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A Comparative Study of the Components of Latex in Some Species and How to Benefit fromThem in Some Biological Application and Biological Techniques

A Thesis

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اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (1) خَلَقَ الْإِنسَانَ مِنْ عَلَقٍ(2) اقْرَأْ وَرَبُّكَ الْأَكْرَمُ (3) الَّذِي عَلَّمَ بِالْقَلَمِ (4) عَلَّمَ الْإِنسَانَ مَا لَمْ يَعْلَمْ (5)

صدق الله العلي العظيم

سورة العلق (1-5)

Dedication

To...

Who gave me the strength and support , (My Beloved Father)

Whom thather praying was the secret of my success ,to my guardian angel, (My Beloved Mother)

The one who supported me and helped me, (My Beloved Husband)

Whom that their presence aquires me force and love my lovely daughter and son, (Mariam and Zaid)

My Dear brothers and sister

All member of my family.

Rula

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Finally, "I would like to thank every one who helped me with a kind word, and I did not mention his name.

Thanks to them all……

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Summary

The current study deals with conducting a chemical and molecular study of five samples of the latex of five producing plant species (*Ficus carica* L., *Ficus elastic* Roxb. ex Hornem,*Calotropis Procera* (Aiton) W.T. Aiton., *Nerium Oleander* L. and *Euphorbia tirucalli* L.) using gas chromatography-mass spectrometry (GC-MS) and Random Amplification replication technique (RAPD),respectively and then the formation of a nano-material from this latex used in some activities which applied for biological application, including as Antibiofilm, anti-bacterial, Hemolysis and DNA Defragmentation.

This study has been conducted in the advanced plant laboratory at the College of Science for Women/ University of Babylon in cooperation with the laboratories of the Ministry of Industry and Minerals and AL-Ameen center for Research and Advance Biotechnology at AL-Najaf province to conduct some tests for the chemical, molecular aspects and nanotechnology for the period from November, 2020 to March, 2021.

The phytochemical analysis of latex-producing plant species included in this study showed the presence of a number of biologically important compounds, some of which were common to a number of plant species and this study confirms the variation between the chemical constituents of latex among plant species, which also showed their different potential of therapeutic activities.

The results of molecular studies were carried out by genetic variation among latex-producing species using five random primers (OPB18, OPC2, BH10, BH11, BH14), after the success of the method for extracting DNA from secondary metabolites represented by latex proved the possibility of confirming the genetic fingerprint of the close relationships between the

species *Ficus carica* and *Ficus elastica* that belong to the same family (Moraceae), as well as in the species *Calotropis procera* and *Nerium oleander* in Apocynaceae and the singular species *Euphorbia tirucalli* which belong to the family (Euphorbiaceae).

This study also includes the successful preparation of silver nanoparticles using the latex of the five studied plant species as a silver reducing agent, this is inferred by the color change that occurred and by testing UV-visible Spectroscopy, given the absorption spectra of nanoparticles created in the reaction mixture display an absorption peak at 400 nm for AgNPs, and SEM Analysis which utilized to confirm the size and morphology of the latex AgNPs. The findings of scanning electron microscopy research indicated that various latex studied of plant species were varied biogenic AgNPs properties, besides X Ray Diffraction XRD and Fourier Transform Infrared Spectroscopy (FTIR).

Some Biological applications of silver nanoparticles are investigated such as the Antibiofilm effect where the production of biofilms was significantly reduced by 40–99% by increasing in Ag-NPs concentration which reduced the quantity of biofilm development in all situations.

The study of the Antioxidant effect revealed that the ability of nanoparticles to reduce DPPH free radicals improved as the concentration of biogenic latex AgNPs increased, it ranged between 65-78% which is the lowest and the highest percentage respectively. In Hemolysis Effect, Latex AgNPs with all concentrations (64, 128, 256, 512 μg/ml) showed a hemolysis rate ranging from (0-4.9) for the whole tested blood, which is considered null, except for *Ficus elastica* where the rates of hemolysis were high at the first concentration.

Summary

The study of the Antibacterial effect showed the activity of silver nanoparticles against gram negative bacteria (*Escherichia coli* , *Klebsiella pneumoniae*) and gram positive bacteria (*Staphylococcus aureus* , *Enterococcus faecalis*), The occurrence of clear inhibition zones and revealed activity against all bacterial species used in this study, with different diameters of the inhibition zone, and this activity was also attributed to the presence of chemical compounds with antibacterial properties that were detected in the latex of the five plant species under study when it was conducted GC-MS analysis, Also the Minimum Inhibitory Concentration (MIC) was determined and there was an inverse proportion between the size of the nanoparticles and the value of the minimum inhibitory concentration.

DNA Fragmentation assay, the results demonstrate that DNA treated with AgNPs exhibits particular DNA smearing, which is a hallmark feature of cell death, whereas, untreated DNA did not exhibit fragmentation.

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List of Abbreviations

Abbreviation	Complete term
RAPD	Random Amplified Polymorphic DNA
PCR	Polymerase Chain Reaction
GC-MS	Gas chromatography–mass spectrometry
APGII	Angiosperm Phylogeny Group
AgNPs	Ag Nanoparticles
SEM	Scanning Electron Microscopy
XRD	X-Ray Diffraction
FTIR	Fourier Transform Infrared Spectroscopy
FABG	Favorgen Blood Genomic
FATG	Favorgen Tissue Genomic
ADHD	Attention deficit hyperactivity disorder

Chapter one

Introduction and Literatures Review

Chapter One: Introduction and Literatures Review

1.1: General Introduction

Plants have two types of defensive systems: primary and secondary defences. Primary defences, which include structurally defensive features on the surface such as hairs, trichomes, thorns, spines, or even thicker leaves, are the initial line of protection against herbivore assaults.

