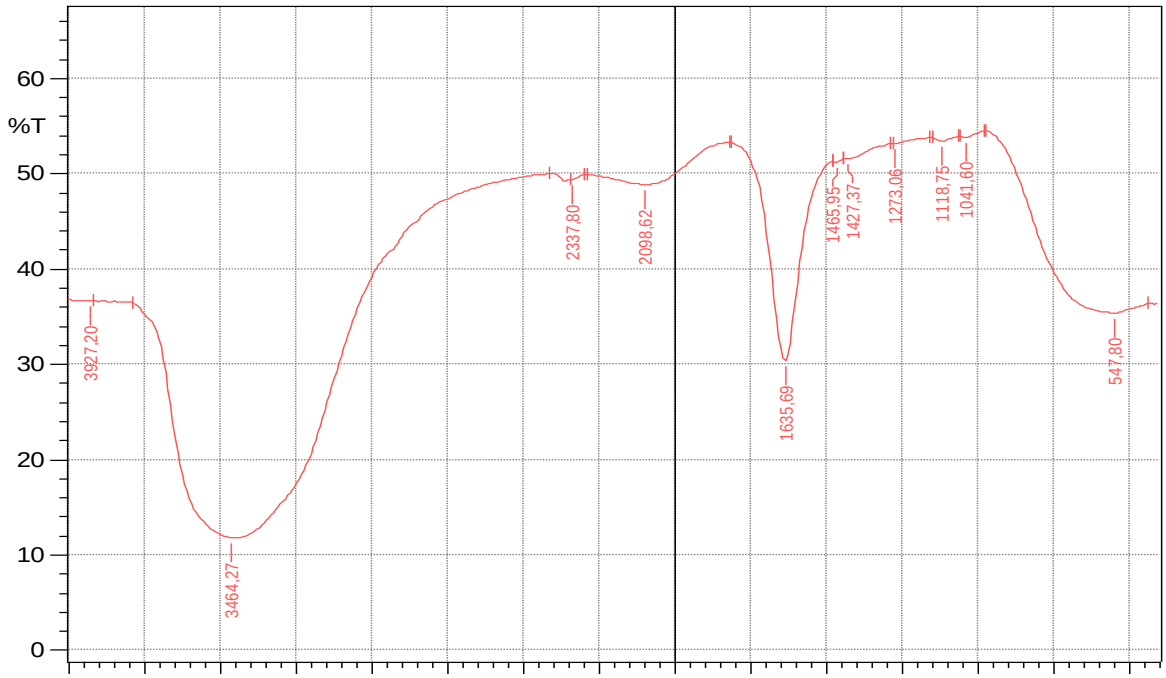


Figure III-10 *FTIR spectrum of silver nanoparticles synthesized by aqueous aerial parts of F. communis extract*

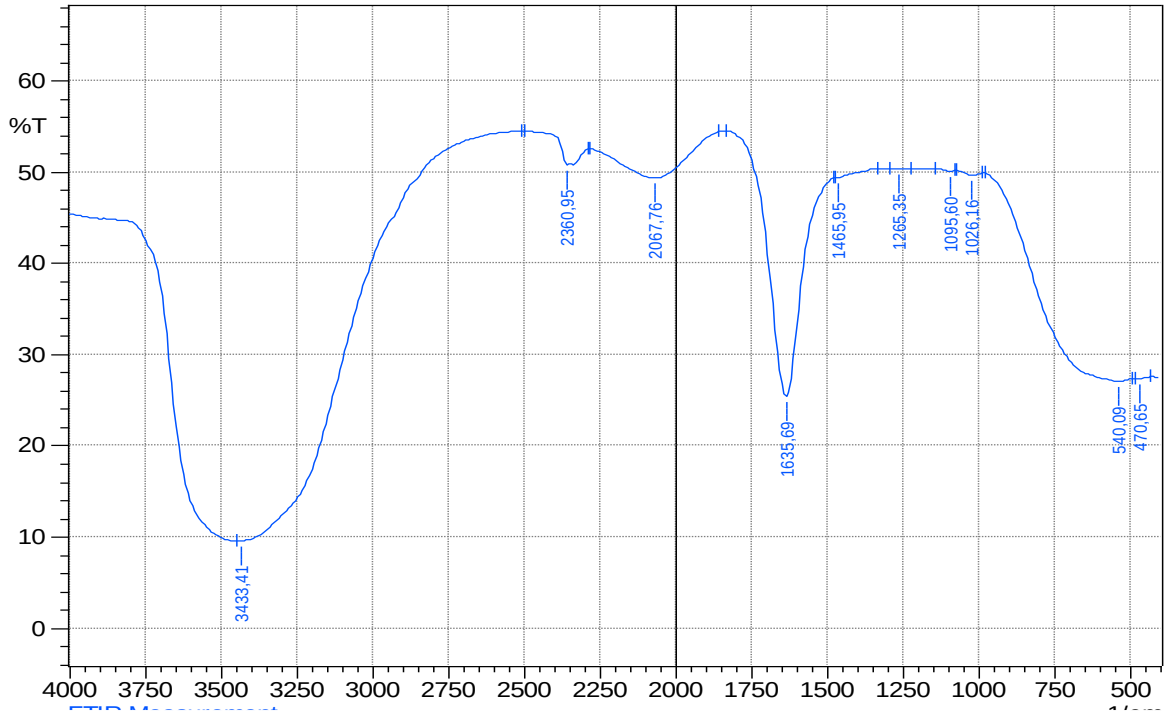


Figure III-12 *FTIR spectrum of silver nanoparticles synthesized by aqueous aerial parts of T. polium*

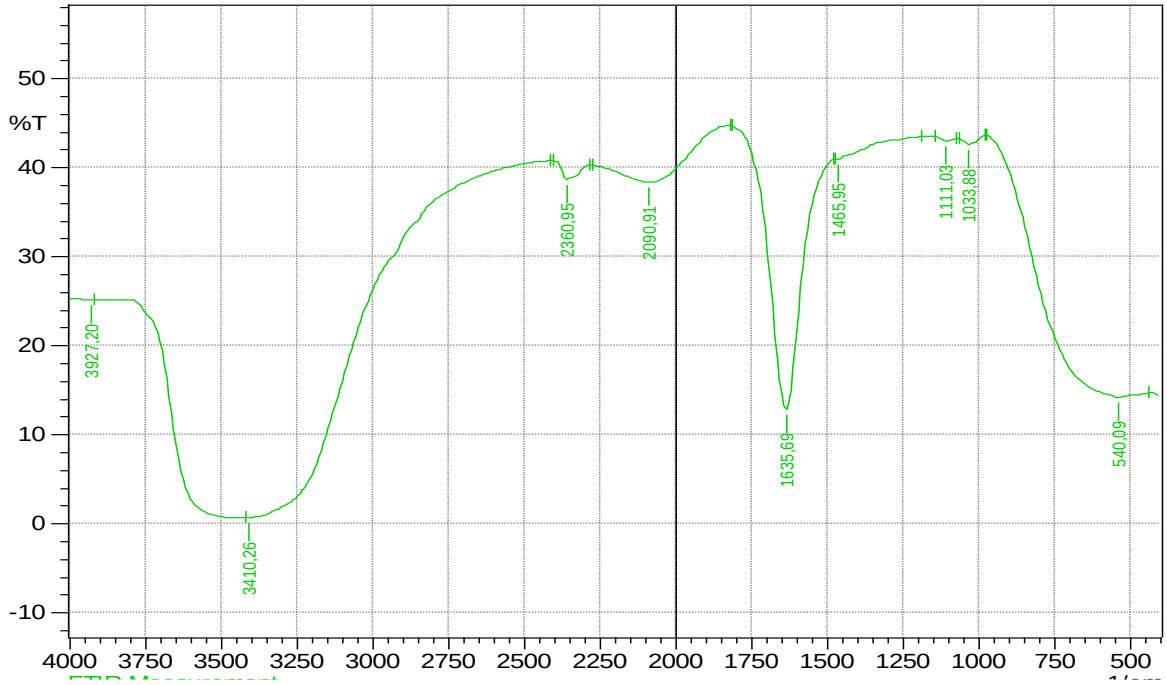


Figure III-11 *FTIR spectrum of silver nanoparticles synthesized by aqueous aerial parts of P. harmala*

