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ONDOKUZ MAYIS UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF NANOSCIENCE AND NANOTECHNOLOGY

THE PRODUCTION OF METAL NANOPARTICLES WITH PLANT EXTRACTS BY USING LASER ABLATION METHOD

Master's Thesis

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Supervisor

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ACCEPTANCE AND APPROVAL OF THE THESIS

The study entitled **"THE PRODUCTION OF METAL NANOPARTICLES WITH PLANT EXTRACTS BY USING LASER ABLATION METHOD"** prepared by **Alya Abdulkhaleq Mohammed AL-ANBAGI**, and supervised by **Assist. Prof. Dr. Sinem ÇEVİK**, was found successful and unanimously accepted by committee members as Master thesis, following the examination on the date 28.12.2023 .

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ÖZET

LAZER ABLASYON YÖNTEMİ KULLANILARAK BİTKİ EKSTRAKTLARINDAN METAL NANOPARTİKÜLLERİN ÜRETİMİ

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Elettaria cardamomum şunları içerir: Glikozitler, Alkaloidler, Saponinler, Tanenler ve Uçucu yağlar, çeşitli bitki ekstraktlarında yüksek konsantrasyonlarda bu bileşik suda çözünür. Antikanser aktiviteye sahip olduğu gösterilmiştir. Ancak çözünürlüğü ve biyoyararlılığı nedeniyle terapötik hedeflerine ulaşmak için bir dağıtım sisteminin kullanılmasını gerektirir. Bu çalışmada, şerit nanoparçacıkları (Car@AgNPs) üzerine yüklenen Elettaria kakule, indirgeyici ve örtücü ajanlar olarak sıcak eşdeğerler Elettaria kakule, gümüş nanopartikülleri ile lazer ablasyon sentezi ile hazırlandı. Mevcut biyosentez yöntemi basit ve düşük maliyetliydi. Gümüş nanoparçacıkların hazırlanması ve hazırlanan çözeltinin renginin sarıdan açık kahverengiye değişmesiyle Elettaria kakule (çözünmüş soluk sarı) eklenmesi sonrasında gümüş nanopartiküller (Car@AgNPs) üzerine yüklenen Elettaria kakule oluşumu gözlendi. Cae@AgNP'lerin sentezini doğrulamak için UV, FTIR, XRD, TEM ile tanımlanan Elettaria kakule yüklü gümüş nanopartiküllerini (Car@-AgNP'ler) üretmek için lazer ablasyon kullanıldı. Bu testler, gümüşten Elettaria kakule karışımını doğrulamak için kullanıldı. nanopartiküller. Oysa Elettaria Cardamomum'un şerit nanopartikülleri üzerindeki absorpsiyon spektrumları 412 nm'lik kavisli bir absorpsiyon gösterdi. Gümüş nanoparçacıklara yüklenen Elettaria cardamomum'un moleküler ve fonksiyonel gruplarını değerlendirmek için FTIR kullanıldı. XRD ve TEM Çalışmaları, gümüş nanoteknolojik parçacıklara yüklenen Elettaria cardamomum'un varlığını doğrulamış ve ortalama 4-45 nm boyutunda düzenli küresel parçacıklar bulunmuştur. Cae@AgNP'lerin antioksidan aktivitesi DPPH'ye karşı test edilmiş ve sonuçlar Car@'ı ortaya çıkarmıştır. AgNP'lerin güçlü bir antioksidan etkisi ve önemli bir yüksek radikal çekim aktivitesi vardı ve bu antioksidan aktivite odaklanmaya dayalıydı, verimlilik oluştuğunda daha fazla odaklanma vardı. ve Escherichia coli de incelenmiştir. Sonuçlar, Elettaria cardamomum ve AgNP'lerle işlenmiş numunenin sentezinin inhibisyonunda bir artış olduğunu ve bu aktivitenin Car@AgNP'lerde daha güçlü olduğunu göstermektedir. Ayrıca Gram-negatif ve Gram-pozitif bakteriler arasında daha sonra tartışılacak olan çeşitli nedenlerden kaynaklanan inhibisyon farkı gözlendi. Car@AgNP'lerin insan meme kanseri hücre dizisi (MCF-7) üzerindeki sitotoksik etkisi değerlendirildi. Hücre canlılığı, MCF-7 hücrelerinde Car@AgNP'ler tarafından konsantrasyona bağlı bir şekilde bastırıldı.

Anahtar Sözcükler: Gümüş nanopartikülleri ve Elettaria kakule; Antioksidan; Antibakteriyel; Sitotoksisite; MCF-7 hücreleri.

ABSTRACT

THE PRODUCTION OF METAL NANOPARTICLES WITH PLANT EXTRACTS BY USING LASER ABLATION METHOD

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Elettaria cardamomum contains: Glycosides, Alkaloids, Saponines, Tannins, and Volatile oils,in high concentrations in several plant extracts this compound is Soluble in water.hat has been shown to have anticancer activity. However, due to its solubility and bioavailability, necessitating the use of a delivery system to achieve its therapeutic goals. In this study, Elettaria cardamomum loaded on sliver nanoparticles (Car@AgNPs) was prepared by laser ablation synthesis with hot equaes Elettaria cardamomum, silver nanoparticles as reducing and covering agents. The current biosynthesis method was simple and low cost. The formation of Elettaria cardamomum loaded onto silver nanoparticles (Car@AgNPs) was observed after preparation of silvernanoparticles and addition of Elettaria cardamomum (dissolved pale yellow) by changing the color of the prepared solution from yellow to light brown. laser ablation was used to generate Elettaria cardamomum-loaded silver nanoparticles (Car@-AgNPs) identified by UV, FTIR, XRD, TEM, to validate the synthesis of Cae@AgNPs.These tests were used to confirm the mixture of Elettaria cardamomum from silver nanoparticles. Whereas, the absorption spectra of Elettaria cardamomum on sliver nanoparticles showed a curved absorption of 412 nm. FTIR was used to evaluate the molecular and functional groups of Elettaria cardamomum, which were loaded onto silver nanoparticles. XRD and TEM Studies confirmed the presence of Elettaria cardamomum loaded on silver nanotechnology particles, and regular spherical particles were found with an average size of 4-45 nm.The antioxidant activity of Cae@AgNPs was tested against DPPH, and the results revealed thatCar@AgNPs had a strong antioxidant effect and a significant activity of high radical attraction and that this antioxidant activity was based on focus, with the greater the focus whenever efficiency occurred.The antimicrobial activity of AgNPs, Elettaria cardamomum and Car@AgNPs on resistance to Staphylococcus aureus and Escherichia coli has also been studied. The results show an increase in the inhibition of the synthesis of Elettaria cardamomum and AgNPs treated sample, and this activity was more robust in Car@AgNPs. Also, the inhibition difference was observed between Gram-negative and Gram-positive bacteria, which is due to several reasons that were discussed later.The cytotoxic effect of Car@AgNPs on the human breast cancer cell line (MCF-7) was assessed. Cell viability was suppressed in a concentration-dependent manner by Car@AgNPs in MCF-7 cells.

Keywords: Silver nanoparticles and Elettaria cardamomum; Antioxidant; Antibacterial; Cytotoxicity; MCF-7 cells.

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Alya Abdulkhaleq Mohammed AL-ANBAGI

CONTENTS

SYMBOLS AND ABBREVIATIONS

Ag^+	: Silver ion
AgNPs	: Silver Nanoparticles
Car@AgNPs	: Sliver nanoparticles conjected with E. cardamomum
D.W	: Distilled water
DNA	: Deoxyribonucleic acid
DPPH	: 1,1-Diphenyl-2-picryl-hydrazyl
E. cardamom	: Elettaria cardamomum
E.coli	: Eisherishia coli
FCC	: Face Centered Cubic
FTIR	: Fourier Transform Infrared spectrometer
MCH-7	: Breast cancer cell line
NPs	: Nanoparticles
ROS	: Reactive oxygen species
S. aureus	: Staphylococcus aureus
SPR	: Surface Plasmon resonance
TEM	: Transmission electron microscopy
XRD	: X-Ray diffraction
ʎmax	: Lambda max

FIGURES LEGENDS

TABLES LEGENDS

1. INTRODUCTION

Nanotechnology is a new and evolving field of applied science that combines chemistry and physics for a variety of applications (Khashan et al., 2021). Nanotechnology is a young and developing branch of applied science with a wide range of uses, including packaging for wastewater treatment, electronics, and food processing (Krishnan et al., 2018). The application of nanotechnology in pharmaceuticals and healthcare, or Nano medicine, has raised hopes for its potential as novel therapeutics and diagnostic tools (Aw-Yong et al., 2018).

Liquid-phase pulsed laser ablation (LP-PLA) is a promising technique for the manufacture of metal oxide NPs because this method provides suitable processing conditions (i.e. high temperature, high pressure, and high density) for the formation of metastable states and even novel nanomaterials. Moreover, LP-PLA is a fast, simple, and low-cost technique, whose processing parameters are easy to control. The laser components include an effective modern medium, a perfect reflective mirror, electronic pumping source, partially reflective mirror, and laser beam outlet (Jawad, et al .,2022 ; Jawad ,2023).

Due to their unique properties and multi-surface functions, Sliver nanoparticles have been widely used in biotechnology. It is widely used in drug delivery systems due to its biocompatibility properties and high surface area ratio .(Xu L et al .,2020), the surface area greatly increases drug density on the surface, allowing to increase drug concentration or load multiple drugs onto single molecules (Aisida et al., 2019). Some studies currently suggest that using Sliver nanoparticles as drug carriers for cancer treatment yields significantly better results than anticancer drug therapy . As a result, extensive research on the development of sliver nanoparticles has been conducted. The nanocarrier and its potential uses in cancer biology and nanomedicine (Zahoor et al .,2021).

Elettaria cardamomum a herb with a leafy stem and a perennial root stalk, is grown in marshy areas alongside mountain streams in Nepal and India (Ela-Segaey et al., 2007). The biochemical analysis revealed that watery extracts of *Elettaria* cardamomum contains: Glycosides, Alkaloids, Saponines, Tannins, and Volatile oils, this compound is Soluble in water (Mahmood et al., 2010). Much research has been conducted in recent years with the goal of discovering more effective and safe natural and synthetic compounds that can be used in the prevention and/or treatment of diseases. Several plant and animal extracts demonstrated various biological activities. According to (Ela-Segaey et al. 2007), E.cardamomum is a sizable perennial herbaceous, rhizomatous monocot that is a member of the Zingiberaceae family and the cardamomum species (Maton) (Li G et al .,2010) . The biochemical analysis revealed that watery extracts of Elettaria cardamomum contains: Glycosides, Alkaloids, Saponines, Tannins, and Volatile oils, this compound is Soluble in water (Mahmood et al., 2010).

It is well known for its bioactive compounds and flavoring ingredients found in dried ripe fruit (pods or capsules), which are valuable to the cosmetic, food, and pharmaceutical industries (Noumi et al., 2018). Cardamom is frequently used in the food industry to flavor processed foods, beverages, and confections. It has a variety of uses in the cosmetic industry, including fragrances, aromatherapy, and hair and skin care products (Guzmán and Lucia, 2021). Additionally, cardamom has been employed in the pharmaceutical industry among other things as an antimicrobial, antibacterial, and antioxidants (Ashokkumar, et al.,2020). Biocompatibility and antibacterial properties are the most important factors in designing novel biomaterials.in these study we designed sliver nanocomposite and Cardamom by laser ablation that contains: (A) sliver nanoparticles, positively charged, hydrophilic. (B) Cardamom Soluble in alcohol and other organics solvents and insoluble in water. that functionalize the surface of the Ag and bioactive carrier of the functionalized Ag nanoparticles. The principal aim of this contribution is to develop an inexpensive, effective, and aqueous procedure for synthesis of AgNPs-E.cardamomum composite and evaluate their antibacterial performance ,antioxdant ,and anticancer activity.

1.1 Aim of the Study

The Laser ablation techqnue of this study is to improve medicine efficiency in biomedical applications by loading E.cardamomum seeds on sliver nanoparticles by Laser ablation in liqued , so several approaches were used to accomplish this:

1) Hot aqueous extract of the E.cardamomum seeds plant
2) Preparation of sliver nanoparticles using Laser Ablation Method.
3) Conjugates (E.cardamomum) onto sliver nanoparticles by Laser ablation in liqued.
4) Characterization of sliver nanoparticles conjugates with E.cardamomum inclued by X-ray diffraction (XRD), UV/Vis

spectrophotometry(UV-is), Fourier infrared spectroscopy (FTIR), and Transmission electron microscopy (TEM)

5) DPPH assays were used to determine the free radical scavenging activity of E.cardamomum - conjugates sliver nanoparticles.
6) Antibacterial activity of sliver nanoparticles conjugates with cardamom against Staphylococcus aureus and Escherichia coli.
7) MTT assay in vitro studies and the effect on morphological appearance changes of human breast (MFC-7) cancer cell line.

1.2 Objectives

The objectives of the current study were:

1) Using a natural product in many medical and biological applications.
2) Studying several biological and medical applications of these products, such as being anti-bacterial and anti-cancer.
3) Experimenting with the use of available and inexpensive materials to convert them into materials that contribute to the treatment of some diseases
4) The introduction of natural materials and products, in addition to medicines and antibiotics, in the treatment and diagnosis of diseases.
5) Finding the properties of E.cardamomum -loaded sliver nanoparticles and the compound used in the research based on several tests such as FTIR, UV, XRD, and TEM.