Plant compounds, particularly specialized metabolites, were thought to mediate secondary defences (e.g., phenolics, terpenoids, and alkaloids). Herbivores and phytopathogenic bacteria can be killed or delayed by the metabolites produced during plant defence (Kant *et al*., 2015).

The traditional view of secondary components in plants as waste products has been supplanted by the assumption that their worth lies in their ability to shield plants against herbivorous and microbial assault. On the other hand secondary routes may still be physiologically significant as a mechanism of channelling and storing carbon molecules gained during photosynthesis at times when nitrogen is scarce or when leaf development was restricted. Secondary metabolite levels can skyrocket in stressed plants, as well as those that have been subjected to mechanical or insect damage (Harborne, 2007).

Plant secondary metabolites were substances that play a significant part in the plant's interaction with its environment for adaptation and defence. They play no vital role in the maintenance of life processes in the plant. (Akula and Ravishankar, 2011). As well as, they are a wide range of active compounds and are biosynthetically derived from primary metabolites, they are more limited

in their distribution in the plant kingdom. (Jain *et al*., 2019). Secondary plant metabolites were classified according to their chemical structures into several categories including Phenolics, Alkaloids, Saponins, Terpenes spidrn, (Hussein and El-Anssary, 2019).

Laticifers produce and store a large number of secondary metabolites; yet,secondary metabolites differ between species, even when they were taxonomically related. Chemical composition, structural properties, and activities of latex molecules have been all examined, revealing that latex and laticifers play a vital role in plant defence (Ramos *et al*., 2020). Latex includes a variety of secondary metabolites that are harmful to herbivores, insects, and diseases, including terpenoids (diterpenes, triterpenes, etc.), glycoside, alkaloids, flavonoids, cardenolides, lignan, coumarins, and phenols (Park, 2021).

The milky latex is used as traditional medicine, as a result, new technology was required to combine latex biomass with metal nanoparticles for usage in catalytic and biological applications. (Venugopalan *et al.*, 2020). Because of their interesting features, silver nanoparticles have gotten a lot of attention.
The biological process has gained a lot of interest among the several ways available for the manufacture of silver nanoparticles. (Ismail *et al.*, 2021). Organic compounds are used as ligands, although they can be poisonous and have difficulty with water solubility (Sharma *et al.*, 2020).

Medical devices, sensors, photonics, water purification, electronics, cosmetics, surface coatings, antibacterial agents, and home appliances are just a few of the goods that employ silver nanoparticles. As a result, the fabrication of silver nanoparticles has piqued the interest of researchers. (Chandhru *et al.*,2019).The biological technique uses a basic synthetic pathway. Furthermore, due to the plant extract or latex used as a ligand and resultant agent, this is inexpensive, easy, and quick to make on a wide scale. so, latex is utilized in the production of silver nanoparticles as a reducing and capping agent (Farooqi, *et al.*, 2019).

More than 12,000 plant species (10 % of flowering plants) leak latex when their tissues were injured, according to Castelblanque, *et al*., (2021).

Despite their morphological differences, were important and useful signs in identifying plant species; however, they have a number of drawbacks, such as being affected by environmental conditions that cause DNA indicators to appear, and polymorphisms providing a higher level of of individuality, so they become a useful tool for detecting genetic relationships and variances between genetic patterns (Heywood, 2002). The RAPD indicators are partial PCR-based markers that may be used to determine genetic kinds without knowing the genome's DNA sequence. (Khalaf *et al.*, 2020)

1.2: Aims of the Present Study

Due to the lack of available information on latex of plants and its biological applications in Iraq, the current research aims to:

1- Identify the latex-containing plant species.
2- Chemical analysis of plant latex by GC-MS technique
3- Attempt to form nanoparticles from latex and comparing between them also study them in biological applications.
4- Find the evolutionary relationship between species genetically by RAPD markers.

Literatures Review

1.3: Plant Latex:

Laticifers, which are highly specialized cells produce latex, is a natural plant polymer. Latex is a milky fluid generated by the laticiferous tissue's channels. (Hagel *et al*., 2008). All flowering plants have these tissue in their roots, stems, leaves, and fruits (Pickard, 2008). After minor tissue injury, it produces an emulsion-like sticky fluid from diverse plant regions. Latex has squirted out of the plant bark like white glue in the majority of plant species. It's a complex mixture of proteins, alkaloids, carbohydrates, and other chemicals (Thomas *et al*., 2008).

Latex is white, yellow, orange, or crimson in most plants, but it changes color when exposed to the air. When exposed to air a polymer microparticle dispersion in an aqueous solution coagulates. Few plants produce latex, which contains an elastic polymer identical to rubber and allows for the creation of films without the release of an organic solvent (Yagami *et al*., 1994; Pickard, 2008). It also contains catalytic enzymes such as cysteine proteases, profilins, and chitin-related proteins, which defend against phytopathogenic fungi and other bacterial infections (Domsalla and Melzig, 2008; Sequeira *et al*., 2009). Furthermore, plant latex includes toxic chemical components which that cause allergic reactions and immediate-type hypersensitivity in the humans. Latex from plants also includes a variety of bioactive chemicals with characteristics that are anti-carcinogenic, anti-proliferative, anti-inflammatory, antioxidant, antibacterial, anti-parasitic, and vasodilator (Mesquita *et al*., 2005).