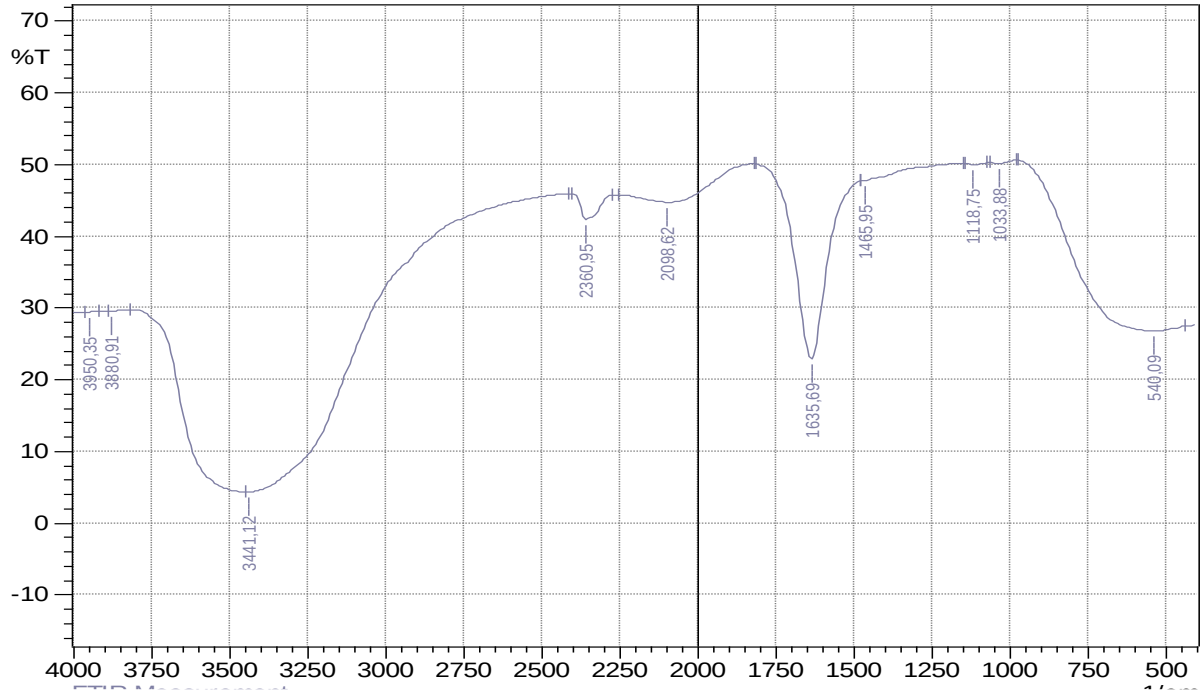


Figure III-13 *FTIR spectrum of silver nanoparticles synthesized by aqueous aerial parts of A. halimus*

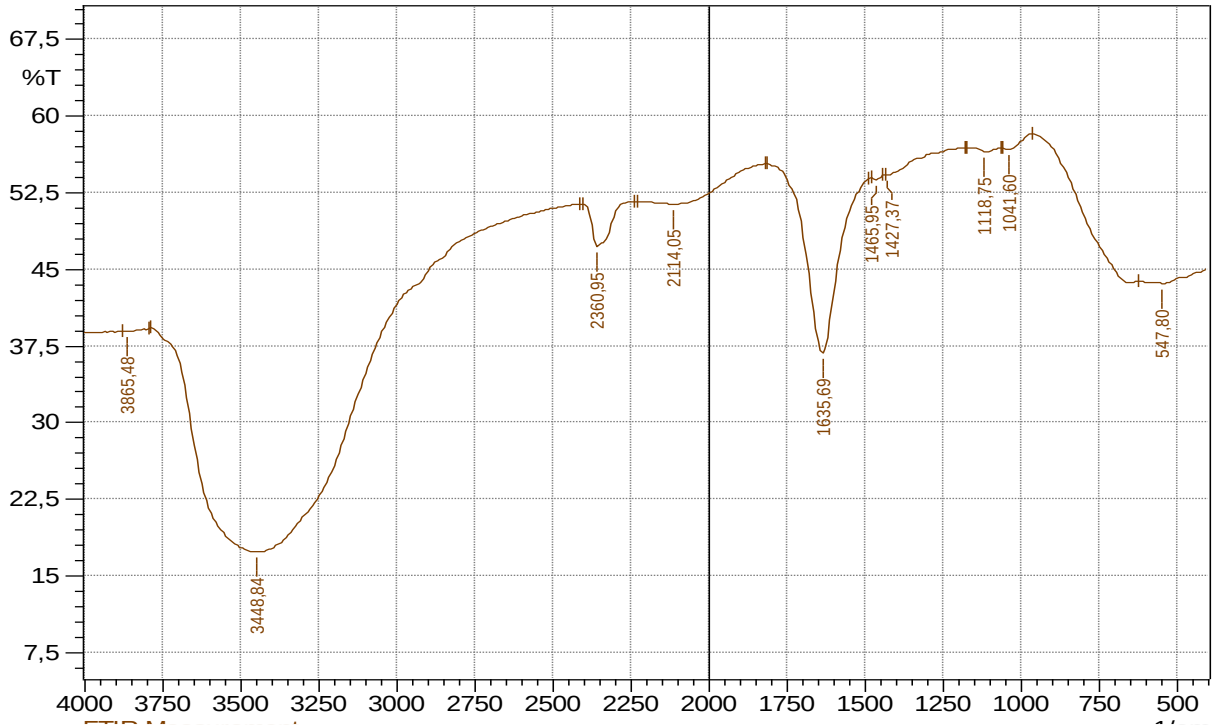


Figure III-14 *FTIR spectrum of silver nanoparticles synthesized by aqueous aerial parts E. arvense*

III.2.2 Biological Activity

III.2.2.1 Antibacterial Activity

- **Results**

The antibacterial activity of green silver nanoparticles (Ag-NPs) was assessed using the agar well diffusion method against several bacterial strains, including *Escherichia coli* (E. coli), *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus*. The zone of inhibition (ZOI) was measured for different concentrations of Ag-NPs (100, 70, 50, and 25 µg/ml), as well as for standard antibiotics streptomycin and AgNO3 as positive controls.