2. LITERATURE REVIEW

2.1 Nanotechnology

One billion parts per billion (10-9) is denoted by the prefix "nano," which comes from the Greek word "nanos," which means "small." Nanoparticles are small, uniform particles with one or more dimensions and sizes between one and one hundred nanometers. The unique characteristics of nanoparticles (NPs) are mostly influenced by their size. The NPs' size and form were controlled by using a variety of stabilizers, solvents, reducing agents, and other agents during the preparation process. (Loeve, 2010). Nanotechnology has been used mainly in Nano medical products as it has proven that it is non-toxic to human cells at low concentrations, but is effective against cancer cells, bacteria, viruses, and fungi. It is also used in many industries such as the textile industry, and food preservation. There are many current and anticipated developments in nano-science and nanotechnology with applications in agriculture, electronics, medicine, etc (Pramanik et al.,2020).

Nanoparticles have unique characteristics that come from their surface shape, such as a high surface area to volume ratio qualities, antibacterial properties, anticancer capabilities, electrical properties, and antioxidant properties, as compared to their bulk counterparts (Vala, et al., 2021). NPs can be produced in various sizes and shapes, such as spheres, cubes, cylinders, rods, triangles, or disks of different dimensions (Husain .,2017). Today, metals with nanoscale sizes are used in many applications of physical, chemical, industrial sciences, as well as biological sciences. As a modern technology known in the nanoscience industries, the products of nanoscale silver particles have gained importance among industrial products, although approximately 30% of the products currently present in Markets have been registered in the nanomaterials database. Because it contains nano-materials within the composition (Namasivayam et al.,2015).

The binding of biomolecules on the surface of nanoparticles, such as proteins, peptides, and other target bonds containing specific molecules that recognize receptors on different cell lines, is important not only in their interactions with biological tissues, but also in their biological functions and cellular absorption, which can facilitate their use in drug delivery, treatments, and diagnosis (Shruti et al., 2013). Controlling the shape and size of metal nanostructures has received a lot of attention because the size and shape affect all of the catalytic, magnetic, optical, and electrical properties of metal nanostructures (Arif and Uddin, 2020). Nanoparticles are widely used in biomedicine for a variety of purposes, including the delivery of pharmaceuticals and diagnostic procedures as well as for therapeutic purposes (Obaid et al., 2022). There are numerous ways to create particles at the nanoscale, which can be categorized into two groups: the first involves creating atoms or molecules that interact with one another to create nanoparticles of the desired shape and size, and the second involves removing nanoparticles from bulk materials using various techniques, such as laser ablation. Due to the requirement for numerous applications in various fields, it is necessary for nanoparticles of various sizes and shapes to form, including nanoshells, nanodisks, nanowires, nanocomposites, and hybrid nanostructures, among others (Nadeem et al.,2021). Nanoparticles have been used to increase the efficacy and selectivity of the drug delivery system because they act as mediators for drug release (Naqvi et al., 2020). Nanoparticles can now have their surfaces further modified with electrical, hydrophobic, anionic, cationic, or other environmentally neutral materials that have many applications in the biological sciences because of their small size and large surface area (Vancha et al., 2022). Nanoparticles that cost a lot of money and contain chemicals bad for the environment have been produced using a variety of physical and chemical techniques. Due to their high surface area bio-pairing with molecular probes and a variety of optical properties centered on local Plasmon resonance (PR) metallic nanoparticles are frequently used in biotechnology and biomedicine (Oleksandra et al., 2020).

2.2. Sliver nanoparticle

Recent developments in nanotechnology and medical science have led to the emergence of many nanoparticles and nanomaterials from various bulk elements, including gold, silver, iron, copper, cobalt, platinum, and so on. There is a wide range of applications for using nanoparticles in drug delivery, image contrast agents, and diagnostic procedures thanks to our ability to manipulate the physical, chemical, and biological properties of nanoparticles (Yaqoob et al., 2020). Silver nanoparticles are particles with a size range of 1 to 100 nm, meaning that they are 10,000 to 15,000 times smaller than an ordinary silver atom and they are prepared by engineering silver metal into very small particles, as the properties of the NPs are determined by its size, composition, crystallization, and its structural form (Adapa.,2020). Physical, chemical, and biological techniques are utilized to create AgNPs. For example, silver has been utilized in numerous forms and different objectives for a long time. AgNPs have received a lot of interest recently because of their unique physical, chemical, and biological properties, examples of these properties are high electrical and thermal conductivity, and chemical stability, that led to their use in different consumer products such as plastics, soaps, food, and textiles (Prabhu and Poulose, 2012). Nowadays, AgNPs play a unique role in a wide of fields, such as in the medical area because of their ease of entry into cells and capacity to interact efficiently with biomolecules existing within or on the cell surface. They can act as antimicrobial agents, therapeutic agents, and as anticancer agents (Jeevanandam et al., 2022). Physical methods typically require a lot of energy to synthesize. AgNPs have been produced biologically using fungus, plants, and bacteria without the use of any harmful reducing agents (Lee and Jun., 2019). As shown in (Figure 2-1).

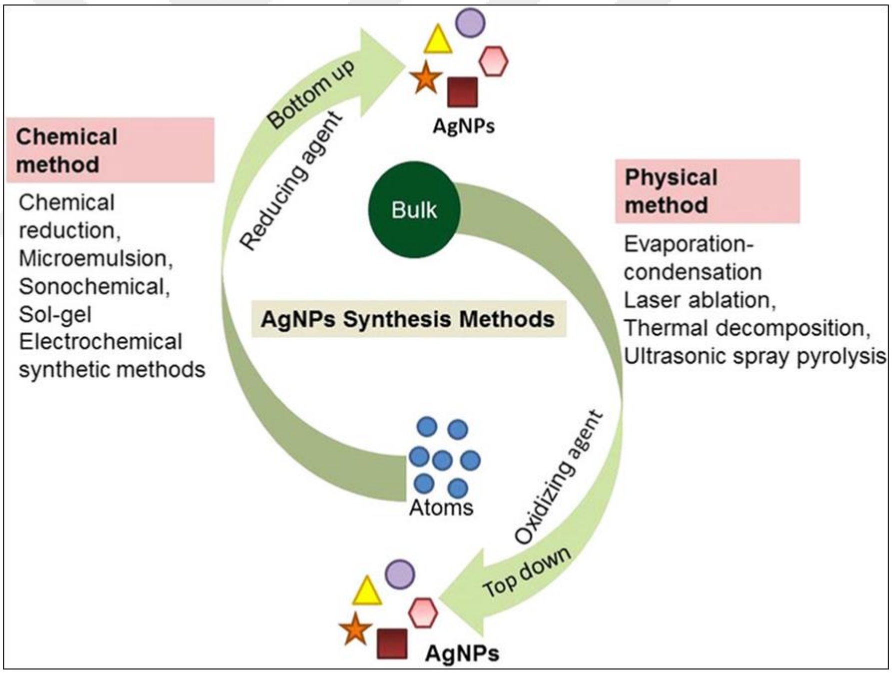


Figure 2.1 The properties of AgNPs synthesis, this figure is adapted (Jabeen et al., 2021).

2.2.1 Characterization of silver nanoparticles:

The physic-chemical features of NPs are essential for their behavior, bio-distribution, safety, and efficacy. As a result, AgNPs characterization is critical for evaluating the functional features of the particles. Several analytical methods are used to characterize the samples, including:

2.2.1.1. UV-vis Spectroscopy:

Surface Plasmon Resonance (SPR) is a phenomenon that occurs on the surfaces of all metals, especially gold and silver and occurs when the light ray strikes the surface of the metal at a certain angle. It is easy to detect SPR of the nano-scale usin UV-spectroscopy in the NPs formation process, as it is indicated when changing the higher absorption (λ max) indicating the nano size (Tajdidzadeh et al., 2014). Seen in figure (2-2)

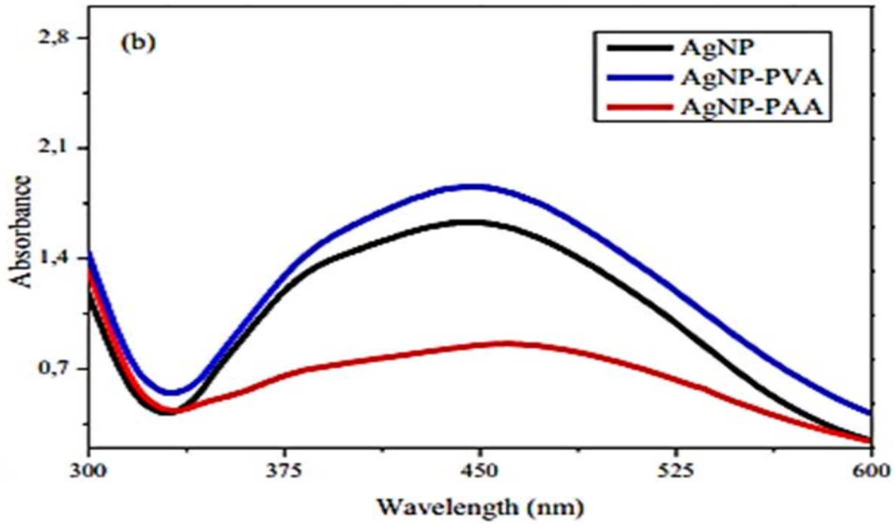


Figure 2.2 UV-Vis spectrum for Ag NPs (Wahaba et al., 2023).

2.2.1.2 Fourier transforms infrared spectroscopy (FTIR):

It is one of the fllowed methods through which it is possible to determine the active bonds present in the substance that forms the compound. The basis of this technology depends on the vibrations of the atoms in the formed molecules to penetrate under the rays, then, the refraction of the light that has been absorbed is determined. The curves that appear represent the absorption and refer to the wavelength of the vibrations of the molecule (Singh et al., 2018). Shown in figure (2-3).

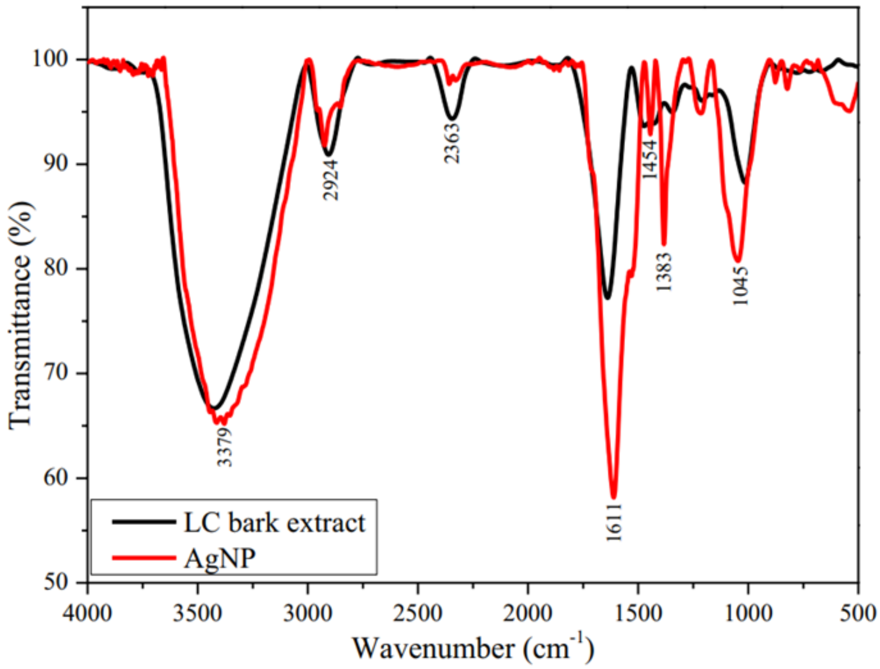


Figure 2.3 FTIR for Ag NPs (Wahaba et al., 2023).

2.2.1.3 X-Ray diffraction (XRD):

One of the characteristics of X-rays is that they have the power to penetrate materials and the absorption rate is dependent on the density of the metal, this method is used to identify the crystal structure of a mineral. They are photons with a wavelength shorter than the length of the ultraviolet rays, when the rays hit the metal to be examined; the X-rays are produced, as it includes observing the patterns and materials deviating from the rays after their union with the model (Jawad et al., 2023).

2.2.1.4 Transmission electron microscope (TEM):

The shape of the particles can be determined through electron microscopes, which are one of the important technologies. The electron rays can draw a picture of the surface of materials. The mechanism of the microscope depends on the firing of electrons from the source (the electron gun) as in the transmission electron microscope (TEM) and that these electrons penetrate the model, where they are dispersed during penetration and then gathered using a blunt lens and are amplified by a magnifying lens, which produces the desired image (Jawad et al.,2023). Figure 2-4.

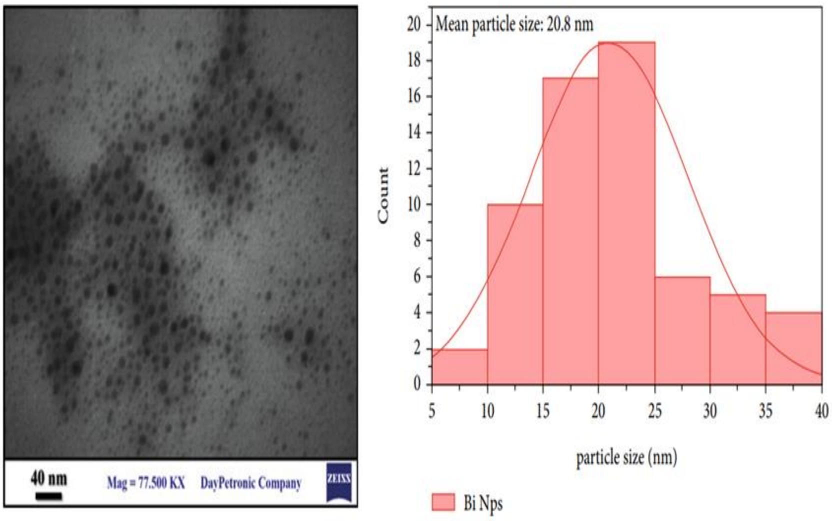


Figure 2.4 TEM image (Jawad et al., 2023).