1.4: Composition of Plant Latex and medical importance:

In many diverse places and civilizations across the world, Plants that produce latex have a lengthy history of improving human health and therapeutic usage. According to a recent study, the Opium poppy (*Papaver somniferum* L.) were already being domesticated around the end of fourth millennium B.C. (Jesus *et al.*, 2021), and the early domesticated forebears of *Cannabis sativa* L. diverged on the subject 10,000 years BC (Ren *et al*., 2021). These are two of the most commonly utilized species of laticiferous plants for therapeutics, and they, along with *Hevea brasiliensis* Muli. Arg., the primary and having the best-known and defined latex composition, and are an invaluable natural rubber source.

Secondary metabolites, which are present in these complex fluids, include terpenes, alkaloids, and phenolics, as well as a wide spectrum of proteins, constitute the initial level of protection for plants herbivores. Greater Celandine (*Chelidonium majus* L.),an opium poppy's related, is another laticiferous medicinal plant that has been extensively studied. It contains a large number of biologically active compounds that have been used in traditional folk medicine such as antiviral, antibacterial, antifungal,and anticancer agents (Zielińska *et al*., 2018; Nawrot *et al*., 2020). Latex chemicals are active in both eukaryotic and prokaryotic species (Salomé *et al*., 2019).

Various phytochemicals, alkaloids, sterols, fatty acids, starches, sugars, oils, tannins, resins, and gums, as well as many enzymatic proteins, such as proteases, chitinases, lipases, peptidases, esterase, thrombins, plasmins, peroxidases, papain hevein, lectins, and allergens, are present in latex (Santos and Van Ree, 2011).

C_3H_3N is the chemical formula for latex (carbon, hydrogen, and nitrogen). The constituents include glycolipids, alkaloids, acids, laticifer proteins, and acid, to mention a few Latex phosphatases of *Euphorbia characias* L. (Pintus *et al*., 2011), Glycolipids such as steryl glucosides (SG), esterified steryl glucosides (ESG), monogalactosyl diacylglycerols (MGDG), and digalactosyl diacylglycerols (DGDG) are found in *Hevea brasiliensis* Müll.Arg. latex. It also contains the sterols stigmasterol and -sitosterol (Liengprayoon *et al.*, 2011).

Glycosides, tannins, phytosterols, flavonoids, acetogenins, and saponins are all components of latex, and they have a variety of biological effects on bacteria, viruses ,fungi, insects , protozoans, nematodes,cancer and tumor cell lines. Profilins, which are found in latex from fruits and pollens, cause allergy sensitization in humans and develop large numbers of cross-reactive IgE antibodies against plant foods (Upadhyay, 2011).

Latex possesses antioxidant properties, according to Shahinuzzaman,*et al.,* (2020). Potential cell wall inhibitors found in plant latex contribute to the development of antiviral drugs. *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Candida albicans* have all been found to exhibit antibacterial activities in crude latex (Prastiyanto *et al*., 2020). Latex also has anti-inflammatory properties due to its ability to prevent the generation of nitric oxide, prostaglandin E2(PGE2), and cytokines (Ramos *et al*., 2020). Antiproliferative Activities are also available (Basu *et al*., 2020). Cancer-fighting properties (Ahmed *et al.*, 2020).

1.5: Exuding canal anatomy: laticifers

Latex-producing cells, or a network of cells that create systems that flow through the plant's numerous tissue are known as laticifers. Several categories have been developed to best understanding of the morphological variety of laticifers. The most often used classification is based upon laticifer ontogeny, in which laticifers can be either nonarticulated or articulated (Evert, 2006). Laticifers are call either articulated or laticiferous vessels if their terminals are articulated or laticiferous vessels. During the creation of the laticifer, walls may be remain solid, become porous, or may be disintegrate totally. A single multinucleated cell that expands as it grows of the plant. These cells are known as nonarticulated laticifers or laticiferous cells (Hagel *et al*., 2008).

Considerable the vacuoles, which occupy a large fraction of laticifer cells and develop during laticifer development, they are considered to be the source of the majority of extruded latex. (Cai *et al*., 2009). However, In some cases, the extruded latex may contain fluid from the cytoplasm of laticifer cells because the cytoplasm of laticifer cells degrades and is combined with vacuoles in the later stages of laticifer growth in some laticiferous plants. (Zhou and Liu, 2010).

1.6: Families Which Include Laticiferous Plants

Latex was found in about 20 000 species from over 40 angiosperm families, accounting for around 8% to 10% of all terrestrial angiosperms (Konno, 2011; Huber *et al*., 2015). Latex was more commonly seen in tropical plants as a general feature. Plant families and species that thrive in tropical areas, for example, have a high percentage of laticiferous families and species (12.2 percent for families and 14 percent for species), whereas plant families and

species that thrive in the temperate areas have a lower percentage of laticiferous families and species (4.9 percent for families and 5.9 percent for species) (Lewinsohn, 1991).

According to some studies, 15–30 % of plant species in tropical Africa (Reitsma, 1988) and 20–35 % of those in Tropical America (Amazon) exude latex, compared to 8.9% globally (Farrell *et al.*, 1991). Because plant-herbivorous insect interactions are more intense in tropical area than in temperate area, the frequent presence of laticiferous plants is compatible with latex and laticifer's protective activities against herbivorous insects.