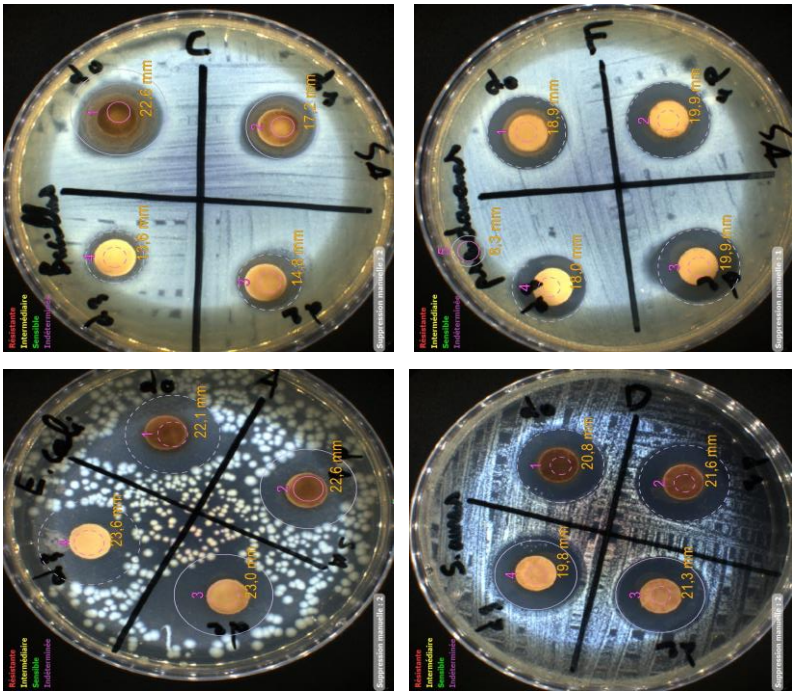


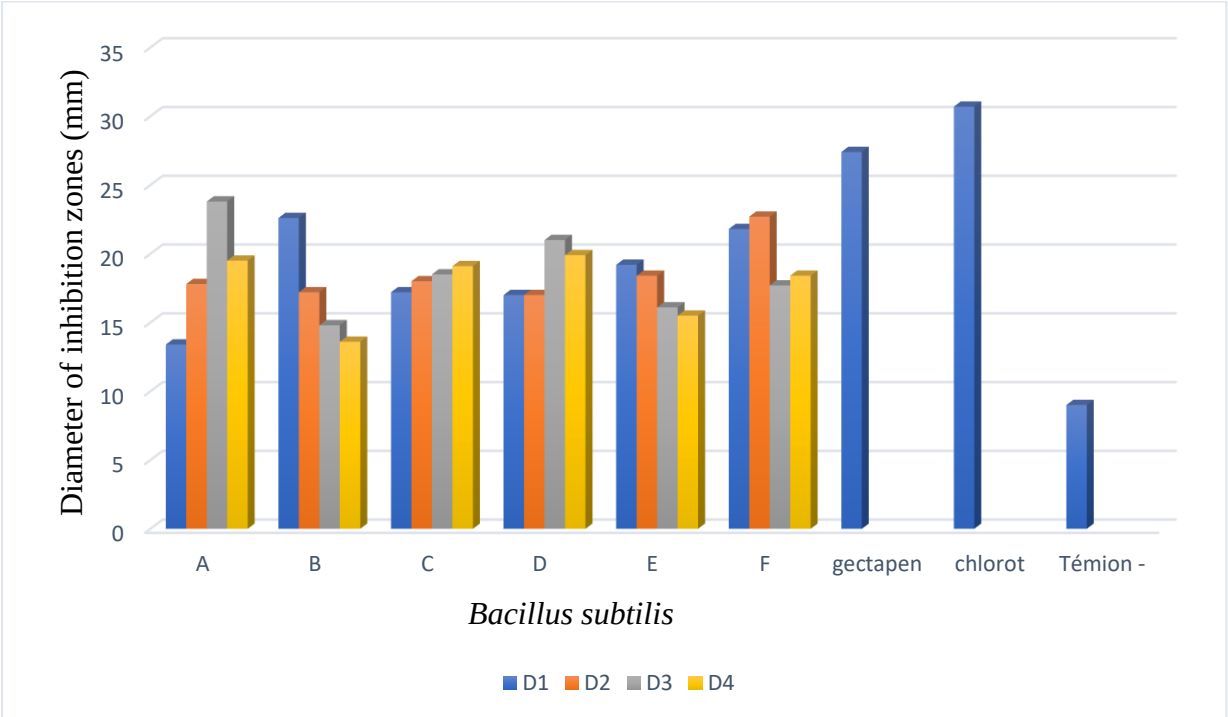
Figure III-15 *The effect of AgNPs of the plants studied on bacterial strains*

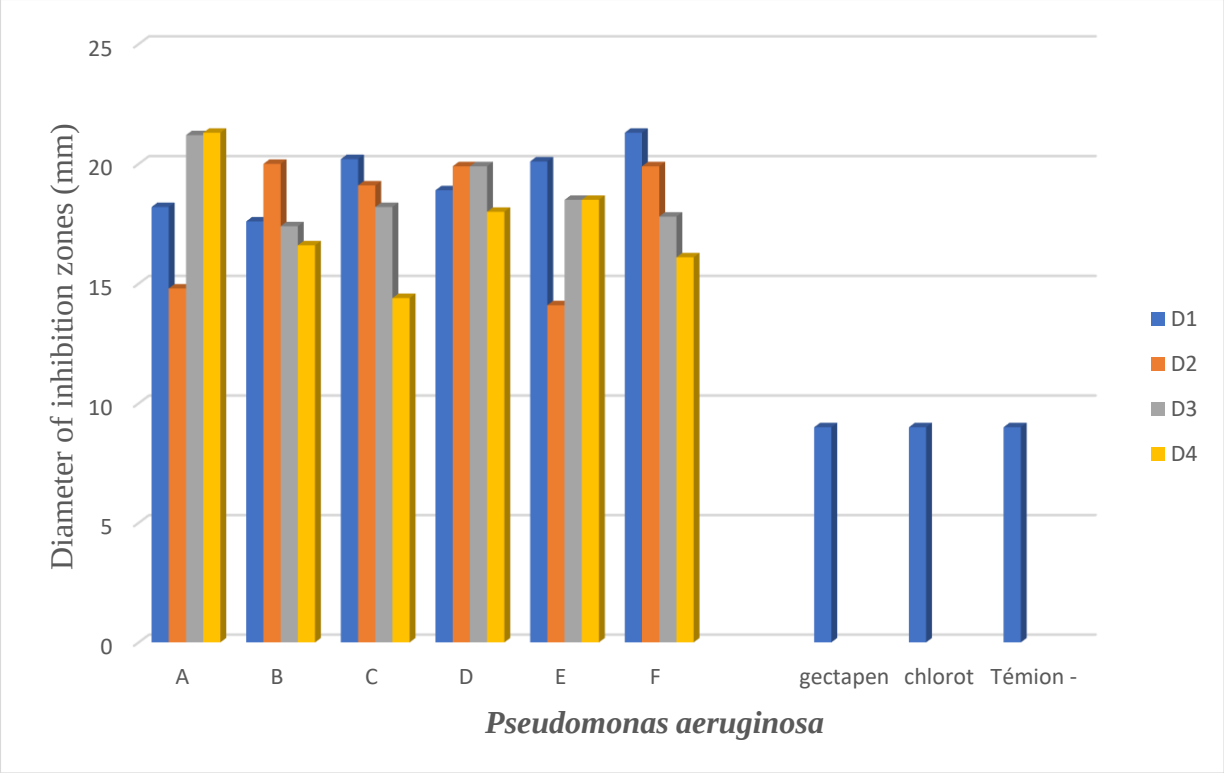
The study's findings show that green silver nanoparticles, or Ag-NPs, which are made from various plant extracts have a strong antibacterial effect on a range of bacterial species. The zones of inhibition differed depending on the bacterial strain and kind of plant extract, suggesting that the target bacteria and nanoparticle supply can affect how effective Ag-NPs are.