2.3. General Laser Ablation in Liquids Setup

An alternative technique to produce and synthesize nanoparticles NPs with desired properties is laser ablation of solid targets in various environments. The development of nanoparticles by laser ablation of solid targets in various environments, such as vacuum, gaseous, or liquid, has received significant attention in recent years due to the increasing interest in nanotechnology (Yu-jin et al., 2015). As shown in Figure (2-5), various nanostructures can be synthesized in various experimental configurations. In a typical setup, a target is illuminated by a high power pulsed laser source, such as Nd:YAG, that has been focused by a lens to a very small spot. The target is contained in a holder and submerged in variable liquid depths (a few millimeters). To avoid splashing, the vessel holding the liquid may be open or have a window flush with the liquid. After that, the target is bombarded with laser pulses for a time that can range from minutes to hours in order to cause ablation (Munaf et al., 2023). The primary mechanism of laser ablation is light interaction with the solid target surface, which results in the vaporization of the solid target and a small amount of the surrounding liquid. The highly excited, ablated species can then interact chemically, electronically, and internationally with the molecules in the liquid. The result of this interaction is a suspension of distinctive nanoparticles in the liquid made up of atoms from the surrounding liquid and the ablated target. The prolonged interaction of these NPS with the laser may lead to additional changes in the NPs composition (size or morphology) as a result of the accumulation of these nanoparticles in the surrounding liquid (forming a colloidal solution) (Rashid et al., 2022).

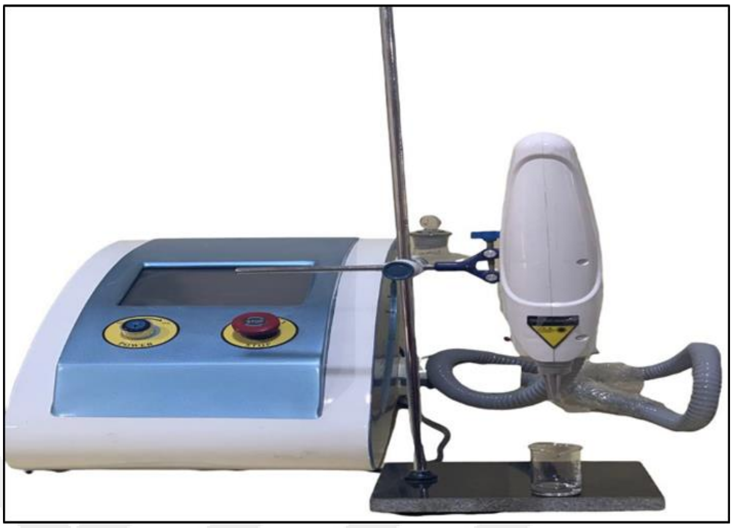


Figure 2.5 photo Nd: YAG laser.

2.4. Pulsed laser ablation in liquid (PLAL) mechanisms

Three crucial elements make up the PLAL experimental setup: a laser source, a solid target, and a liquid medium. Focusing a powerful laser beam on a solid target whose surface is submerged in a liquid is known as laser ablation. As shown in Figure (2.6), some of the laser light that strikes the solid target is reflected, while the rest is absorbed and interacts with the target surface to heat, melt, and vaporise it, creating an ablation plume that contains high kinetic energy particles like ions, atoms, and clusters. According to (Freeland et al. 2020). these species interact and collide with the molecules in the surrounding liquid to create new compounds made up of atoms from both the target and the surrounding liquid. Nanoparticle synthesis using PLAL is a rapid and intricate progressive technique. It involves the formation of a plasma plume, the generation of a shock wave in a liquid as a result of the plume's expansion, the generation of another shock wave in a target as a result of the plume's recoil, cavitation bubbles, and finally the dynamic progression of nanoparticles.

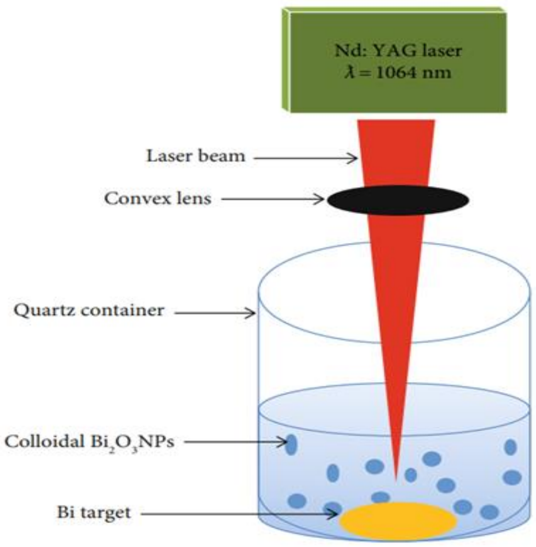


Figure 2.6 Setup for nanoparticle synthesis. (Jawad et al., 2022).

2.5. Advantages of pulsed laser ablation in liquid

PLAL is a promising method because it can create a variety of nanoparticles, including metals, noble metals, semiconductor insulators, nanoalloys, and oxides. It also has a number of benefits, including (i) PLAL demonstrating easier and less expensive experimental setups that do not necessitate expensive hoover systems and intricate experimental setups. Two ideas (Enza et al., 2020) can be used to control the PLAL process; (1) Chemical methods, which employ various liquid medium (organic solvents, chemical solutions, etc.) (2) Physical methods, which regulate the liquid's temperature and pressure. (iii) PLAL produced highly stable charged nanoparticles (NPs). (iv) Because no chemical precursors are required, PLAL produced colloids are of high purity (100%) and free surfaces NPs are from without any chemical contamination. The availability of more NPs sites and surfaces for functionalization, drug loading, and environmental sensing has increased as a result (Xi-Feng et al., 2016).

2.6. Parameters Effecting on PLAL

The shape, size, and morphology of nanoparticles produced by laser ablation in liquid are strongly influenced by a variety of ablation parameters, including laser wavelength, laser energy, ablation time, frequency, pulse duration, repetition rate, temperature, height of liquid above target, target type, and liquid environment (Anugop et al., 2022).

2.7. Silver nanoparticles applications

Silver nanoparticles has wide range of applications in an industries, including the medical and pharmaceutical sectors, food production, agriculture, water technology, and many other applications. A summary of the crucial applications is provided below, in Figure (2.7).

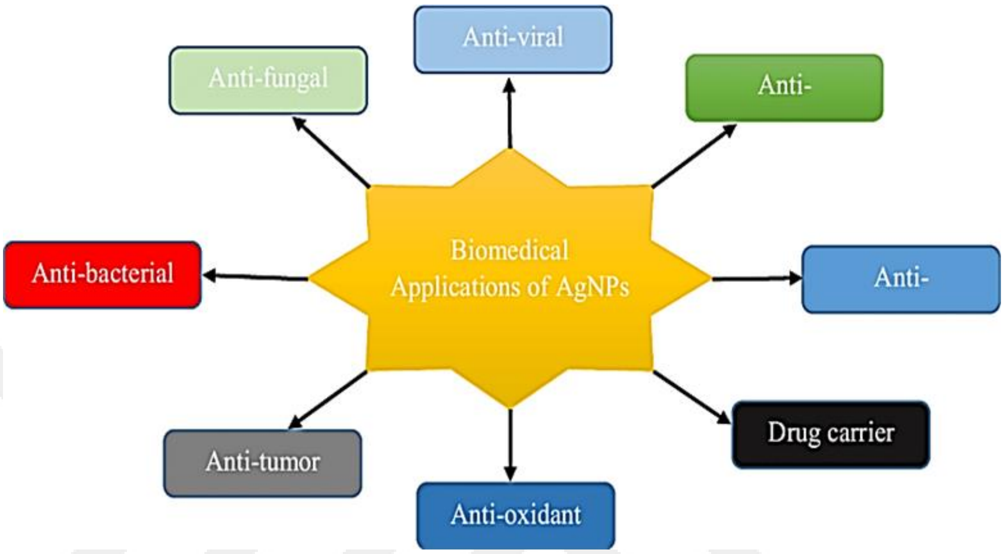


Figure 2.7 Applications of AgNPs, this figure is adapted from (Sunday et al., 2021).

2.7.1. The antibacterial action of AgNPs:

AgNPs have attracted the interest of researchers due to its broad spectrum of antifungal and antibacterial properties. It is the most common nanomaterial used as antibacterial (Rajawat and Qureshi., 2013), due to its ability to combine with sulfur groups (SH) found in bacterial respiratory enzymes, studies have proven that AgNPs can bind with components containing phosphorous such as deoxyribonucleic acid (DNA) and thus prevent its duplication process, and it has been scientifically proven that AgNPs have a lighter weight than other NPs. Several theories have been put forward to explain the antimicrobial mechanism of AgNPs on microorganisms, by interaction with the cell membrane of the bacterial cell causing a change in permeability of cell wall and plasma membrane degradation, interaction with enzymes, amino acids, and with nucleic acids (DNA and RNA), induction of toxicity and oxidative stress by the production of reactive oxygen species (ROS) and free radicals (Jabbar et al., 2020). As shown in Figure (2.8).

Antibacterial properties of AgNPs have allowed reducing the antibiotic resistance, which has emerged as a major health problem in recent years (Heras et al., 2015). Research focuses on finding compounds that have direct antibacterial action or may work in tandem (synergy) with traditional pharmacological drugs (Sun et al., 2014). Metal NPs, particularly those made of silver or gold, have a wide range of applications. It has been demonstrated to be veridical against a variety of viruses and to decrease viral infectivity considerably (Bruna et al., 2021).

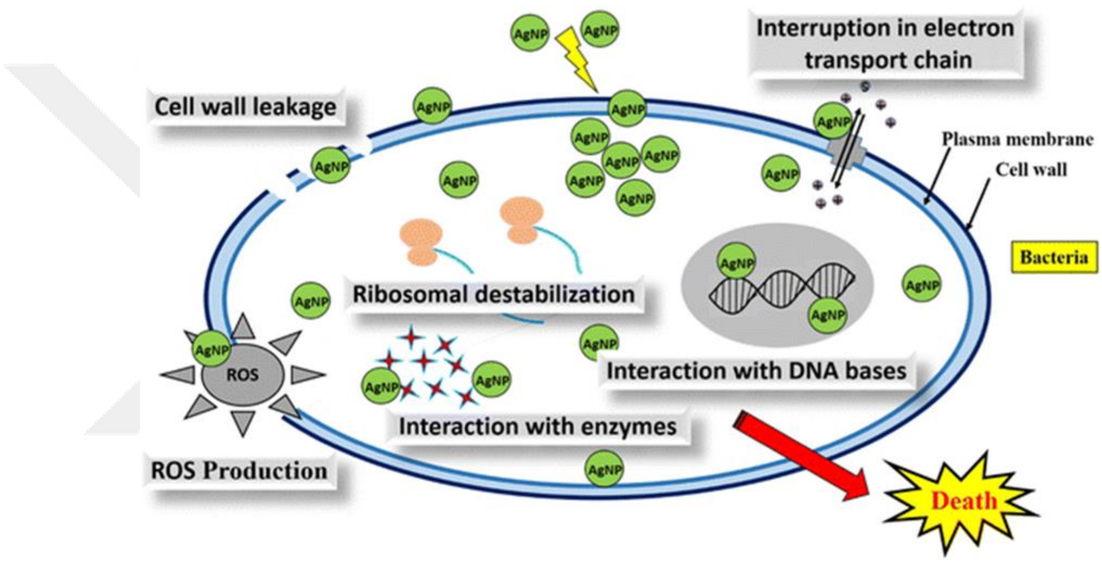


Figure 2.8 Mechanism action of AgNPs as antimicrobial, this figure is adapted from (Maheshkumar et al., 2017).

2.7.1.1. General characteristics of S. aureus:

S. aureus is a non-spore-forming, non-motile Gram-positive, facultative anaerobe, commonly encapsulated, sphere-shaped cells around 0.5–1.5µm in diameter. It appears as an irregularly grape-like cluster or sometimes individually or in pairs, and typical colonies are smoothly elevated yellow to golden yellow and hemolytic on 5% sheep or horse blood agar (Abeck .,2022) The bacteria can grow at temperatures ranging from 15 to 45˚C, their pH growth ranging from 4.5-9.3 with an optimum between 7-7.5. S. aureus can increase in the presence of 10% NaCl and poorly in 15% NaCl but are gladly inhibited using sure chemical substances (e.g. 3%

hexachlorophene) (Missiakas and Schneewind ,2013). Biochemically coagulase and catalase-positive. S. aureus produces the coagulase enzyme that interacts with prothrombin in the blood leading to plasma clotting by converting fibrinogen to fibrin. The aforementioned coagulation assay is used to distinguish S. aureus from other members of the genus Staphylococci, which are called coagulase-negative staphylococci (Abeck., 2022). *S.* aureus is a ubiquitous, flexible, and highly adaptive pathogen that invades the skin and mucous membrane of the anterior nares, GIT, GUT and pharynx tracts, in addition to its presence in people with several diseases, including DM and chronic skin diseases (Mason et al., 2018).S. aureus is classified according to (Gulzar and Zehra. 2018) as follows:

Kingdom: Bacteria

Class: Bacill

Phylum: Bacillota

Order: Bacillales

Family: Staphylococcaceae

Genus: Staphylococcus

Species: S. aureus.