Lewinsohn (1991) pointed to families that include laticiferous plants are (Araceae Juss. , Cactaceae Juss. , Cannabinaceae Martinov. , Caricaceae Dumort. , Nymphaeaceae Salisb. , Olacaceae R.Br., Alismataceae L., Amaryllidaceae L., Celastraceae R.Br., Compositae L., Convolvulaceae L.) as well These are the families under study:

1.6.1 Moraceae Gaudich.

Often called as Mulberry family or fig family, the discovery of the family Johann Heinrich Friedrich Link (1767-1851) He was a German naturalist and botanist. The majority of this family's members live in the tropics and subtropics, however, a few genera, such as *Morus* (Mulberry) and *Broussontia papyrifera*, are typically found in temperate areas.

There are around 28 genera and 1150 species (Punt and Malotaux, 2007). Trees or shrubs have a milky latex, whether deciduous or evergreen (rarely herbs), Simple or palmately lobed leaves alternating (rarely opposite). Two Stipules are frequently elusive and leave a visible scar, Regular flowers, monoecious or dioecious, considerably reduced and inconspicuous, in close heads or spikes, or the pistillate arranged in an enlarged and discoid or hollow receptacle.

In Iraq, there are four genera of **Moraceae** (two natives, two introduced), A significant economic family with valuable timber trees and others with edible fruits, bark that produces paper and fabric, latex that produces rubber, and other useful items (Townsend and Guest, 1980).

Species of Moraceae under study:

A- *Ficus carica* L.

Geographical distribution is one of the first fruit trees to be grown (Solomon et al., 2006). Ficus species number over 500 and may be found throughout Asia and Australia, In Egypt, Malaysia , south China, Turkey, and India (Nawaz and Nawaz, 2019). It is endemic to the nations of South-West Asia and the Mediterranean area. *Ficus* species are mostly grown scattered in gardens or home yards. (Chawla and Sharma,2012) .

Description: sometimes known as the fig tree, is a deciduous plant. It is 1.5–2 meters tall, has several branches, and secret milky white latex. The plant's root system is usually shallow and spread out, Carica refers to the papaya-like. Leaves are huge, brilliant green, alternating, more or less deeply lobed with 1–5 sinuses, rough hairy on the upper surface and soft hairy on the underneath. (McGovern, 2002).

Flowers emerge from the axils of old leaves and are seen in receptacles. Female flowers occupy the top half of the receptacle, whereas male flowers occupy the lower section. syconium, the mature receptacle, has a great number of little white seeds. The number of seeds per fruit can range from 30 to 1600 and can be big, medium, tiny, or minute. Unless fertilized, the edible seeds are abundant and hollow. Dried figs have a distinct nutty flavor that comes from pollinated seeds. A white inner ring contains a seed mass bonded with jelly-like flesh on the inside.

Classification of *Ficus carica:* (Chawla and Sharma, 2012)

Kingdom: Plantae
Subkingdom: Tracheobion
Superdivision: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Hamamelididae
Order: Urticales
Family: Moraceae
Genus: *Ficus*
Species: *carica*

The economic and medical importance

Ficus species were consumed by humans as a dietary supplement The roots, stem, bark, leaves, latex, fruit, and pulp of the plants are medicinally significant due to their high phytochemical content and great antioxidant potential, which includes Flavonoids (Vaya, 2006), Phenolic acids, Coumarins, and Phytosterols, among others (Jeong and Kim, 2009). Antimicrobial , hepatoprotective , anti-obesity, antidiabetic, cardioprotective, renal-protective, and anticancer effects are all demonstrated by these plants (Nawaz and Nawaz, 2019). Some chemical compounds and their medicinal use were summarized according to different sources in Table (1-1).

Table 1-1: The chemical composition and medicinal uses in *Ficus carica* according to different references

No	Chemical composition	Medical important	References
1	phenolics	antioxidant activity	(Abdel-Aty, *et al*.,2019)
2	polyphenols and flavonoids.	antibacterial, antitumor, as well as anti-inflammatory activities	(Bouyahya, *et al*.,2016)
3	4-hydroxy-3 methoxbenzoic acid, methyl ester	Antioxidant, anti-cancer and anti-apoptotic	(Kumar, *et al*.,2003)
4	Psoralen	Antioxidant, anticancer, antimicrobial, Bone-modifying therapeutic agent for bone metastases treatment	(Wu, *et al*., 2013)
5	Phthalic acid	Antioxidant, cytotoxic on human cancer cell lines and anti-apoptotic, anti-inflammatory	(Kok, *et al*., 2018)
6	Dibutyl phthalate	Antioxidant, potent antimicrobial agent	(Roya, *et al*., 2006)
7	Coumarins	Antimicrobial activities	(Lazreg-Aref, *et al*.,2012)
8	Xanthotoxin	Anti-leukodermal and antitumor properties	(Abdel Hafez, *et al*.,2009)
9	Benzoic acid, 2-hydroxy-, methyl ester	Antioxidant, antimicrobial and antiproliferative agent	(Jaganathan and Mandal, 2009)
10	Proteolytic enzymes, diastase,esterase, lipase, catalase, and peroxidase	Enzymes	(Aref HL, *et al*., 2010)
11	Ficin (nonspecific sulfhydryl protease)	Antibiofilm	(Baidamshina, *et al*., 2017)
12	Proteinase	Proteolytic	(Upadhyay, 2011)

B- *Ficus elastica* Roxb. ex Hornem

Geographical distribution of rubber tree: It grows in humid, warm, tropical climates, where it may reach heights of 7.5 to 10 meters and extend its branches widely. It appears to be localized in the Asian-Australasian area, with inhabitants in the northern portion of Central America and the northern Andean region being quite well-represented (Starr and Loope, 2003).