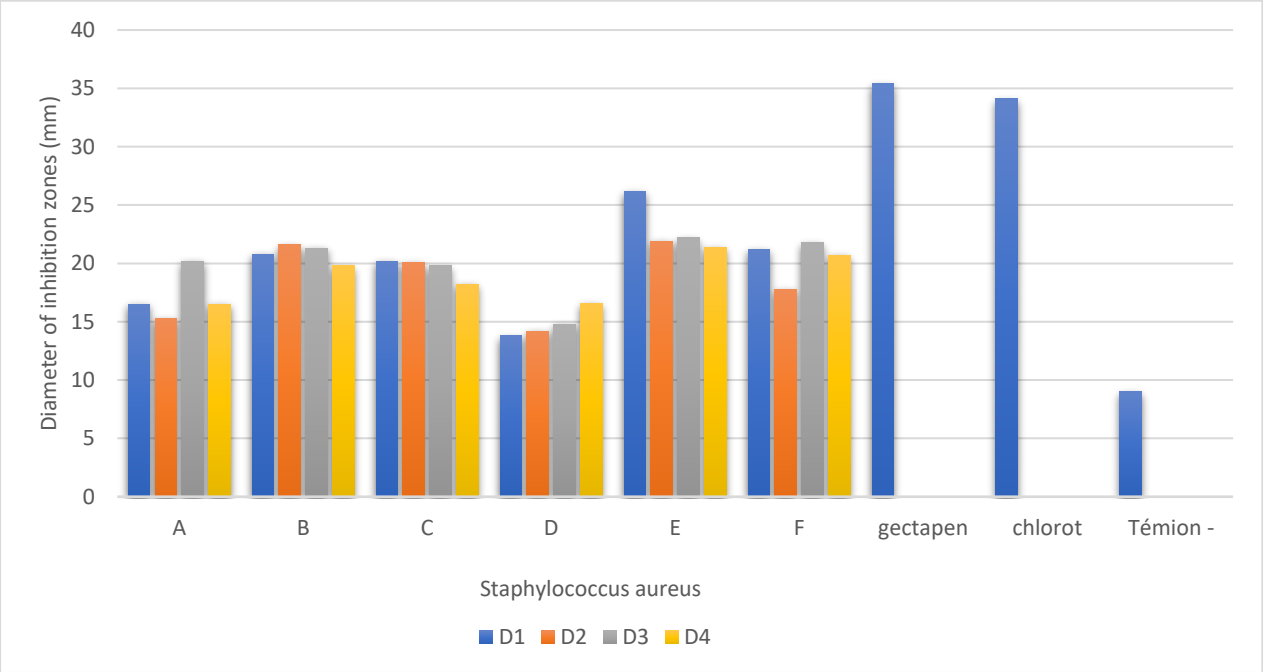
The experimental conditions were validated by the expected high zones of inhibition displayed by the positive control antibiotics, $AgNO_3$ and streptomycin. Nonetheless, some Ag-NPs generated from plants were just as effective as conventional antibiotics, demonstrating the potential of these nanoparticles as substitute antibacterial agents.

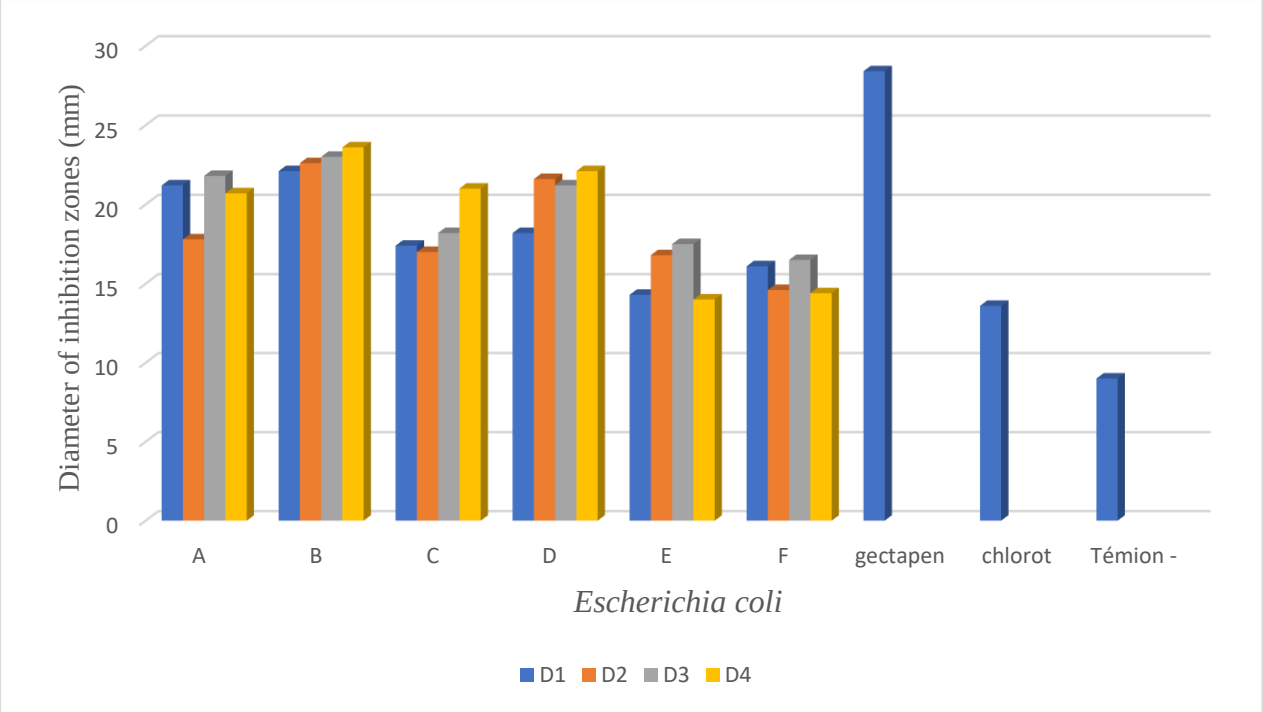
According to the study, utilizing plant extracts for the green synthesis of Ag-NPs is a feasible approach to create potent antibacterial drugs. The fact that the antibacterial activity varies according to the plant source highlights how crucial it is to choose the right plants for the manufacture of nanoparticles in order to efficiently target particular bacterial strains. Deeper understanding of the useful uses of these discoveries in the environmental and medical domains may be obtained by further investigation into the mechanisms of action, synthesis technique optimization, and in vivo studies.

A: *S. olusatrum*	D1: Dose 1
B: *F. communis*	D2: Dose 2
C: *T. polium*	D3: Dose 3
D: *P. harmala*	D4: Dose 4
E: *A. halimus*	
F: *E. arvense*	




25
20
15
10
5
0
Diameter of inhibition zones (mm)
A
B
C
D
E
F
gectapen
chlorot
Témion -
D1
D2
D3
D4
Pseudomonas aeruginosa


40
35
30
25
20
15
10
5
0
Diameter of inhibition zones (mm)
A
B
C
D
E
F
gectapen
chlorot
Témion -
Staphylococcus aureus
D1
D2
D3
D4



➢ **Discussion**

Atriplex halimus and *Ferula communis* showed excellent efficiency against a variety of pathogens, including *E. Coli* and *Staphylococcus aureus*. Strong antibacterial qualities were also demonstrated by *Smyrnium olusatrum* against various strains.

Compared to the plants with high potency, *Teucrium polium* and *Equisetum arvense* showed less consistency in their moderate to high activity and efficiency against many bacterial strains. *Peganum harmala* displayed variable but good antibacterial efficacy.