2.7.1.2. General characteristics of Escherichia coli

E.coli is a gram-negative, non-sporulating, rod-shaped, facultatively anaerobic, and coliform bacterium that typically lives in the environment, food, and the lower gut of warm-blooded animals (Feng et al., 2007). On a variety of substrates, Escherichia coli can survive. E. coli niche is determined by the nutrients that are present in the host species' intestines. The primary niche for E. coli is the (GI) tract of humans and many other warm-blooded animals. Warm-blooded animal intestines and the environment (water, sediment, and soil) are its two main habitats, and they are both very different in terms of their physical characteristics, range of nutrient availability, and quantity of nutrients available. Between E. coli and its host, a mutual relationship develops. While the host provides an ecological niche and nutrients, E. coli in the colon produces K and B complex vitamins and guards the GI tract against invasion by pathogenic microbes. The majority of facultative anaerobes found in the intestine are E.coli, accounting for approximately 0.1% (Elbing et al., 2019).

Scientific classification is according to (Castellani & Chalmers .,2022) as follows:

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gamma proteobacteria

Order: Enterobacterales

Family: Enterobacteriaceae

Genus: Escherichia

Species: Escherichiacoli (E. coli)

2.7.2. Antioxidant activity of silver nanoparticles:

Reactive oxygen species (ROS) are produced by many cells and include irritant carbonyl (C=O), single oxygen (O_2), and hydrogen peroxide (H_2O_2), which can harm DNA, proteins, and lipids, leading to cell death. ROS has been combated effectively with AgNPs. Antioxidants are well known for their capacity to neutralize free radicals and shield the body from a range of diseases, including cancer and heart disease. AgNPs were used to combat ROS yielded novel outcomes (Rai, and Raghavendra .,2016).

2.7.3. Anticancer activity

Breast cancer is the most common type of cancer among women worldwide, and is the second leading cause of death for women in developed countries. It accounts for about 23% of all gynecological cancers and 14% of cancer deaths(Jemal et al., 2011). MCF-7 is one of the most widely studied human breast cancer cells worldwide. MCF-7 is a human breast cancer cell line that was discovered in 1970 in the malignant breast tissue of a 69-year-old woman(Lee et al., 2015). Because MCF-7 cell lineages have different ideal characteristics specific to the breast epithelium, they are useful for in vitro breast cancer research.   MCF-7 cells are also sensitive to cytokeratin, and when grown in vitro, epithelial-like cells form domes and grow in monolayers. Several gold NPs with different covering factors were tested for cytotoxicity against a leukemia cell line in a single experiment. According to various studies, spherical gold particles enter cells and are non-toxic to cellular function (Boldeiu et al., 2019). The MTT assay was used to assess cytotoxicity. Several studies show no biologically adverse effects in both in vivo and in vitro experiments, and it has been suggested that some of the observed effects are due to conjugates or admixtures on AgNPs rather than the AgNPs themselves.

2.8. General characteristics of E.Cardamomum (Elettaria cardamomum)

The perennial herb Elettaria cardamomum is indigenous to Bangladesh, Pakistan, and Indonesia in parts of Asia. It belongs to the Zingiberaceae plant family, which also includes ginger, and has white flowers (Yahyazadeh., 2021). Its dried seeds are frequently used as a spice in numerous Asian dishes, such as coffee, cakes, and curries. For thousands of years, the seeds, leaves, bark, roots, and flowers of the cardamom plant have all been used for their potential medicinal properties. (Abu-Taweel., 2018). E. cardamomum has been demonstrated to have antioxidant, anti-inflammatory, antihypertensive, cholesterol-lowering, and blood sugar-lowering effects in preliminary research studies (Delgadillo-Puga et al., 2023). Clinical studies demonstrating these and other health advantages, however, are scarce Figure 2.9.

Figure 2.9 E.Cardamomum (Elettaria cardamomum) image

2.8.1. Potential Benefits of Cardamom

A healthcare professional, such as a registered dietitian, pharmacist, or healthcare provider, should evaluate each individual's supplement use. No dietary supplement is meant to treat, prevent, or cure disease. Cardamom has been used to treat psoriasis, multiple sclerosis, and rheumatoid arthritis in conventional medicine (Cárdenas et al., 2021). Cardamom has also been praised for its medicinal properties. According to some studies, cardamom is (Yahyazadeh et al., 2021).

- Antioxidant activity
- Antimutagenic
- Antibacterial activity
- Anti-inflammatory
- Antidiabetic
- Heart protective
- Liver protective
- Chemoprotective (an agent that may reduce the side effects of chemotherapy)

The majority of studies on cardamom's medicinal uses have been conducted on animals or in lab settings rather than on humans, despite the herb's widespread use. A look at some of the research is provided below.

2.8.2. Antibacterial Properties

It is believed that E. cardamomum has antibacterial qualities that may be helpful in treating some infections. According to a laboratory study, cardamom essential oil is effective in treating Candida albicans, Staphylococcus aureus, Salmonella typhi, Escherichia coli, and Streptococcus mutans, among other common bacterial strains (Abdullah et al., 2017). Another study investigated the potential impact of E. cardamomum on periodontal (gum) bacteria. According to (Souissi et al., 2019), Porphyromonas gingivalis and other types of bacteria frequently found in periodontal infections were resistant to the antibacterial effects of E. cardamomum extract. E. cardamomum needs to undergo more thorough research before it can be suggested for use as an antibacterial.

3. MATERIALS AND METHOD

3.1 Equipment and apparatus

The various tools and instruments used in the current study are listed in Table

Table 3.1 Apparatuses used in the study, manufacturer, and origin.

Equipment and Apparatuses	**Company**	**Country**
Autoclave	Westtlne	Chain
Camera Digital	Cannon	China
Cell culture plates	Santa Cruz Biotechnology	USA
Centrifuge	Gemmy industrial CORP	Taiwan
CO_2 incubator	Cypress Diagnostics	Belgium
Cooling centrifuge	Sorvall	USA
FTIR	Shimadzu	Japan
Laminar flow hood	K & K Scientific Supplier	Korea
Magnetic stirrer	Hy-hm	Korai
Micropipette	Cypress Diagnostics	Belgium
Microtiter reader	Gennex Lab	USA
Sensitive balance	Ohaus	Germany
Sonicator	Trade mark	China
UV-VIS spectrophotometer	Shimadzu-1	Japan
Vortex	Strat scienti fied	UK
Water bath	Menmort	UK
X-ray diffraction	Sigma	Germany

3.2. Chemicals and reagents

The different chemicals and reagents were used in present study are recorded in table 3.2.

Table 3.2 The equipment used in the study, manufacturer, and origin

Chemicals and Reagents	Company	Country
2,2-Diphenyl-1-Picryl-Hydrazyl (DPPH)	Sigma	Germany
3-(Dimethylthiazol-2-yl)-2,5Diphenyltetrazoliumbromide (MTT)	Sigma	Germany
Balance 4 digits	Hangzhou	China
DMSO	Santacruz Biotechnology	USA
Elettaria cardamomum	BDH	India
Ethanol	BDH	England
Fetal bovine serum	Capricorn	Germany
Glass Erlenmeyer Flask	Pyrex	Germany
HACH4.3H2O	Glentham	UK
Hot plate Magnetic stirrer	Four E's	China
Mueller Hinton Agar	Accumix	Spin
NaBH4	Sigma	Germany
Nutrient broth	Sigma- Aldrich	USA
Round Flask	labmax	Germany
RPMI 1640	Capricorn	Germany
Sodium Citrate	US-Biological	USA
Trypsin/EDTA	Capricorn	Germany

3.3. Laboratory experiments

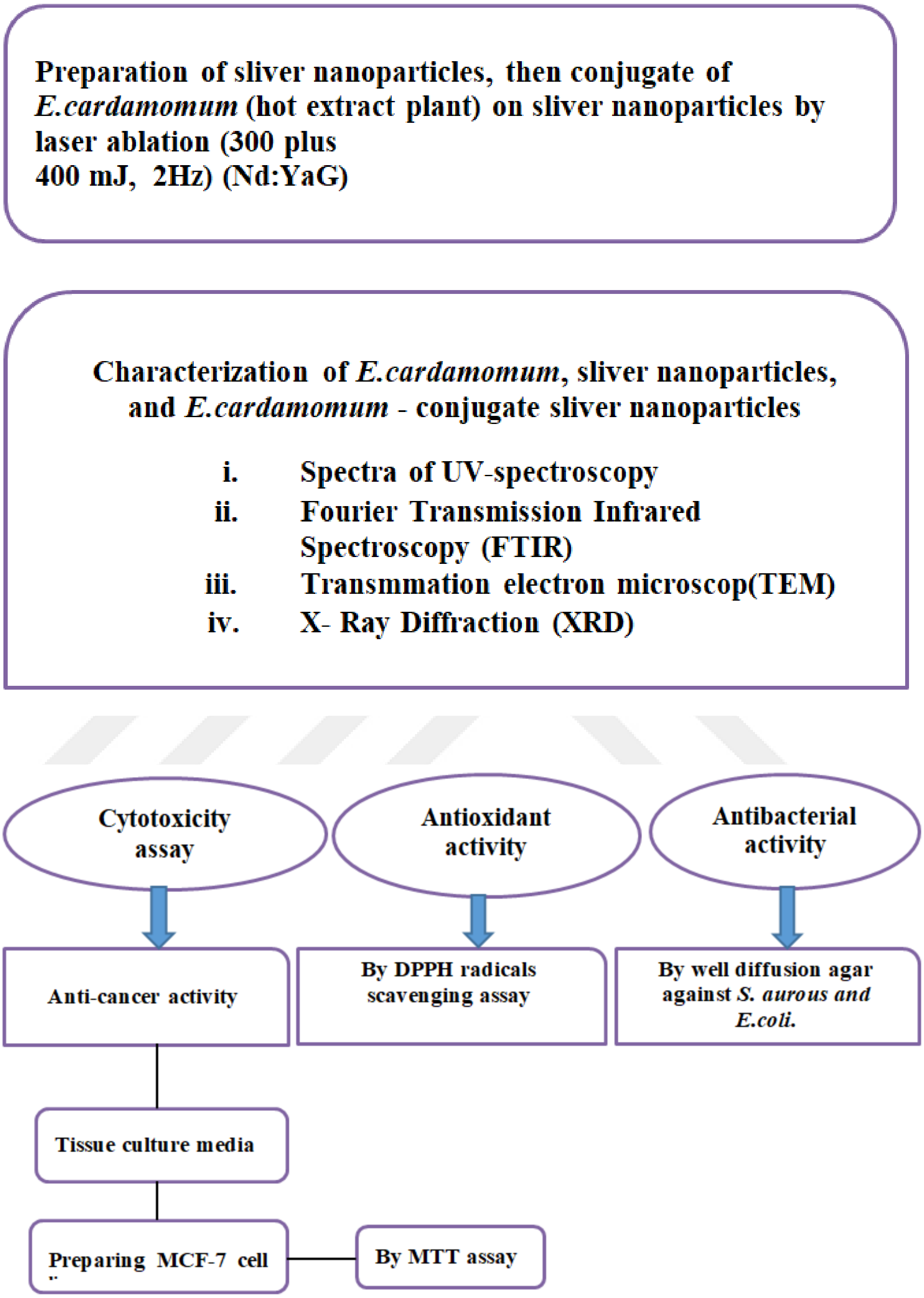


Figure 3.1 Diagram of experimental work.

3.4 Preparation of E.cardamomum - conjugate sliver nanoparticles

3.4.1 Synthesis of sliver nanoparticles by laser ablation

Synthesis of NPs synthesis was achieved through the application of LASER ablation in DI water; first, sliver pellets was of 99 % purity (Merck, Kondapur, India), was made into pellets, each with a diameter of 15 *15 mm. The sliver target was irradiated using a Nd:YAG laser (light amplification by stimulated emission of radiation) with a pulse duration of 7 ns (nanoseconds) Figure (3.2). In a plastic beaker with a sliver target fixed at the bottom, 3 millilitres of water with a 1 cm water level over the target were used. The laser's 400 mJ/cm2 fluence was used for ablation, and the ablation process took 30 minutes. As seen in Figure (3.1).

3.4.2 E.cardamomum (hot extract plant)

Secondly steps plant samples the powder of E. cardamom plant was obtained from local market of Baghdad. E.cardamomum seed were used 10 g. Grinded thoroughly and boiling for 1 h at 100 °C in 100 ml of sterile water to obtain hot aqueous extract. The collected extracts were filtered with Whitman filter papers. Filtered extracts were further diluted to reach a concentration of 70%, then was kept in oven at (50 ºC) for drying, and stored at 4 °C for further use. The color changes extract to brown. (HH Hussein 2017). shown in Figure (3.2).

3.4.3 Conjugation of E.cardamomum –with sliver nanoparticles

Thirdly steps for the synthesis of creating a nona-conjugated system , by the dissolved E.cardamomum was combined with the sliver nanoparticles that had previously been prepared (each 1/2 gram with 5 ml of D.W) at room temperature and the same as the steps followed for producing the conjugated of E.cardamomum on sliver nanoparticles by LASER ablation in liquid. we noticed the color changed to a light yellow.