Description : Terrestrial or hemiepiphytic trees up to 30 m tall. glabrous or (slightly) puberulous leafy twigs Spiral-arranged leaves; lamina elliptic to oblong, coriaceous, apex acuminate, base cuneate to obtuse (or rounded); both

surfaces glabrous; cystoliths on both sides; waxy gland at the base of the midrib (sometimes faint); petiole glabrous; stipules glabrous or white puberulous, caducous Figs in pairs (or solitary) axillary or slightly below the leaves; basal bracts early caducous; receptacle ellipsoid to cylindrical. Ostiole round, covered with three bracts, glabrous when dry, yellow at maturity, apex somewhat umbonate, glabrous. Staminate blooms are scattered, sessile or pedicellate, with a single stamen and oblong to lanceolate tepals that are free. Sessile or pedicellate pistillate flowers, ovary white, tepals ovate to lanceolate, free (Chantarasuwan *et al*., 2016).

Classification of *Ficus elastica*:

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Hamamelididae

Order: Urticales

Family: Moraceae

Genus: *Ficus*

Species: *elastica*

The economic and medical importance:

Rubber is made from the white latex derived from the bark of the *Ficus elastica* tree, it's also used in medicine to treat skin infections, allergies,

anaemia, neurological disorders, and hepatic issues, as well as acting as a diuretic. Herbalism is a type of traditional or folk medicine that uses plant extracts. (Acharya and Shrivastava, 2008). Astringents, carminatives, stomachics, Vermonicides, and anti-dysentery drugs have all been utilized in medicine. Ficus plants are also used to treat a variety of ailments, not just

cancerix. *Ficus elastica* and *Ficus bengalensis* Linn. are analgesic and anti-inflammatory (Iqbal, 2018). Jain,*et al*., (2015) showed the presence of carbohydrates, phenolic, flavonoids, proteins and tannins, which might be responsible for the medicinal properties. Some chemical compounds and their medicinal use are summarized according to different sources in Table (1-2).

Table 1-2: The chemical composition and medicinal uses in *Ficus elastica* according to different references

No	Chemical composition	Medical important	References
1	Coumarins: α-amyrin; bergapten	natural anti-inflammatory and anti-tumour agent	(Abdel-Wahab, *et al*.,1989) (Ashraf, *et al*.,2021)
2	α-amyrine: β-amyrine; lupeol	Anti-inflammatory and Anti-cancer	(Van der Bie1,946) (Ashraf, *et al*.,2021) (Saleem, 2009).
3	Ficin E (a serine centred protease)	antibiofilm	(Lynn KR, 1986) (Ashraf, *et al*.,2021)
4	phenols	plant disease resistance	(Augustus, *et al*.,2011)
5	Polyphenols	manufacture various adhesives, phenolic resins, and antioxidants	
6	steroids and alkaloids	analgestic, emetic, vermifuge, diaphoretic, and anti-tumourous	
7	proteases	antifungal and antimicrobial activity	(Musidlak, *et al.*,2020)

1.6.2 Asclepiadaceae Burnett According to the APG II (2003) classification system, the Asclepiadaceae family is a former plant family that was currently classified as a subfamily (Asclepiadoideae) inside the Apocynaceae family (Kumar, 2020).

The Asclepiadaceae family, also known as the milkweed family, contains around 250 genera and 2000 species. Many medicinal plants in this family may be found in the damp parts of India (Patel *et al*.,2020).

Shrubs with upright, scrambling, or twining branches (little trees) or perennial plants with erect, decumbent, or twining branches, and occasionally tiny succulents with milky sap. Leaves are opposite decussate, simple, usually whole but sometimes serrated and infrequently lobed, vary in size, sometimes reduced to scales or obsolete, and Exstipulate. Cymose inflorescence, commonly umbelliform, occasionally racemose branching, occasionally sessile, and rarely solitary. Regular, pentamerous, and gamopetalous flowers (Townsend and Guest,1980).

Species of Asclepiadaceae under study:

***Calotropis procera* (Aiton) W.T. Aiton.**

Geographical distribution: *Calotropis procera* was native to Africa and Asia's tropical and subtropical regions, but it is more prevalent in the Middle East (Rahman and Wilcock 1991). This species is naturalized in various Pacific islands, Mexico, Central ,Australia, Indonesia, and South America . (Rahman and Wilcock 1991) and northern Brazil (Ellison and Barreto 2004). In Iraq, there is just one rare species that is infrequently farmed and grows wild in several regions of the country's lowlands (Townsend and Guest,1980).

Description*:* *Calotropis procera* is a remarkable medium-sized shrub or small tree that can grow up to 4 meters tall. It has grey-green stems that are smooth and crooked, and older stems have a soft, thick corky bark. Branches near the base of the plant are frequent. When stems are injured, they release enormous amounts of sticky latex. Lactifer cells in the stems and roots create latex (Ogundipe, 1993).

The opposite leaves are oblong obovate to nearly orbicular, leaf blades are bright to dark green with almost white veins. (Nandkarni, 2000). They are seven to eighteen cm long and five to thirteen cm wide, with a delicate of coating of silky hairs that rub off, and are slightly leathery. Umbelliform cymes develop near the ends or at the ends of branches and produce flower

clusters, The blooms are shallowly campanulate, with four to five mm long sepals that are fleshy and colors range from white to pink, frequently dotted or tinged with purple, when fully ripe, the fruits are inflated, obliquely ovoid follicles that split and invert, releasing flat, brown seeds with a tuft of white hairs at one end (Murti *et al*., 2010).