III.2.2.2 Antifungal Activity

➢ **Results**

Using the well diffusion method, the antifungal activity of green Ag-NPs against *Aspergillus brasiliensis* and *Candida albicans* was assessed. A range of Ag-NP concentrations (150, 200, 250, and 300 µg/ml) were examined in conjunction with AgNO3 and Lamidaz serving as positive controls.

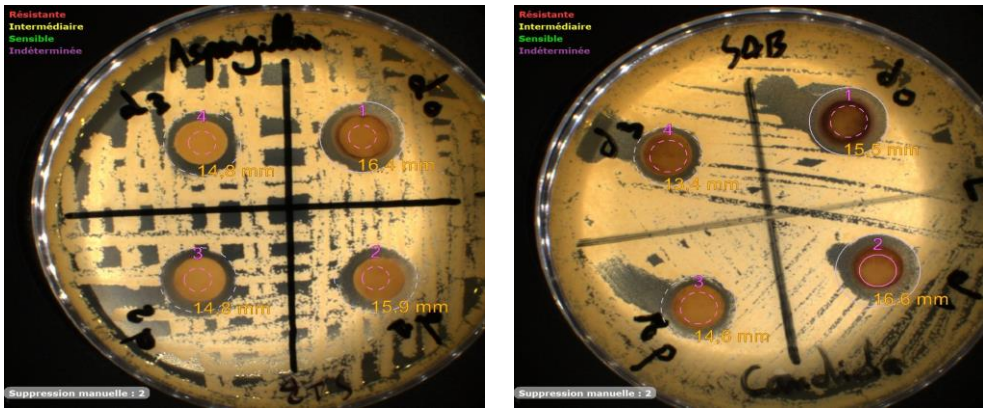
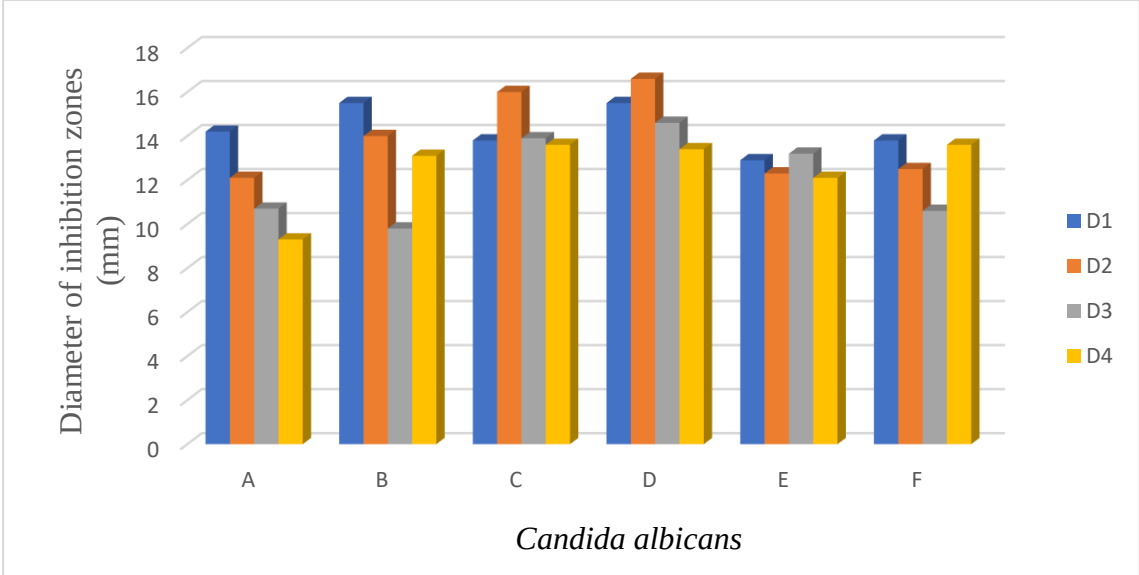
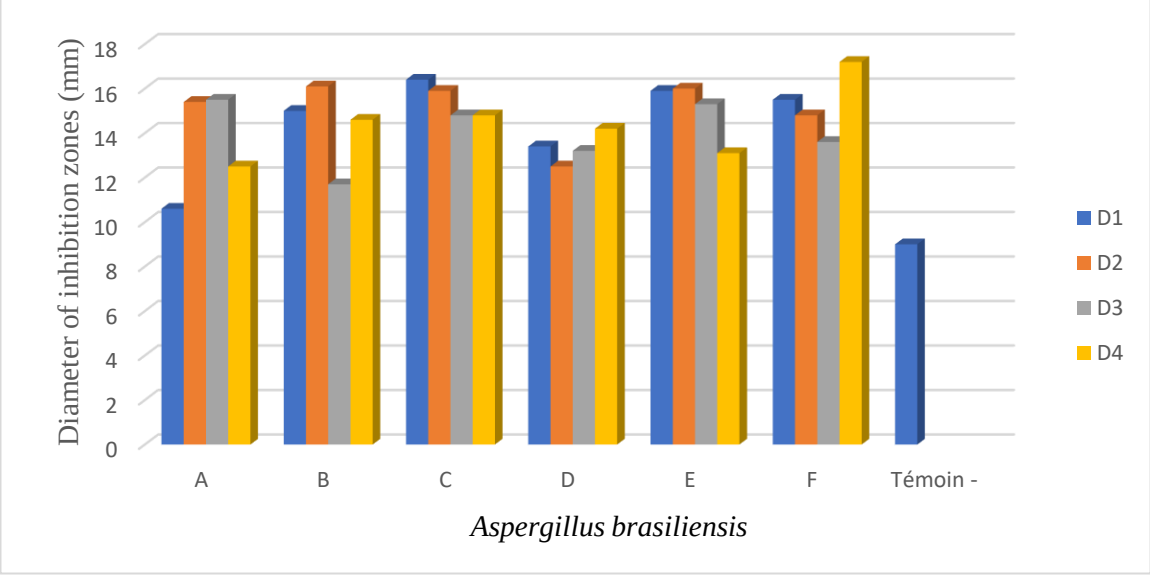


Figure III-16 The effect of AgNPs of the plants studied on bacterial strains





Discussion

All six plant extracts showed varying degrees of antifungal activity against *Candida albicans* and *Aspergillus brasiliensis.* ***F. communis*** and ***T. polium*** were the most effective against both fungal strains, indicating their potential as broad-spectrum antifungal agents. ***E. arvense*** and ***A. halimus*** also demonstrated strong activity against *Aspergillus brasiliensis*. Further research is needed to isolate and characterize the active compounds responsible for these antifungal properties and to assess their potential for therapeutic use.