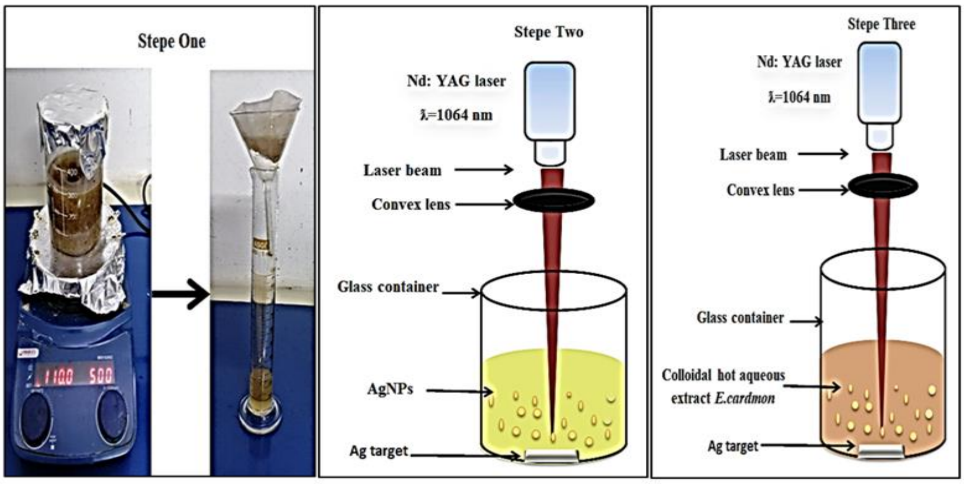


Figure 3.2 Steps of conjugation of E.cardamomum –with sliver nanoparticles.

3.5.Characterizations of prepare E.cardamomum, AgNPs and E.cardamomum –AgNPs

3.5.1 UV-VIS spectroscopy analysis

The absorption spectra of E.cardamomum , sliver nanoparticles , and E.cardamomum with sliver nanoparticles were measured by UV/Vis spectrophotometer with ranges from (200–800 nm). Ministry of Science and Technology in Baghdad.

3.5.2. Infrared spectroscopy analysis using Fourier transforms (FTIR)

FTIR analysis was used to identify the functional group present in E.cardamomum, sliver nanoparticles, and E.cardamomum with sliver nanoparticles with wavelengths ranging from (400-4000 cm^{-1}). The FTIR test was conducted at the Ministry of Science and Technology in Baghdad.

3.5.3. X-Ray Diffraction analysis

X-ray diffraction measurements were performed after sample deposition on a 1 cm 2 glass slide. The structure of E.cardamomum, sliver nanoparticles, and E.cardamomum –with sliver nanoparticles were recorded by XRD equipped with Cu-kα radiation at a wavelength (λ = 0.154056 nm) generated at 45 kV. The domain size was calculated from the width of the XRD peaks, assuming they are free of irregular strains, using Scherer's formula:

$$D = K \lambda / \beta \cos$$

where D is the crystal particle size, K is the absolute constant (0.94), the X-ray wavelength (0.154056), the width of the XRD peak at half height, θ is the Bragg diffraction angle. XRD examination was performed at the Ministry of Science and Technology in Baghdad.

3.5.4. Transmmation electron microscop (TEM)

E.cardamomum , sliver nanoparticles , and E.cardamomum –with sliver nanoparticles The assay was performed in Ministry .of Science and Technology, Baghdad, Iraq.

3.6. Medical Application of E.cardamomum -Sliver nanoparticles

3.6.1. Bacterial isolates

Nanoparticles were tested for antibacterial activity against E.coli and S.aureas. Bacterial strains were obtained from the University of Nehrein postgraduate laboratory, biotechnology division, Iraq.

3.6.2. Media culture preparation

3.6.2.1. Muller Hinton Agar preparation

It is applied in the assessment of antimicrobial susceptibility. By dissolving 28 g of Mueller-Hinton in 1000 ml of D.W., it was created in accordance with the manufacturer's instructions. The medium was put into 10 ml tubes and autoclaved at 121°C for 15 minutes. 2% of Mueller-Hinton agar medium is added for every 100 ml of D.W. (Hasan D M. et al., 2022).

3.6.2.2. Nutrient Broth Preparation

A wide variety of non-microorganisms can grow in nutrient agar, which is an all-purpose medium. Popular because it promotes the growth of various types of bacteria and fungi and contains many of the nutrients required for bacterial growth is nutrient agar. According to the manufacturer's instructions, it was made by combining 1 liter of distilled water with 38 grams of Nutrient Broth. The medium was put into 10 mL tubes and autoclaved at 121°C for 15 minutes. 2% per 100 ml D.W. of Nutrient agar medium is added (Hussein et al., 2019).

3.6.2.3. McFarland standard solutions

According to (Hussein NN et al. 2019), the McFarland standard solution this solution was created as the following: a suspension with an OD of (0.8) and (1.510^{8}) CFU/ml.

Solution (A) - One gramme of barium chloride ($BaCl_2$) was dissolved in one hundred millilitres of D.W.

Solution (B): One millilitre of pure sulfuric acid (H_2SO_4) was added, and D.W. completed the volume to one hundred millilitres. (99.5) ml of solution (B) and (0.5) ml of solution (A) were combined and kept in a dark bottle until needed.

3.6.2.4. Antibacterial activity

The antibacterial activity of the (E.cardamomum and AgNPs and E.cardamomum -AgNPs) was tested against E.coli, and S. aureus, using the following protocol. A sterile wire loop was used to capture the bacteria from their stock cultures. The bacterial strains were cultured on M-H agar plates at 37° C, the collection freshly cultured plates inoculations composed of 50 mL of nutrient agar in sterile Petri dishes, and the bacterial species E. coli and S. aureus were then cultured on the culture media to determine how (E.cardamomum and AgNPs and E.cardamomum -AgNPs) affects the growing curve of bacteria. A 0.5 McFarland standard density adjustment was made to the suspension. Using a sterile swab, the bacterial isolates were evenly inoculated onto the Muller-Hinton Agar (MHA) plate surface. A sterile cork borer with a diameter of 5 mm was used to help create the wells. The wells were filled with 50 µl of E.cardamomum and AgNPs and E.cardamomum -AgNPs. Negative controls were processed via the addition of 50 µl of D.W. Plates were incubated at 37C° for 24 hr. The inhibition zone was recorded by mm (Soud, A S. et al., 2020).

3.7. Anti-oxidant activity

3.7.1. DPPH radical scavenging assay

Materials and Solutions

A- Ethanol: Absolute ethanol was used.

B- DPPH solution: It was prepared by dissolving 2.366 mg of 2, 2-diphenyl 1-picrylhydrazyl in 100 mL of absolute ethanol to obtain 60 µM DPPH. (Sabah T H.,et al ., 2023)

C- E.cardamomum, AgNPs and E.cardamomum –AgNPs solution: The solutions were prepared according to 3.4.1 and 3.4.2 section.

D- Ascorbic acid : It was prepared by dissolving 1 mg of ascorbic acid in 100 mL D.W (Jawad K H. 2023).

The Method is The antioxidant activity of E.cardamomum, AgNPs and E.cardamomum -AgNPs

was evaluated with DPPH assay (Jawad et al ., 2021). The DPPH radical has a deep violet color due to its unpaired electron and radical scavenging capability can be followed spectrophotometrically by absorbance loss at 517 nm when the pale yellow non radical form is produced. Based on this assay, equal volumes (0.5 mL) of DPPH and 10 ml of E.cardamomum and AgNPs and E.cardamomum -AgNPs were mixed in a cuvette and allowed to wait for 30 min at room temperature.

Then, the absorbance was read at 517 nm in a UV/VIS spectrophotometer. The DPPH with ethanol only was used as negative control and ascorbic acid (Vitamin C) as positive controls. The absorbance of the control (DPPH solution) was also read. The percentage inhibition of the sample was calculated using the following formula:

$$\text{DPPH Scavenging Activity (\%)} = (AC - AS / AC) \times 100$$

Where AC is the peak intensity for DPPH and AS is the peak intensity for E.cardamomum and AgNPs and E.cardamomum -AgNPs sample solvent.

3.8.Cytotoxic effects of E.cardamomum and AgNPs and E.cardamomum –AgNPs

3.8.1. Tissue culture media

3.8.1.1. Roswell park memorial institute (RPMI)-1640 medium

To prepare it about 16.4 g. of RPMI-1640 powder was resolved in 800 mL of D.W., and the following components were added and mixed by magnetic stirrer (Al-Ziaydi, A. et al., 2020; Al-Salman, H. et al 2020).

- 100 mL of Fetal bovine serum (FBS).
- 0.5 mL of Streptomycin (100 µg mL-1).
- 0.5 mL of Benzyl penicillin solution (100 µg mL-1).
- 5-10 mL Sodium bicarbonate (4.4%) to reach final pH 0f 7.6.

The pH was regulated to 7.2, after that the volume was completed to 1 L and the latter solution was filtrated by 0.22 µm Millipore filter (Jawad KH et al., 2022).

3.8.1.2. Cancer cell line

3.8.1.2.1. Maintenance of MCF-7 cells

Al-Mustansiriya University in Baghdad, Iraq, provided the MCF-7 human breast cancer cell lineages. The cells were incubated at 37 °C for 24 hours after rapid thawing in a water bath at 37 °C. Cells were centrifuged for about 10 minutes at 800 rpm and resuspended in 5 ml of culture medium (RPMI-1640). The medium was then separated and replaced with fresh medium supplemented with 10% FBS (Al-Musawi, S., et al ., 2020).

3.8.1.2.2. MCF-7 cells

MCF-7 cells were suspended in RPMI-1640 medium and cultured in T-50 culture flasks at 37 °C in an incubator with 5% CO and 95% air. Once the cultures had reached 70-80% confluence, cell sub-strains were cultured. Remove the growth medium and wash it in PBS solution twice more. Trypsin-EDTA solution was used to detach the cultured cells from the flask surfaces and collect them. After suspension, trypsin activity was stopped by adding FBS-containing medium, and cells were distributed to other flasks. Subcultures were cultured every three days to keep cell lines in exponential growth. (Ibrahim A .A et al ., 2021).

3.8.2. Anticancer activity

3.8.2.1. MTT assay (Materials and solutions)

(MTT) Solution It was made by dissolving 0.2 gm MTT in 100 mL PBS to make 2 mg mL-1 MTT solution. The solution was then filtered through a 0.2 m Millipore filter to remove any blue formazan that had formed in the solution and stored at -20 degrees Celsius in a sterilized black glass container. Within two weeks of preparing the solution, it should be used (Jabir, M. et al., 2022).

The method is in accordance with freshen, the colorimetric MTT method was used to determine the proliferation of cancer cells. MCF-7 cells were seeded at a density of 10^7 cells per well in RPMI-1640 culture medium supplemented with 10% heat-activated fetal bovine serum (FBS) and incubated at 37°C in a 96-well plate with a flat bottom in an incubator with 5% CO_2. The MCF-7 cells were exposed to various concentrations (6.25, 12.5, 25, 50, and 100 µg/mL-1) for an additional 24 hours after entering the exponential growth phase after 48 hours. After 24 hours, 100 mL of yellow-colored Methyl Thiazolyltetrazolium (MTT) solution was added to each well. The microplate was then incubated at 37°C for 4 hours to allow metabolically active cells to reduce MTT. The supernatants were then removed. 100 mL DMSO was added to each well as a solubilisation solution for dissolving the MTT crystals, and the experiment was incubated for 5 minutes. Using an ELISA reader with a 550 nm absorbance, the optical density of each well was determined. The following formula was used to calculate the rate of cell inhibiter:

$$\text{Inhibition Rate (\%)} = (OD^{A} – OD^{B} / OD^{A}) \times 100$$

When A is the negative control's, OD is optical density, and B is the samples'.

3.9. Statistical analysis

The obtained data were statically analyzed using an unpaired t-test with Graph Pad Pris.The values were presented as the mean ± SD of triplicate measurements(Jawad K.H ., 2023).

4. RESULTS AND DISCUSSION

4.1.Sliver nanoparticle (AgNPs) and their conjugate characterizations

4.1.1 UV-VIS Test

The optical characteristics of the NPs were studied using UV–Visible spectrometer, Figure (4.1) shows the absorbance's of AgNPs and their conjugates in a spectral range from 200 to 800nm Figure 3. The peak at 408 nm is characteristic of AgNPs, The peak at 270 nm is characteristic of E.cardamomum, while that at 412 nm indicated the formation of AgNPs-E.cardamomum , and the difference in absorbance peaks is due to the increased particles size (Hussein, and Muslim,2019). As for AgNPs-E.cardamomum, the characteristic SPR peak appeared at 412nm, this increase is due to color change, the surrounding chemical environment, and the size increase of the NPs because of the adsorption layer, thereupon representing a large shift to the red spectrum following the E.cardamomum addition, from 408 to 412 nm, the absorption peak of free E.cardamomum, was at 270 nm, as obtained previously in (Tran, and Le and 2013; Veronika.et al., 2017). The result which indicates that drug molecules can bind to the surfaces of AgNPs and change their optical properties. This is due to the phenomenon of clay surface resonance (SPR) in precious metals, which occurs as a result of the vibration of both electrons in metallic NPs in time with the light wave (Veronika.et al.,2017; Jawad et al.,2022).