Classification of *Calotropis procera:* (Khairnar *et al*.,2012)

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision : Spermatophyta

Division : Magnoliophyta

Class: Magnoliopsida

Subclass: Asteridae

Order: Gentianales

Family : Asclepiadaceae

Genus: *Calotropis*

Species : *procera*

The economic and medical importance:

Calotropis procera contains physiologically active chemical groups such as cardenolides,steroids,tannins,glycosides,phenols,terpenoids,sugars,flavonoids, alkaloids, and saponins. Antimicrobial, anthelmintic, anti-inflammatory, analgesic, and antipyretic effects were observed, as well as anticancer, anti-angiogenic, immunological, cardiovascular, hypolipidemic, gastroprotective, hepatic protective, renal protective,antidiarrheal, antioxidant, anticonvulsant, wound healing enhancement, antifertility, and smooth muscle relaxant effects (Al-Snafi, 2015).

According to phytochemical studies, alkaloids, flavonoids, glycosides, saponins, and terpenes were found in *C. procera* root extracts (Samy and Chow, 2012).

Various glycosides, such as calotropin, uscharin, calotoxin, and calactin, were discovered in the leaves (Meena *et al*., 2010). Processterol, a new steroidal hydroxy ketone, was discovered in the fresh and undried flowers of *C.procera.* Some chemical compounds and their medicinal use are summarized according to different sources in Table (1-3) according to different references

Table 1-3: The chemical composition and medicinal uses in *C.procera*

No	Chemical composition	Medical important	References
1	flavonoids	antioxidant activity	(Joshi, et al.,2009)
2	caloptropaine.	antimicrobial proteolytic enzyme	Sharma A.K.; Kharb R. and Kaur R. (2011)Pharmacognostical aspects of Calotropis procera (Ait.) R. Br. International Journal of Pharma and BioSciences. 2(3):480–8.
3	1-(2′,5′-dimethoxyphenyl)-glycerol,	treatment of parasites	(Cavalcante,*et al* ., 2016)
4	cysteine proteinase and dismutase	A bacteriolytic enzyme and oxidant enzymes	(Freitas,*et al* ., 2007)
5	Tannins Saponins	anti-inflammatory properties, antioxidant, antibacterial,	(Kawo, *et al*., 2009)
6	Alkaloids	Antimicrobial property	(Shobowale, *et al*.,2013) (Bello, *et al*.,2020)
7	Phenyl butazone and atropine	Anti-diarrhea	(Quazi, *et al*.,2013)
8	Terpenoids	anti-bacterial, antifungal, antimalarial	(Farooq, *et al*.,2017)
9	Osmatin and thumatin proteins	Effective against fungi Fusarium solini	(Larhsini M, *et al*.,1997)
10	Calanolide	Inhibt growth of phytopathogens	(Upadhyay, 2011).
11	A-D mannosidase	Anti-fungal	(Upadhyay, 2011).
12	Steroids	use in arthritis	(Manoorkar, and Gachande,2015)

1.6.3: Apocynaceae L.

Apocynaceae is sometimes known as the Dogbane family, consists of roughly 300 genera and 1300 species, It is predominantly found in the tropics and subtropics, with temperate regions being underrepresented (Wongm *et al.*, 2013). Rauvolfioideae, Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae are five subfamilies of Apocynaceae. It's worth noting that the Apocynaceae family is closely connected to the Asclepiadaceae family (Sultanaa *et al.*, 2013).

Branched lactiferous cells and bicollateral vascular bundles are found in all trees, shrubs, and plants except for herbs. Leaves are normally opposite, although they can also be whorled. They are whole and usually Exstipulate. Flowers were hermaphrodite, actinomorphic, hypogynous, (4-)5-merous, solitary or in cymose inflorescences, and are hermaphrodite, actinomorphic, and hypogynous. In the bud, the gamopetalous corolla is twisted. Anthers convergent on or near the stylar head, connective elaborate, often sagittately

protracted; stamens as numerous as the corolla lobes and alternating with them, epipetalous, with short filaments ,frequently sagittately prolonged. Superior or semi-inferior ovary with two carpels that are typically free below but have a shared style; ovules few to many on the carpels' ventral sutures. A follicular fruit, a capsule, a berry, or a drupe. Endosperm straight, fleshy to firm-fleshy; seeds typically winged or plumed (Townsend and Guest,1980).

Species of Apocynaceae under study:

Nerium oleander L.

Geographical distribution: *Nerium oleander* is a commonly cultivated plant that is assumed to have originated in the Mediterranean area and the Indo-Pakistan subcontinent, and is found in a variety of geographical and ecological settings (Farooqui and Tyagi, 2018) It is widely spread in the Mediterranean region, subtropical Asia, the southern United States, and many other warm locations (Rashan *et al*., 2011)

where it is grown outdoors in parks, gardens, and along the roadside by people who may not be aware of its toxic potential (O'Leary, 1964).

In Iraq, it is distributed occasionally in the lower forest zone and steppe region, cultivated in the desert region, and also cultivated in gardens, notably in our territory (Townsend and Guest, 1980).

Description*:* The dogbane (*Nerium oleander*) is a small tree or shrub that is evergreen.Although it also goes by the names *Nerium indicum* Mill and *Nerium odorum* Soland, it is known as *oleander* due to its superficial resemblance to the unrelated species Olive *Olea*. The variety with white and scarlet blooms is *Nerium indicum* (Santhi *et al*., 2011).