III.2.2.3 Antioxidant activity of silver nanoparticles:

Free radical scavenging ability on 2, 2-diphenyl-2- picrylhydrazyl (DPPH):

The results shown in Figure represent the results obtained from the average inhibition concentrations (IC50) of the different AgNPs synthesized from the aerial parts of six different plants: *Smyrnium olusatrum, Ferula communis, Teucrium polium, Peganum harmala, Atriplex halimus, and Equisetum arvense.*

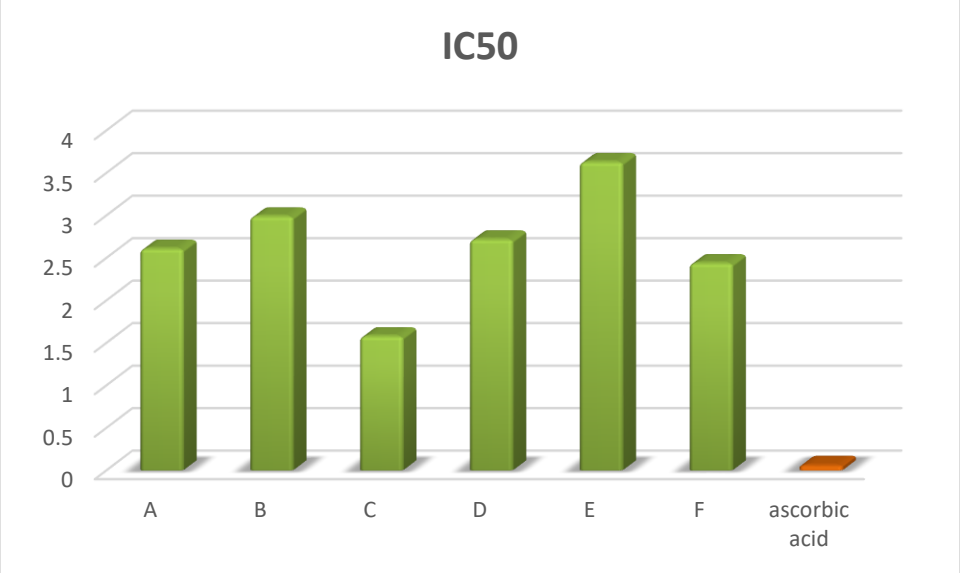


Figure III-17 *Histogram represent the IC50 values of AgNPs of (A) Smyrnium olusatrum, (B) Ferula communis, (C) Teucrium polium, (D) Peganum harmala, (E) Atriplex halimus, and (F) Equisetum arvense.*

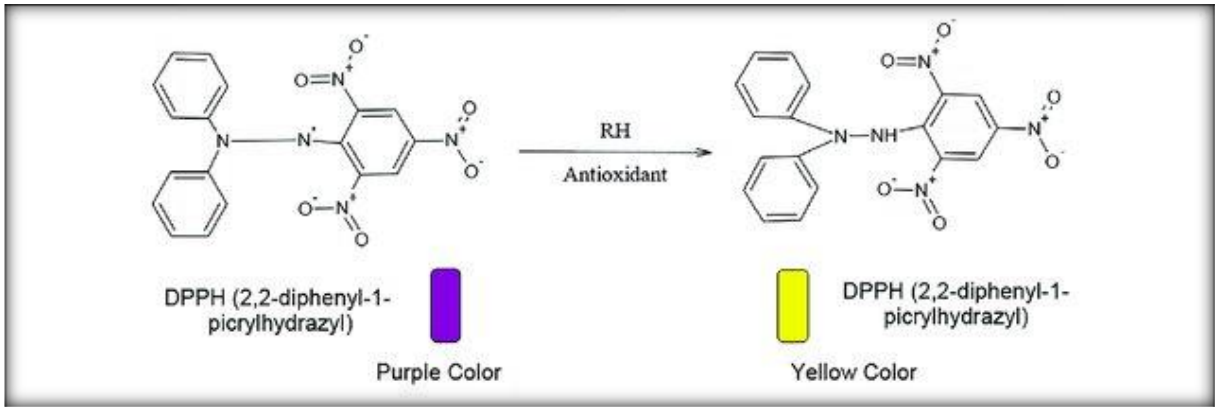


Figure III-18 *Mechanism of antioxidants*

(Shahid et al., 2022).

The antioxidant activity was assessed using the DPPH free radical scavenging method, with ascorbic acid serving as a control (IC50 = 0.06 mg/ml).

- **Results**

The results confirm that while the plant extracts and their synthesized silver nanoparticles exhibit significant antioxidant activities, they are less potent than the control (ascorbic acid, IC50 = 0.06). Among the samples, *Teucrium polium* demonstrated the highest antioxidant activity, followed by *Equisetum arvense* AgNPs, *Smyrnium olusatrum*, Peganum harmala, *Ferula communis*, and *Atriplex halimus*, which indicate the lowest antioxidant activity compared to ascorbic acid. These findings highlight the potential of these plants and their nanoparticles for developing natural antioxidant agents for various applications. Further research and development could explore their use in the pharmaceutical, cosmetic, and food industries.

- **Discussion**

The lower IC50 value indicates a stronger ability of the extract to act as a DPPH scavenger, while the higher IC50 value indicates a lower scavenging activity **(Salari et al., 2019).**

Specifically, the flower extract of *S. olusatrum* had the highest content of total phenolics (48.97 mg GAE/g) and flavonoids (52.63 mg RE/g), which contributed to its potent antioxidant properties **(Hamdi et al., 2024).**

F. communis extracts, particularly the flower extracts, contained high amounts of total phenolics, which contributed to their antioxidant properties **(Rahali et al., 2018).**

T. polium extracts have been shown to possess significant antioxidant activity, which is mainly attributed to phenylpropanoids and flavonoids. The antioxidant activity is proportional to the content of phenolic compounds and flavonoids **(Ljubuncic et al., 2006), (Timizar et al., 2024).**

P. harmala extracts, particularly the methanolic extract, have been shown to possess significant antioxidant activity. The antioxidant activity is attributed to the presence of phenolic compounds, flavonoids, and other secondary metabolites in the plant. The methanolic fraction 2 obtained using bioassay-guided fractionation showed the presence of quinic acid, peganine, harmol, harmaline, and harmine, confirmed by electrospray ionization mass spectrometry and quantified using external standards on high performance liquid chromatography **(Abbas et al., 2021).**

The phytochemical analysis of *A. halimus* extracts revealed the presence of a wide range of bioactive compounds, including flavonoids, polyphenols, alkaloids, saponins, and terpenes, which are known to contribute to the antioxidant properties of the plant. **(Elbouzidi et al., 2022).**

E. arvense extracts, particularly the ethanol extract, have been shown to possess significant antioxidant activity. The antioxidant activity is attributed to the high content of phenolic compounds, flavonoids, tannins, sterols, and saponins in the plant **(Gu et al., 2022).**

III.2.2.4 Cytotoxic activity of silver nanoparticles

The cytotoxic activity of silver nanoparticles was assayed in vitro using human blood erythrocytes (RBCs).

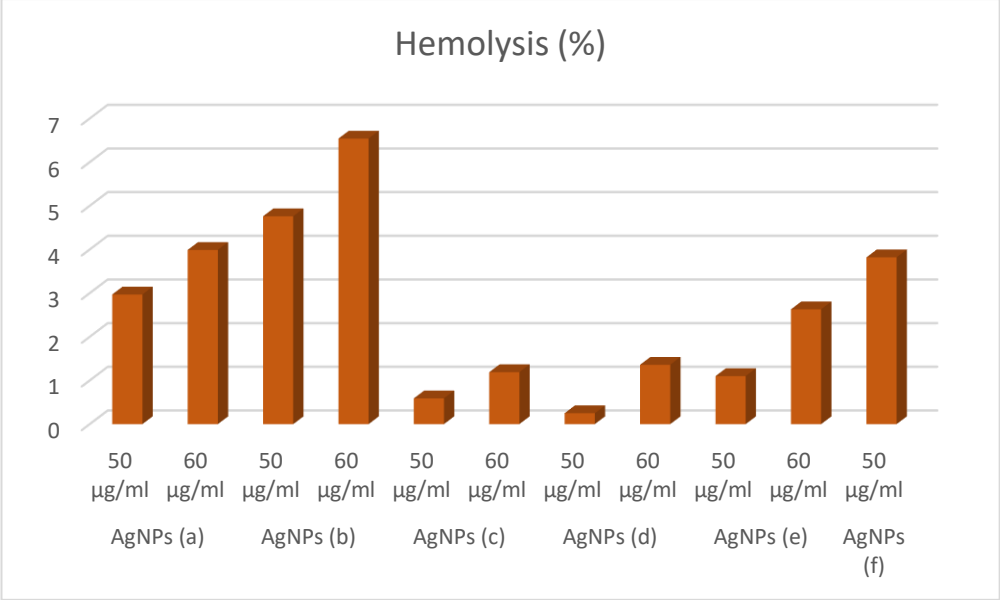


Figure III-19 *Hemolytic activity of AgNPs.*

- **Discussion**

Silver nanoparticles based in medicine will be introduced into the vascular system via injection or orally administration, direct interaction between silver nanoparticles and RBCs may occur frequently. The earlier report on silver nanoparticles have clearly demonstrated that small particles have higher hemolytic activity compared to large particles **(Hashim, 2018).**