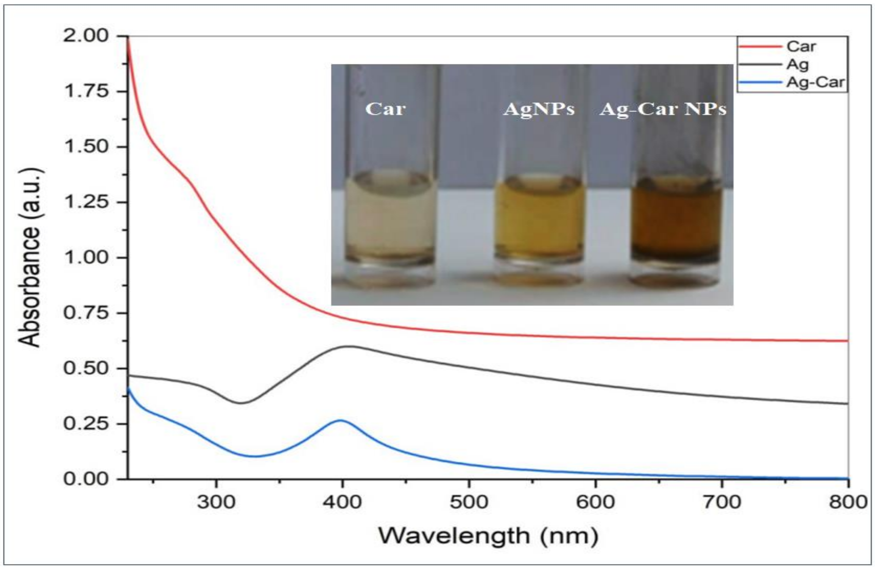


Figure 4.1 UV-VIS spectrum analysis of E. cardamomuum, AgNPs, and their conjugate.

4.1.2 XRD measurements

A Phillips PW 1830 instrument was used to perform the XRD measurements by laser ablation at energy 400 mJ on synthesized silver nanoparticle cast onto glass slides. The diffraction peaks of Ag NPs correspond to (111), (200), (220), and (311) planes .level at 2θ angles 38.64°, 44.54°, 69.38° and 78.28°(Muzamil et al., 2014). All peaks were well matched with hexagonal structure of AgNPs using the standard data (JCPDSile No.04-783). The average crystallite size of the Ag NPs calculated using Scherer formula $D = 0.9\lambda / (\beta \cos \theta)$. Where, λ is the x-ray wavelength (1.504 nm), θ is the Bragg diffraction angle, and β is the full width at half maximum. Based on the results of the plane (111) calculated for the hexagonal wurtzite phase, the mean size of Ag nanoparticles was determined to be 53.354 nm. As shown in Table 1, Figure (4.2) shows, work the same as the steps followed for producing the solution mixture (E. cardamomum and Silver) by laser ablation. We noticed intensity of the peaks decrease because of reduction of silver ions by the E.cardamomum seed extract, by LASER ablation in liquid for 15 min. Aftering we get of isolated silver nanoparticle free from other organic compounds present in solution. The emergence of a new peak of nanoparticles was observed with the disappearance of another as shown in the Figure (4-2), Table 4- 1, 4-2, the size of AgNPs - E. cardamomum is evaluated around ~30.485nm from the expansion of the (111) reflection, which confirms that there is a small change in the size of the silver nanoparticles (Adil et al., 2019).

Table 4.1 The detail peaks for XRD of AgNPs.

2 Theta (degree)	**Hkl (lattice planes)**	**FWHM (deg)**	**2 theta (Rad.)**	**FWHM (Rad)**	**D (nm)**	**Matched by**
38.05325	111	0.15744	0.332	0.003	53.354	01-087-0717
44.27259	200	0.31488	0.386	0.005	27.227	
64.63483	220	0.94464	0.564	0.016	9.947	
77.4383	311	0.3607	0.380	0.0063	30.176	

Table 4.2 The detail peaks for XRD of E.cardamomum +AgNPs.

2 theta (degree)	**hkl**	**FWHM (deg)**	**2 theta (Rad.)**	**FWHM (Rad)**	**D (nm)**	**Matched by**
38.01623	111	0.27552	0.332	0.005	30.485	01-087-0597
44.24557	200	0.23616	0.386	0.004	36.299	
64.5051	220	0.62976	0.563	0.011	14.911	
					27.231	

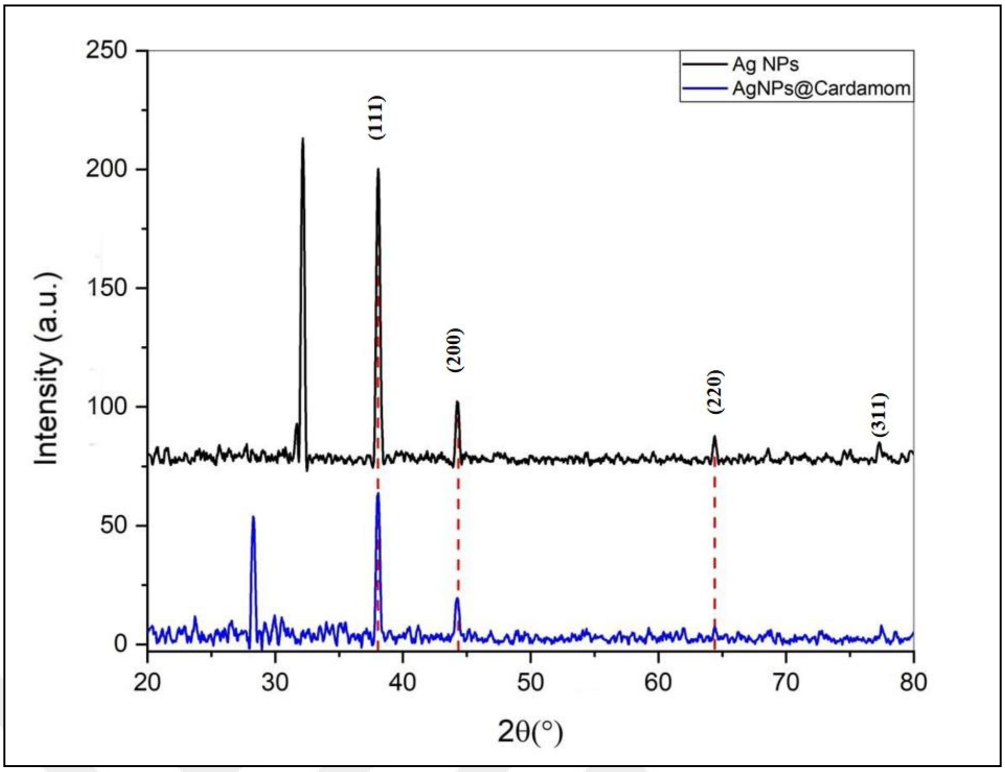
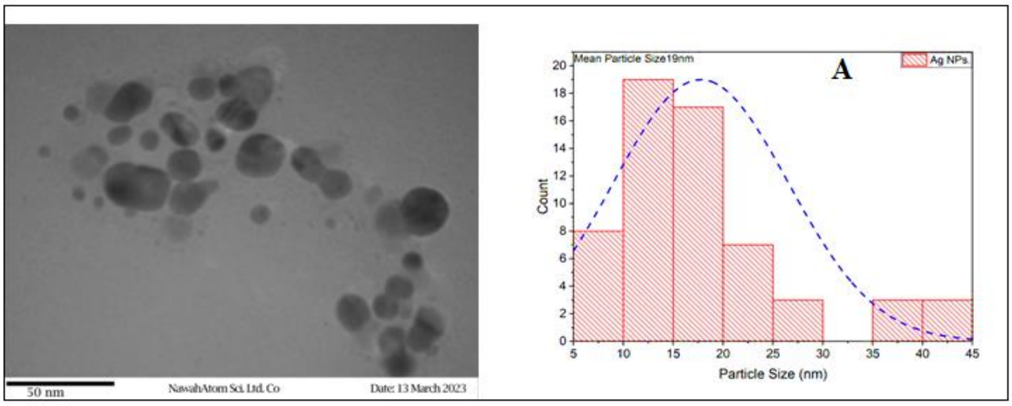


Figure 4.2 XRD for of E.cardamomum +AgNPs.

4.1.3 Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) was used to determine the size distribution and morphology of nanoparticles. Figure (4.3A) shows a TEM image of Ag nanoparticles prepared by laser ablation in a liquid with a 350mJ/pulse with 30 min time. Figure (4.3B) shows the size distribution Ag nanoparticles in diameter in the range of 5 – 45 nm and the nanoparticles are mostly of spherical shape (The mean diameter of prepared AgNPs at lower laser energy 400 mJ was 19 nm), While Figure (4-3B) shows the size distribution AgNPs, E,cardamomum of synthesized nanoparticles the having a diameter in the range 5-45 nm were obtained and spherical shape nanoparticles were dominated. While the higher peak corresponds to nanoparticles with smaller sizes (The mean diameter of prepared AgNPs, E.cardamomum at lower laser energy 400 mJ was 21nm), TEM results recommend a critical dependency of the laser energy and ablation process duration on the size.



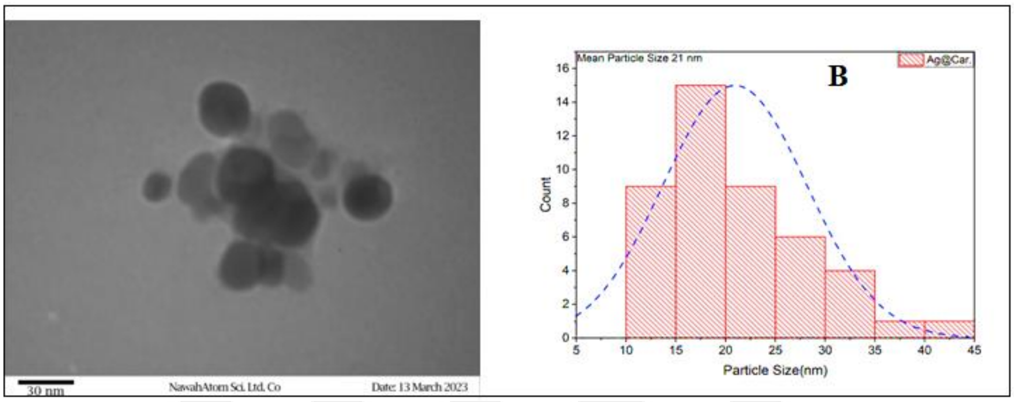


Figure 4.3(A) for AgNPs and (B) E. cardamomum +AgNPs.

4.1.4. Fourier transforms infrared spectroscopy analysis (FTIR)

The E.cardamomum, AgNPs, and AgNPs-E.cardamomum samples were examined by FTIR (Figures 4.4 to 4.6). The AgNPs showed transmittance peaks at ~1001.59, 1087, 1448.59, 1666.55, 2929.97,3315, and 3525 cm-1. The peak at 3525 cm-1 was assigned to O-H (hydroxyl group) and N-H strong stretching because a portion of O-H not create during the oxidation process when some moisture is adsorbed on the highly reactive surface of AgNPs (Muzamil et al.,2014), (Figure 4.4). The E.cardamomum showed transmittance peaks at ~785.05, 1064, 1309.71 ,1398.44 ,16020.2941.54 and ~3227.26 cm-1. The peaks at 785.05 and 1064 .74cm-1 were assigned to C-H, and N-H strong stretching, (Figure 4.5). The final conjugates of AgNPs- E.cardamomum AgNPs- E.cardamomum, numerous peaks were observed, including 5280.51, 781.20, 1074.39, 1311.64, 1398.44, 1616.40, 2937.68 to 3468.13 cm-1. The interaction between AgNPs and E.cardamomum shifted the O-H hydroxyl group peak of AgNPs from 3435.56 cm-1 to 3428.65 cm-1 due to the conjugation with E.cardamomum, and a peak was detected at ~5280.51 to 781.20 cm-1 corresponding to an Halogen compound, and C-H bending. While the peak at

~1074.39 cm-1 was indicated for strong S=O stretching polysaccharides, for peak strong C-O stretching was observed at ~1311.64 cm-1, Furthermore, there is medium bending O-H, band around ~1398.44 cm-1. For peak strong C=C stretching at ~1616.40 cm-1, a peak at ~2937.68 to 3468.13 cm-1 were indicated for CH to N-H stretching seen in Figure 4-6. These findings revealed the production of polysaccharides groups in the obtained AgNPs + E.cardamomum., Furthermore, there is a broad and vigorous characteristic band around ~1078 cm-1. These findings revealed the production of polysaccharides groups in the obtained Silver + E.cardamomum. Furthermore, there is a broad and vigorous characteristic band around ~1078 cm-1, Furthermore, there is a broad and vigorous distinctive band around 2929.97 cm-1 in silver (Muzamil et al., 2014). this is analogous to the stretching folding of C-H that occurs in water between carbon and hydrogen atoms, and a weak band of C=C stretching vibration group has appeared at ~1666.55 cm-1. Finally, a small peak of C-H bending mode (Halogen compound) was shown at ~ 528.51to ~781.20 cm-1. In (Figure 4.6).The FTIR assay is one of the specific laboratory tests that used to identify the chemical elements in the compounds under study, in which the identification of the chemical compounds is determined by how the chemical bonds between the compounds absorb infrared radiation, as each component absorbs differently (Konop et al.,2016).