Oleander grows two–six m in height, first-year stems exhibit a glaucous bloom, whereas elder stems have grey bark The leaves are thick and leathery, dark green, narrowly lanceolate, five–twenty centimeters long and one–three point five centimeters wide, with an entire border, and are grouped in pairs or whorls of three (Siddiqui *et al*., 2012).

Flowers range in color from white to pink and red , and grow in clusters at the end of each branch. They have a deeply 5-lobed fringed corolla encirclement the central corolla tube. They usually have a pleasant scent; however, this isn't always the case. When completely grown, the fruit is a

long, thin capsule that splits apart to release numerous downy seeds (Kiran and Prasad, 2014).

Classification of *Nerium oleander*: (Sinha and Biswas, 2016).

Kingdom: Plantae

Division: Angiosperms

Class: Magnoliopsida

Subclass: Asteridae

Order: Gentianales

Family: Apocynaceae

Genus: *Nerium*

Species: *oleander*

The economic and medical importance of *Nerium oleander*:

In Chinese traditional medicine, *Nerium oleander* is an important medicinal plant. Previously, it was considered that all portions of the oleander plant were dangerous to people, animals, and some insects, but now, various experts have established a variety of pharmacological activities.

Polysaccharides, cardenolides, glycosides, and triterpenoids are the most active ingredients. Antinociceptive, anti-inflammatory, antibacterial, anticancer, and Central nervous system (CNS) depressing action are all essential pharmacological activities (Vikas and Payal, 2010). All plant parts were used medicinally (Khare, 2004).

According to early phytochemical screening by Chaudhary ,*et al*., (2015), indicated the plant included alkaloids, flavonoids, carbohydrates, tannins, phenolics, saponins, cardenolides, cardiac glycosides, pregnanes, triterpenoids, triterpenes, and steroids. Some chemical compounds and their medicinal use are summarized according to different sources in Table (1-4).

Table 1- 4: The chemical composition and medicinal uses in *Nerium oleander* according to different references

No	Chemical composition	Medical important	References
1	Alkaloids	antibacterial	(Manoorkar, and Gachande, 2015)
2	Saponins	Anti-proliferative	(Upadhyay, 2011).
3	Terpenoids	use in arthritis (Cytotoxic)	(Brahmbhatt,2012).
4	Coumarin	treatment of inflammation	(Brahmbhatt,2012). (Alasmari, *et al* .,2009*)*
5	Glycosides	Inhibitors of Na K ATPase Anticancer, antiviral, anti-inflammatory	

1.6.4 Euphorbiaceae Juss.

Euphorbiaceae is one of the biggest flowering plant families. It is found all across the world except for the polar areas and is particularly abundant in both hemispheres' tropics, some people believe it isn't a truly " natural " family, it has affinities with several different families, including Malvaceae and Flacourtiaceae (Townsend and Guest,1980). It is commonly referred to as euphorbias in common English. It is also the name of a family genus (Webster, 2014).

This family is mostly found in the tropics, with the Indo-Malayan area accounting for the bulk of species and tropical America a close second. Tropical Africa has great diversity, although it is not as diverse or prolific as the other two tropical zones. In nontropical locations such as the Mediterranean Basin, the Middle East, South Africa, and the Southern United States, however, the Euphorbiaceae has a large number of species (Mondal *et al*.,2016).

Herbs, shrubs, trees, or succulents. Leaves alternating or opposite; stipules missing, a minute or big and foliaceous, Flowers can be unisexual, dioecious, or monoecious, although they are seldom bisexual, With over 300

genera and 6,000 species. Iraq has seven genera (4 Native, 2 Naturalized and now subspontaneous, and 1 recently introduced) (Townsend and Guest,1980).

Species of Euphorbiaceae under study:

Euphorbia tirucalli

Geographical distribution: It may be found in black clay soils all across Africa, with a strong presence in northeastern, central, and southern Africa, it's possible that it's native to other sections of the continent, as well as certain nearby islands and the Arabian Peninsula, and it's been imported to several tropical countries, including Brazil, India, Vietnam, the Philippines, and Ghana (Upadhyay *et al.*,2010). It thrives in arid environments, particularly the Savanna. *E.tirucalli* is a popular decorative plant in India, and it was brought as a garden plant from Africa to tropical nations. It may be found in Brazil, the Amazon's northeast region, and certain Iranian coastal locations (Swapna *et al.*,2011).

Description: The plant is a tiny tree or shrub endemic to tropical locations with pencil-like branches, thus the name "pencil-tree." *E. tirucalli* is considered evergreen since its stems and branches remain green all year and animals seldom feed on it**.** It's grown up to five m tall with upright branches, with rough and cracked greenish-brown bark that emits a milky sap when fractured, and branchlets that are thin, smooth, cylindrical, polished, whorled. *E.tirucalli* is a branching plant with branches grouped in pseudo whorls10, producing brush-like masses that are the species' most well-known trait (Priya and Rao, 2011).

Leaves are small, few, simple, fleshy, small or minute, thin, and alternating, and fall extremely early or fast (early deciduous). The leaf blades are linear-lanceolate to oblanceolate, one to two point five cm long, three to four mm wide, and two mm thick, acute at the tip, tapering to the sessile base,

spirally organized, and only present at the terminals of juvenile branchlets (Gupta *et al.*, 2013).