The anti-hemolytic activity of the *T. polium* extracts was strongly correlated with their total contents of phenolics, flavonoids, alkaloids, and saponins. These phytochemical constituents are known to possess anti-inflammatory properties **(Sharifi-Rad et al., 2022).** Additionally, a study reported that a methanolic extract of T. polium protected red blood cells against lipid peroxidation induced by hydrogen peroxide **(Boumerfeg et al., 2012).**

bioactive compounds like chlorogenic acid, hesperetin, and rutin present in P. harmala extracts have promising potential as inhibitors of the COX-2 enzyme, which is involved in the inflammatory response **(Djarmouni et al., 2022).** the methanol extract of *P. harmala* seeds exhibited significant protective effects against AAPH-induced oxidative hemolysis of red blood cells **(Djarmouni et al., 2024).**

Another study evaluated the anti-inflammatory and hemostatic activities of the methanolic extract of *A. halimus* leaves collected in Algeria. The results showed that the extract had significant anti-inflammatory effects, as demonstrated by its ability to inhibit hypotonic-induced and heat-induced hemolysis of red blood cells. The extract also exhibited hemostatic

activity, as it decreased the plasma recalcification time in a dose-dependent manner **(Yahia, 2023).**

The methanol extract of E. arvense showed significant anti-hemolytic activity, protecting red blood cells from lipid peroxidation induced by hydrogen peroxide **(Roumil et al., 2022).**

Conclusion

conclusion

In conclusion, the green synthesis of silver nanoparticles using plant extracts provides an eco-friendly and efficient method for producing nanoparticles with potent antimicrobial and antioxidant activities. The study demonstrated that extracts from ***Atriplex halimus, Smyrnium olusatrum, Ferula communis, Teucrium polium, Peganum harmala***, and **Equisetum arvense** are effective in synthesizing AgNPs. The characterization techniques confirmed the successful formation of nanoparticles, and their biological activities were validated through comprehensive antimicrobial and antioxidant assays. These findings highlight the potential application of plant-synthesized AgNPs in various fields, including medicine, agriculture, and environmental protection. Future research could focus on exploring the mechanisms underlying the biological activities of these nanoparticles and scaling up the synthesis process for industrial applications. Overall, this work contributes to the growing body of knowledge on green nanotechnology and its applications in developing sustainable solutions for health and environmental challenges.