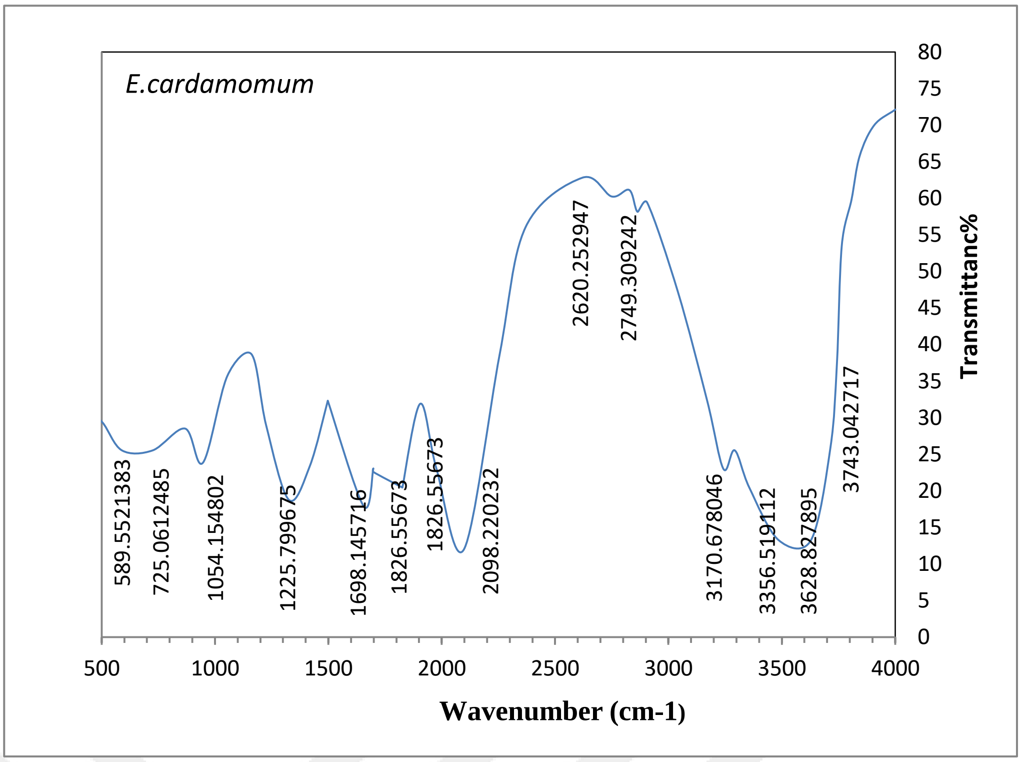


Figure 4.4 FTIR assay for E.cardamomum

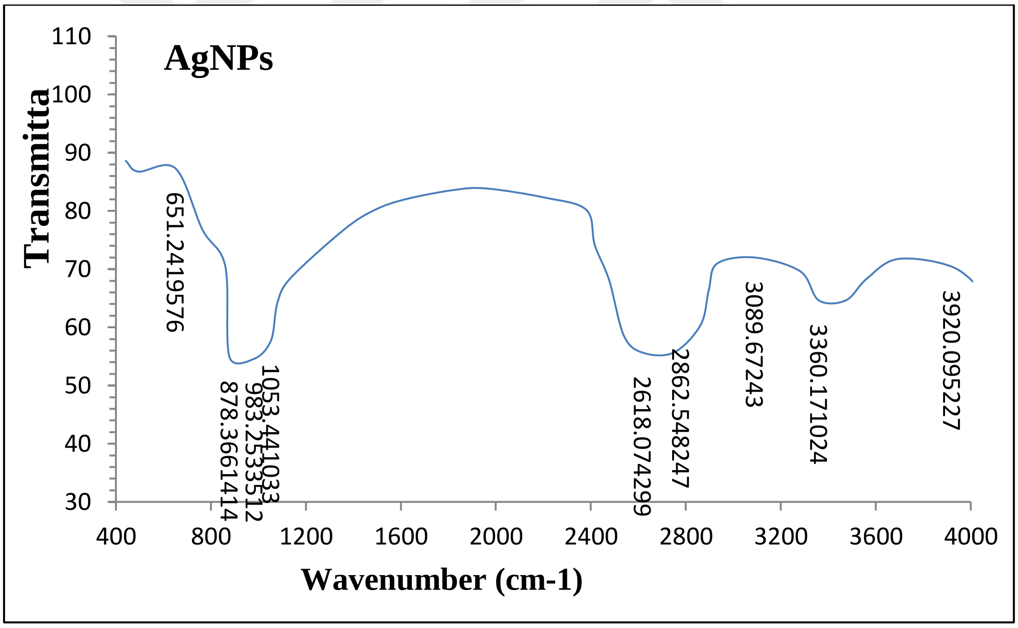


Figure 4.5 FTIR assay for AgNPs.

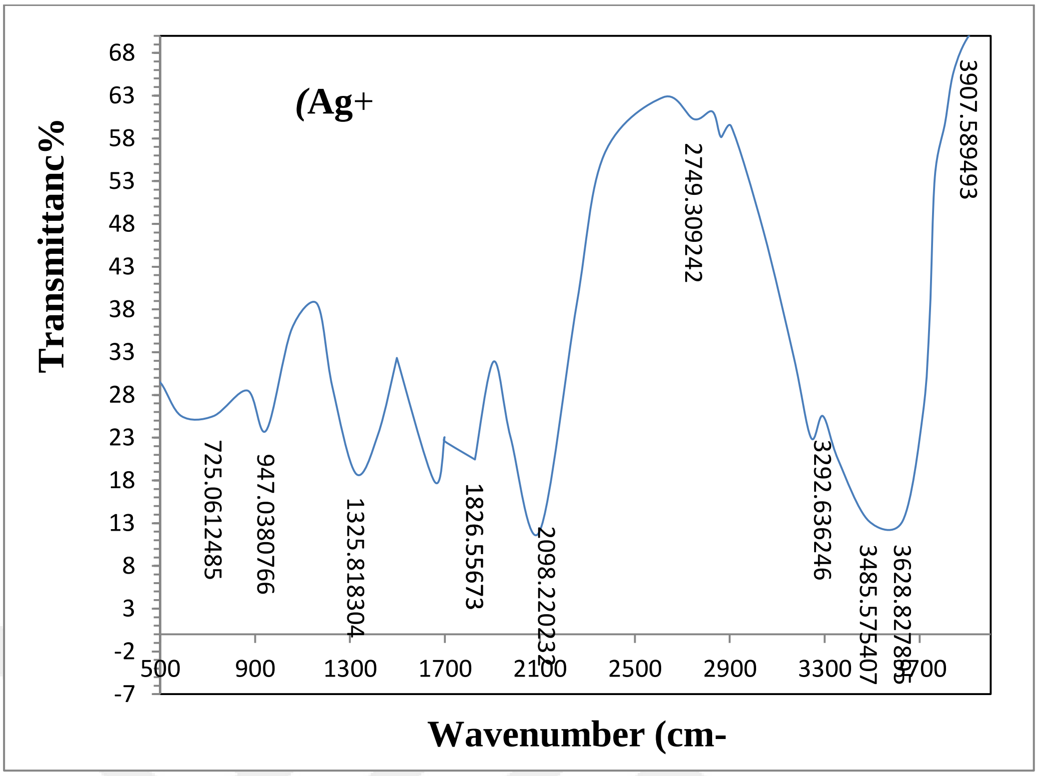


Figure 4.6 FTIR assay for (Silver+ E.cardamomum) NPs.

4.2. Medical application of nanoparticles

4.2.1. Antibacterial activity

It depicts E.cardamomum, Sliver nanoparticles, and E.cardamomum -Sliver nanoparticles antibacterial application against (E.coli and S. aureus). After treatment with concentrations (250µg/mL), E.cardamomum -AgNPs had the most effective blocking and killing of bacterial negative than positive bacterial, followed by sliver nanoparticles and E.cardamomum. From the down results, we note that the inhibition rate of Escherichia coil (Gram-negative) is much higher than that of Staphylococcus aureus (Gram-positive) seen in Figure (4.7), and (4.8). The reasons is due to several reasons, among them is that Gram-negative bacteria have a lower cell wall thickness than Gram-positive, and contain a polypeptidoglycan layer, while the rest Gram have a single layer, and Gram-positive bacteria do not contain purines. In contrast to the negative gram, this is why we note the difference in the percentage of inhibition above.

Along with the aforementioned, the free uncoated NPs demonstrated moderate antibacterial properties, whereas the E. cardamomum exhibited weak inhibitory activity against Gram-negative bacteria. Since many E. cardamomum have previously demonstrated excellent potential against Gram-positive strains, the outer membrane may act as a shield for Gram-negative bacteria against this type of natural product (Jawad et al.,2022). AgNPs' ability to inhibit Gram-negative bacteria may be improved by concentrating E. cardamomum on their surface. In order to reveal their antibacterial properties, we created E. cardamomum - sliver nanopartical conjugates. The E. cardamomum - conjugates sliver-nanocombination was the most powerful and capable of penetrating the Escherichia coli cell wall after we linked NPs and E. cardamomum. One of the two proposed mechanisms for how these nanoconjugates are able to combat Gram-negative bacteria is by making the bacterial membrane more rigid and thereby less functional. concentrating on the bacterial cell.

The proposed antibacterial mechanisms of E.cardamomum mainly include: destroy of DNA synthesis, inhibtion of cytoplasmic membrane function, damage of energy metabolism, cell reduction association and biofilm formation, inhibition of purines on the cell membrane, alteration of membrane permeability, and alteration to the cytoplasmic membrane. All result of antibacterial activity with different material shown by the figures below all details explained by Table 4.3.

Table 4.3 Antibacterial activity of (A), control negative. (B), E.cardamomum. (C), Sliver nanoparticles. D, E.cardamomum -Sliver nanoparticles against E. coli, S.aureus.

Antibacterial analysis(Zone of inhibition)mm				
Microorganisms	A	B	C	D
E. coil	6	9	16	18
S.aureus	6	8	15	17

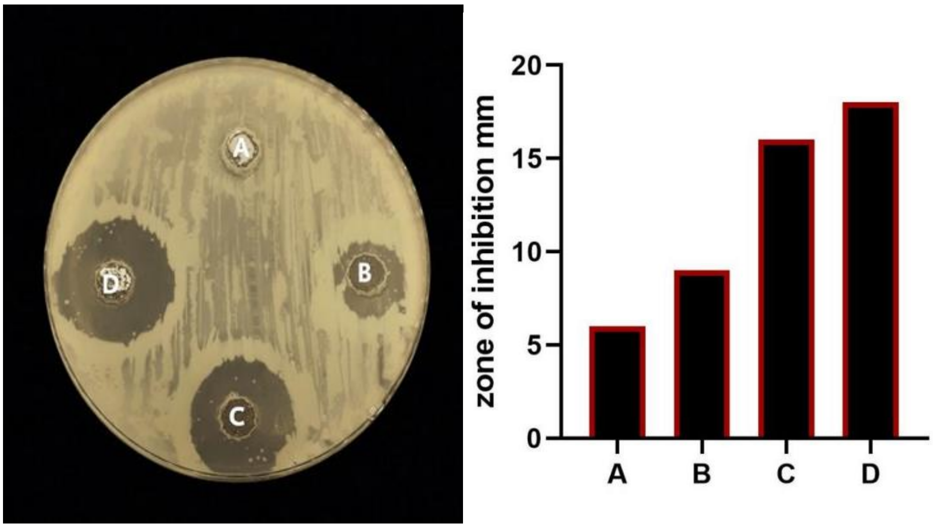


Figure 4.7 Antibacterial activity of (Tested materials) against E. coli. (A), control negative. (B), E.cardamomum (C), Sliver nanoparticles. (D), E.cardamomum -Sliver nanoparticles.

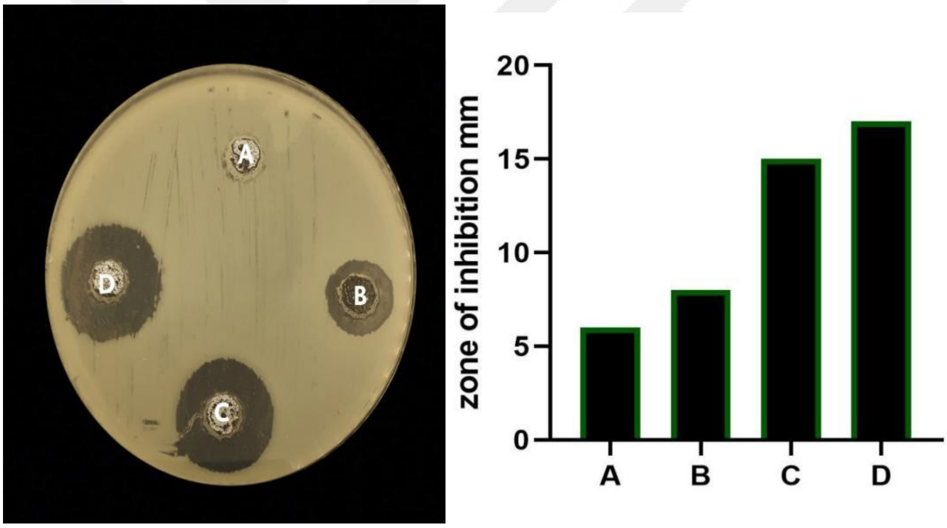


Figure 4.8 Antibacterial activity of (Tested materials) against S. aureus. (A), control negative. (B), E.cardamomum (C), Sliver nanoparticles. (D), E.cardamomum -Sliver nanoparticles.

4.2.2. Antioxidant activities (Free radical scavenging activity)

Figure (4.9) shows the antioxidant activities of E.cardamomum, Sliver-NPs and E.cardamomum –sliver nanoprticales, which were monitored using four different concentrations. The data show that the Antioxidation concentrations differed between E.cardamomum, sliver nanoprticales and E.cardamomum - sliver nanoprticales, wherein the Antioxidation concentrations were (25,50,75 and 100 µg ml-1)The assays conducted for E.cardamomum, AgNPs, and AgNPs-E.cardamomum, showed that DPPH was scavenged proportionally to the concentrations of the AgNPs, i.e., at concentrations of 100,75,50 ,and 25 μg ml-1 of AgNPs, the DPPH free radicals' scavenging were 65.66%,55.65%,45.98%,and 35.46% respectively (Figure 4.9), showed that DPPH was scavenged proportionally to the concentrations of the E.cardamomum, i.e., at concentrations of 25,50,75 and 100 μg ml-1 of E.cardamomum, the DPPH free radicals' scavenging were 22.45%, 31.98%, 40.66%, and 49.67%, respectively .the results showed that E.cardamomum +AgNPs the DPPH free radicals' scavenging were 38.50%, 51.64%, 74.44%, and 88.50%, respectively. The concentration was read, The findings demonstrated that E. cardamomum significantly decreased the level of DPPH in a concentration-dependent manner, with 100 $\mu g/mL^{-1}$ being noticeably superior to the other concentrations. which showed an antioxidant activity of 55.45%. Compared with the activity of sliver nanoprticales, E.cardamomum sliver nanoprticales have higher ability to reduce DPPH radicals than E.cardamonmum which shows sliver nanoprticales 65.43% and E.cardamomum - sliver nanoprticales 88.50% antioxidant activity at a concentration of 100 $\mu g/mL^{-1}$. This can be attributed to the fact that the biological activity of E.cardamomum -sliver nanoprticales can be increased by increasing the specific surface area due to the increased surface energy and catalytic reaction. Importantly, antioxidants can donate electrons to reactive radicals and convert them to more stable (jawad et al., 2023). When dissolved in organic solvents, the free radical DPPH, is stable at 25ºC, takes on a dark violet hue and exhibits an absorption wavelength of 517 nm. The DPPH stability was found to have decreased in the presence of AgNPs, and the violet color changed to yellow due to the presence of phenolic, and OH groups (Deligiannakis et al.,2012). The DPPH method is one of the easy and fast methods used in the evaluation of antioxidants by measuring the absorbance of several substances at the same time. The principle of this method depends on the electron transfer that produces the violet color, where these stable free radicals are reduced in the temperature and the presence of an antioxidant molecule, to combat free radicals which leads to being colorless (Jawad et al., 2022).

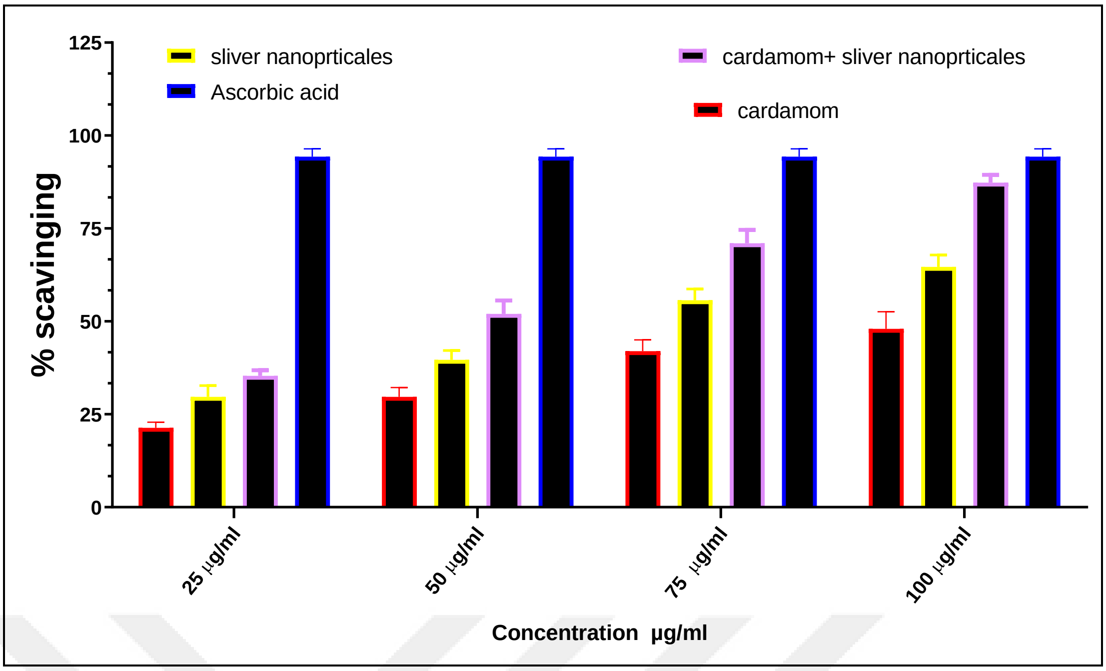


Figure 4.9 E.cardamomum, sliver nanoparticles and E.cardamomum - Sliver nanoparticles increase scavenging of free radicals.

4.2.3. Viability and cytotoxicity assay

One of the most common colorimetric assays for assessing cytotoxicity or cell viability is the methylthiazoletetrazolium bromide (MTT) (3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide) assay (Sabah et al., 2023). The MTT assay clearly explains the cellular response to a toxin, and synthesized E.cardamomum – Sliver NPs have higher anticancer activity against MCF-7 cells than E.cardamomum or sliver nanoparticles in this study. When compared to E.cardamomum or sliver nanoparticles, there was dose-dependent cytotoxicity. After E.cardamomum, sliver-NPs and E.cardamomum –sliver nanoparticles. E.cardamomum –Sliver nanoparticles have the most cytotoxicty effect. Figures (4.10 to 4.13).

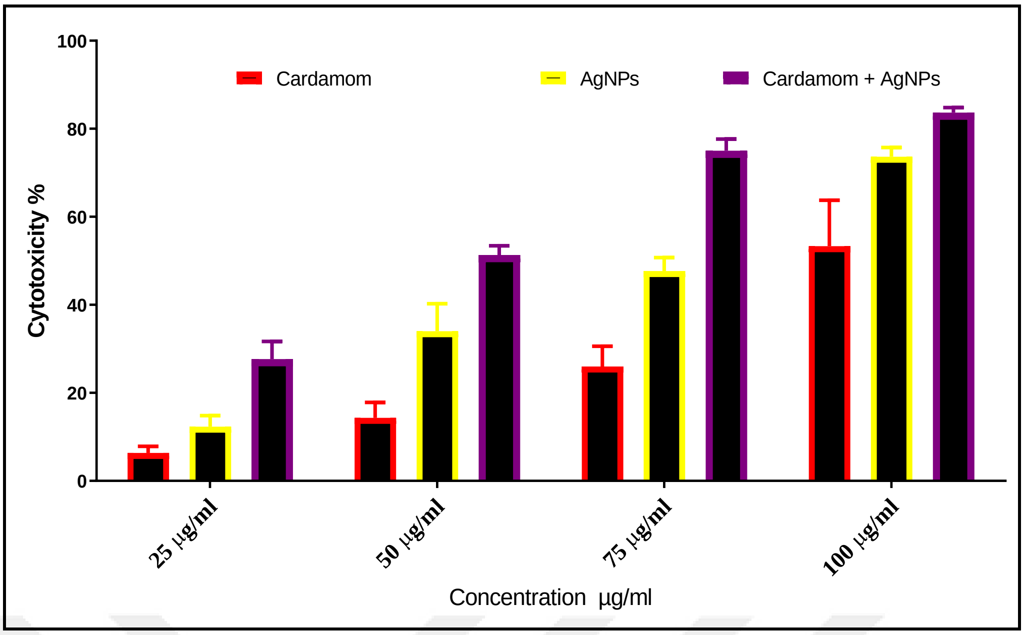


Figure 4.10 Cytotoxicty effect of E.cardamomum, sliver nanoparticles and E.cardamomum -Sliver nanoparticles in MCF-7 cell line.

At 100 µg/ml^{-1} E.cardamomum -Sliver nanoparticles concentration, the highest cytotoxicity (83.17%) was observed, sliver nanoparticles treatment was 73.22 % whereas E.cardamomum treatment was 68.56 % effective. E.cardamomum -Sliver nanoparticles had a much stronger cytotoxic effect than free E.cardamomum, indicating that E.cardamomum anticancer efficacy has improved since it was functionalized with Sliver nanoparticles. It's worth noting that the concentration of synthetic E.cardamomum -Sliver nanoparticles used in this study was significantly higher than that of E.cardamomum. With increasing E.cardamomum - Sliver nanoparticles concentrations, cell viability decreases, indicating that more NPs can accumulate inside cells, causing increased stress and eventually cell death. The biokinetics and toxicity of nanoparticles are influenced by their size, shape, surface area, and surface actuation (Jamal A et al., 2006). In a recent study, it was shown that the synthesis of E.cardamomum -Sliver nanoparticles inhibited cell proliferation and growth in human breast cancer cells in a concentration-dependent manner ($P \leq 0.05$). (MCF-7). As shown in Figs. 4-16 to18. The data of this work may show Cardamom - Sliver nanoparticles cell death by programmed cell death or necrosis. A study is needed on the mechanism by which E.cardamomum -Sliver nanoparticles lead to cell death in cancer cells.

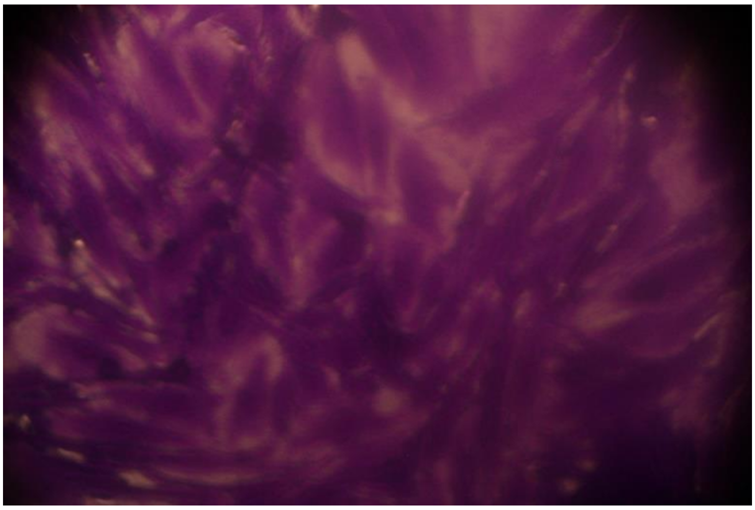


Figure 4.11 Control untreated MCF-7 cells. Magnification power 40x.

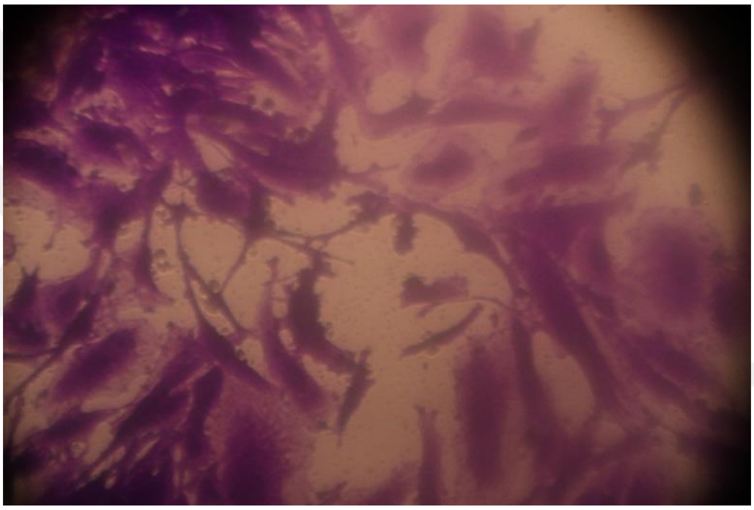


Figure 4.12 Morphological changes in MCF-7 cells after treated with E.cardamomum Magnification power 40x

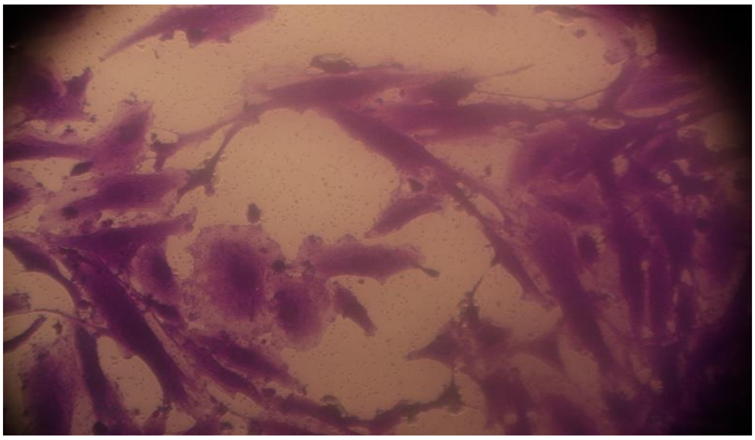


Figure 4.13 Morphological changes in MCF-7 cells after treated with -Sliver nanoparticles Magnification power 40x.

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

Based on the results of the current study:

1) The loaded of E.cardmom onto physcialy synthesized (by laser ablation) AgNPs can produce an effective conjugate AgNPs-E.cardamom against bacterial isolates compared to free E.cardmom.
2) Silver nanoparticles- E.cardamom conjugates showed greater antibacterial activity than Silver nanoparticles.
3) The results find that the best antibacterial nanoconjugate was obtained when using 350mJ as a link bridge and as a stabilizer to prepare AgNPs-E.cardmom
4) AgNPs, and their conjugates showed antioxidant activity by using the DPPH assay.
5) The physcialy synthesized (by laser ablation) AgNPs and their conjugates showed an effect on the anticancer activity on MFC-7cell line.

5.2. Recommendations

1) Further studies are needed for the isolation of another microorganism such as anaerobic bacteria, or fungi.
2) Production of other nanomaterial’s such as gold loaded E.cardamomum.
3) Studying the effect of toxicity of AgNPs, E.cardamomum, and their conjugates on laboratory animals (In vivo).
4) Studying the effect of AgNPs, E.cardamomum, and their conjugates against cancerous lines such as liver and skin cancer, and studying their effect on the normal cell lines.
5) Comparison of the effect of AgNPs, E.cardamomum, and their conjugates synthesizes by different methods (like biosynthesis, physical or chemical.
6) Study the effect of AgNPs, E.cardamomum, and their conjugates on the gene expression of virulence factors genes.

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