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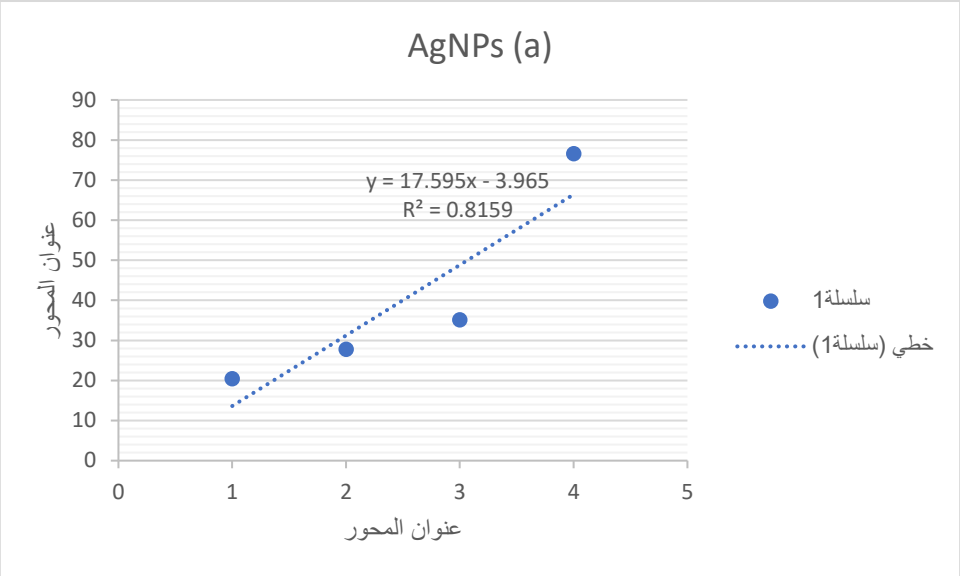
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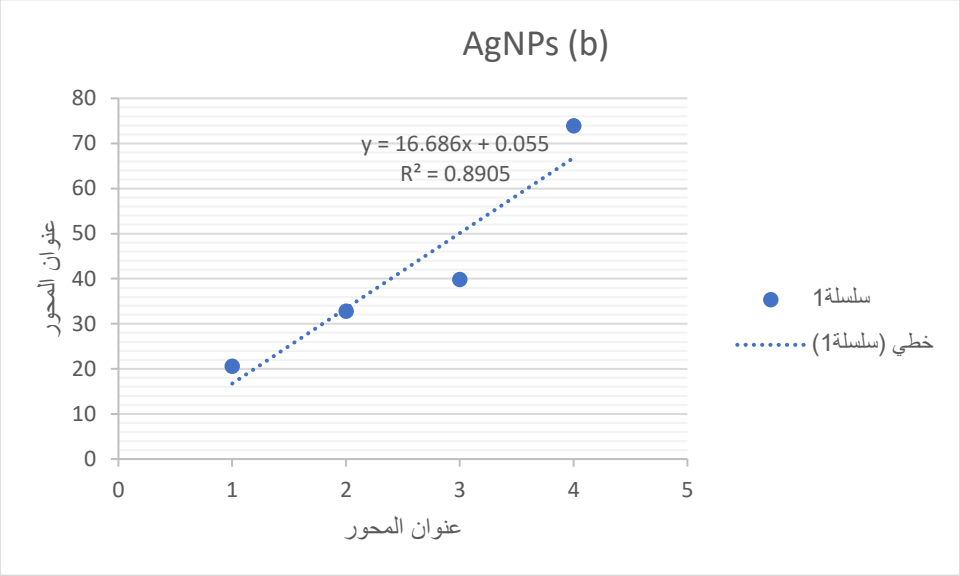
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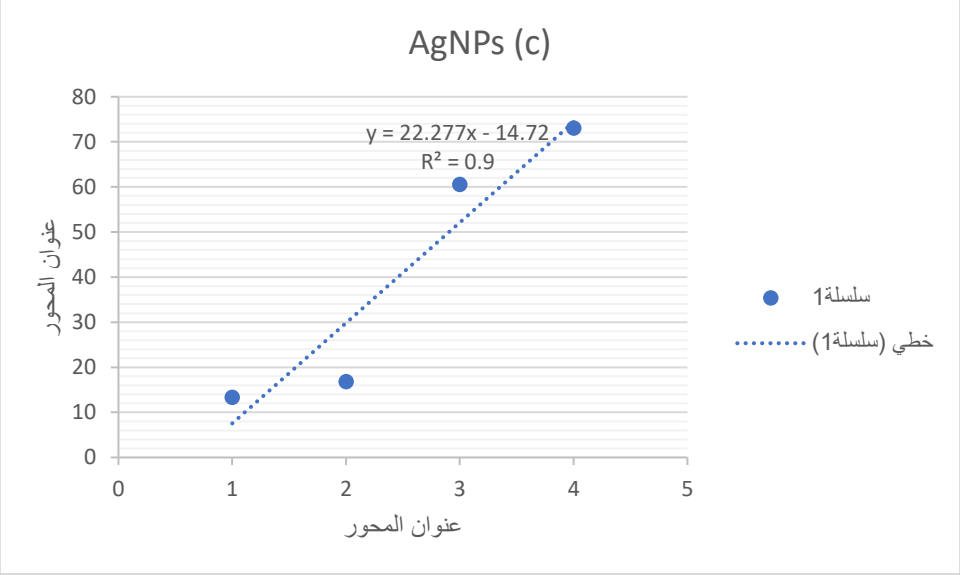
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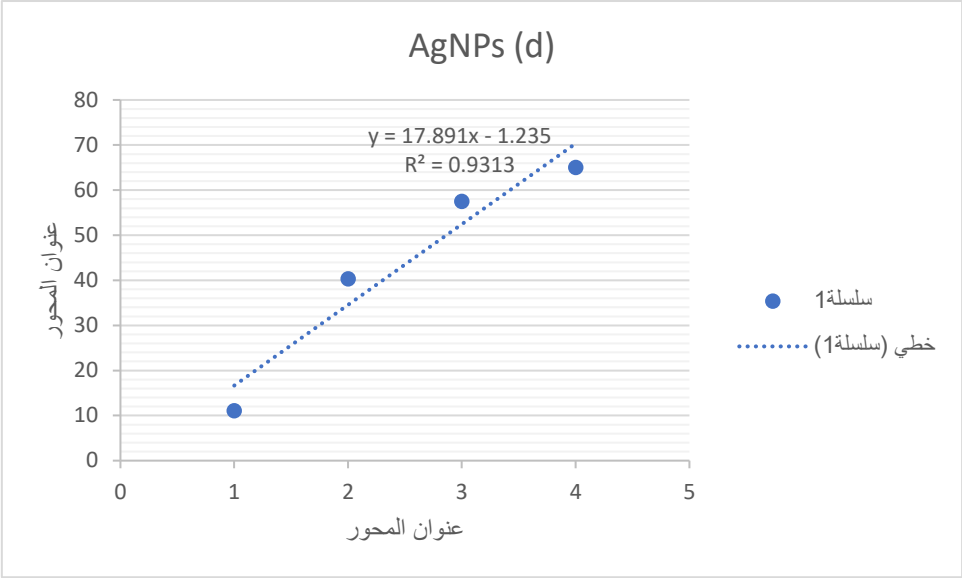
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10. **Equisetum L. | Plants of the World Online | Kew Science**
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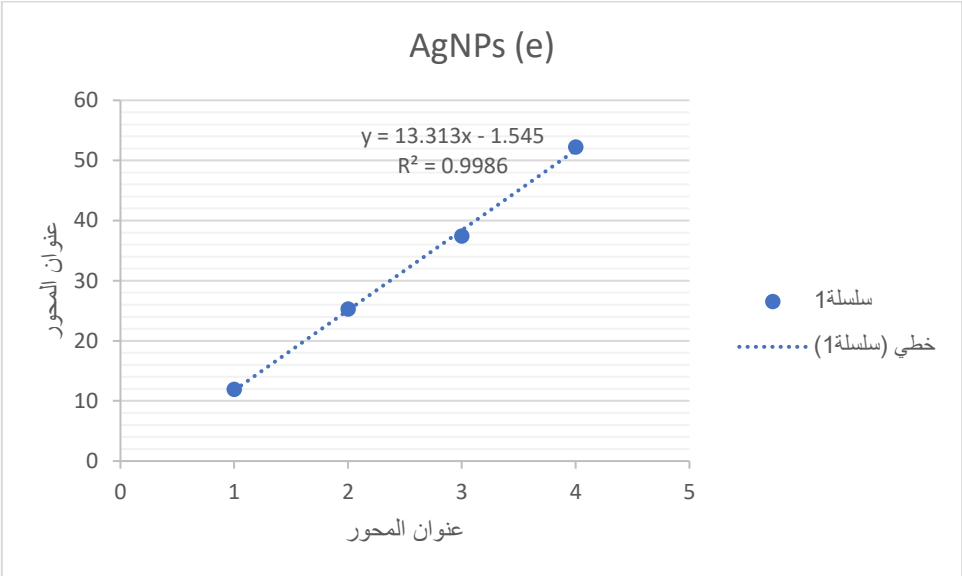
Annexes

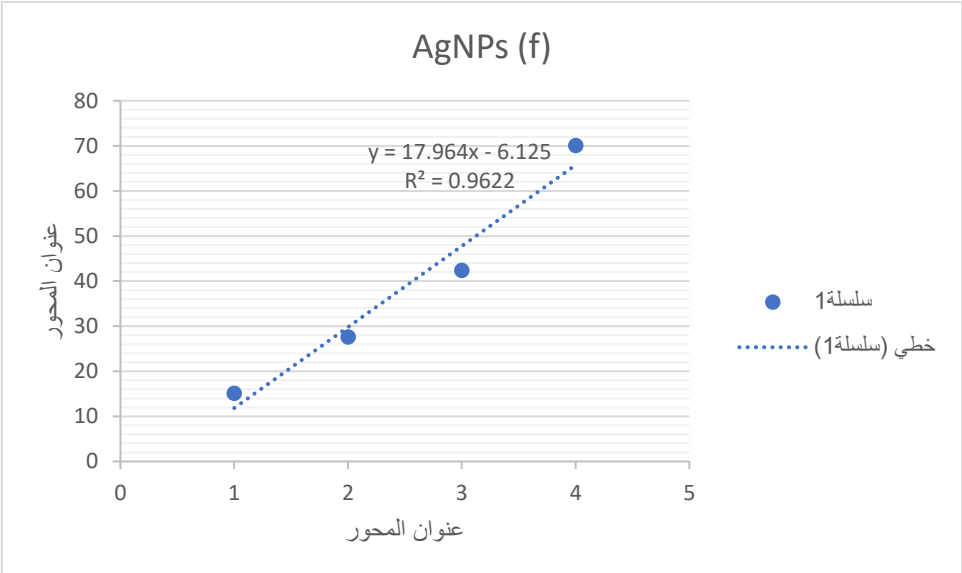

AgNPs (a)
y = 17.595x - 3.965
R² = 0.8159
عنوان المحور
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خطي (سلسلة1)


AgNPs (b)
y = 16.686x + 0.055
R² = 0.8905
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عنوان المحور
سلسلة1
خطي (سلسلة1)


AgNPs (c)
y = 22.277x - 14.72
R² = 0.9
عنوان المحور
عنوان المحور
سلسلة1
خطي (سلسلة1)


AgNPs (d)
y = 17.891x - 1.235
R² = 0.9313
عنوان المحور
عنوان المحور
سلسلة1
خطي (سلسلة1)


AgNPs (e)
y = 13.313x - 1.545
R² = 0.9986
عنوان المحور
عنوان المحور
سلسلة1
خطي (سلسلة1)


AgNPs (f)
y = 17.964x - 6.125
R² = 0.9622
عنوان المحور
عنوان المحور
سلسلة1
خطي (سلسلة1)