Classification of *Euphorbia tirucalli*: (Wal *et al.*,2013).

Kingdom: Plantae.

Division: Magnoliophyta.

Class: Magnoliopsida

Order: Malpighiales.

Family: Euphorbiaceae.

Genus: *Euphorbia.*

Species: *tirucalli.*

The economic and medical importance of *Euphorbia tirucalli*:

E.tirucalli has been shown to have hydrocarbon polymers, which are exploited to make rubber alternatives. Its latex, according to some experts, is a water-based emulsion of terpenes and resins that may be easily converted into rubber at a minimal cost. (Van Damme, 2001).

E.tirucalli is employed in semi-arid places to carry out afforestation and reforestation for the goal of soil conservation due to its favorable agronomic qualities such as drought tolerance. These plants can be used as a soil cover in some area where other plants aren't allowed to grow (even grasses) (Van Damme, 2001).

Flavonoids, diterpenes, tannins, steroids, and alkaloids are known to be important phytochemical constituents in *E. Tirucalli*, Terpenes, alcohol eufol, alfaeuforbol, taraxasterol, tirucallol, cycloeuphornol, n-hexacosanol, terpenic alcohol, and trigliane are also claimed to be present in the plant. Terpenoids and sterols are essential sources of vitamins, steroid chemicals, and other substances in plants, Insecticides and anticancer medications are used in industry (Priya, and Rao, 2011). Some chemical compounds and their medicinal use are summarized according to different sources in Table (1-5).

Table 1-5: The chemical composition and medicinal uses in *E. Tirucalli* according to different references

No	Chemical composition	Medical important	References
1	Phenolics	antioxidant activity	(Abdel-Aty, *et al*.,2019)
2	Pregn-4-ene-3,20-dione, 11- Hydroxy	Steroid compound Therapeutic drug	(Meher, *et al*.,2013)
4	9,19-Cyclo-9. beta. -lanostane-3. beta.,25-diol	Cytotoxicity against prostate cancer cell line	(Shamsabadipour, *et al*.,2018)
5	Lanosterol	Chemo-preventive activity against colon carcinogenesis, cytotoxicity against some human cancer cell lines.	(Ma, L., *et al.*,2013)
6	triterpenes euphol and tirucallol,	anticancer activity	(de Souza, *et al*.,2019)
7	diterpene esters	antitumor potential	(de Souza, *et al*.,2019)
8	Alkaloids	Antimicrobial property	(Vattem, *et al*.,2005) (Manoorkar, *et al*.,2015)
9	Flavonoids	antioxidant activity	
11	Tannins	anti-inflammatory properties, antioxidant, antibacterial	
12	Phenols		
13	Steroids	use in arthritis	

1.7: Gas Chromatography-Mass Spectrometry (GC-MS) Technique:

The GC–MS system is widely regarded as a flexible analytical tool. Its resilience, high separation capabilities, selectivity, sensitivity, and repeatability all contribute to this. Electron ionization (EI) and chemical ionization (CI) are the two basic types of ionization employed in GC–MS. In metabolomics, EI has been used in most GC–MS procedures. Many analysts choose EI-based GC–MS because of the availability of many mass spectrum databases- libraries that correlate to it. Other benefits include simplicity of use (in terms of analysis time and operational expenses) and the ability to give insight into chemical identification (Beale *et al*., 2018).

Gas chromatography can quantitatively evaluate compounds present at extremely low concentrations using the flame ionization detector and the electron capture detector (Mohammed *et al*., 2016). As a result, pollution research and general trace analysis are the second and third most prominent

application areas, Because of its simplicity, sensitivity, and usefulness in separating components of mixtures, gas chromatography is one of the most important devices in chemistry. It's often used to quantify and qualitatively assess mixtures, purify chemicals, and determine their composition. It's commonly used to analyze mixtures quantitatively and qualitatively, purify substances, and determine (Amirav *et al*., 2008).

Knowledge of plant chemical contents is useful not only for the identification of medicinal agents (Bliesner, 2006) but also for revealing new sources of economically viable Phyto-compounds for the synthesis of complex chemical substances and for determining the true relevance of folklore cures. Higher plants continue to play a significant role in human health maintenance as sources of bioactive chemicals. Green plant reports provide a reservoir of potent chemotherapeutics that are non-phytotoxic, systemic, and quickly biodegradable (Andrew, 2007).

Many studies have been conducted using GC MS. Where Abdel-Aty *et al*. (2019) conducted their study about Phytochemical screening, antioxidant and cytotoxic of *Ficus carica, Ficus sycomorus and Euphorbia tirucalli latex* extracts and they found the phenolic extracts of *Ficus carica* showed potent antioxidant activity.

Nielsen ,*et al.,* (1979) carried out their research (Steroids from *Euphorbia* and other latex-bearing plants), The results showed Steroids in latices isolated from 15 *Euphorbia* species and four other latex-bearing plants

were analyzed using computerized GC-MS. Therefore, divided these plants into six groupings.

In 2016 Sharma ,*et al* ., also performed a comparative GC-MS analysis of the bioactive compounds in methanol extract of *Calotropis gigantea* leaf and latex, the results analysis revealed the presence of a total of 46 bioactive compounds (24 from leaves and 22 from latex) with valuable activity.

1.8 Molecular study:

Over the last several years, tremendous breakthroughs in molecular genetics have equipped individuals working in the conservation of plant genetic resources with a variety of novel approaches for identifying plant species quickly and accurately. Many of these methods have been used to investigate the size and distribution of diversity in species gene pools, as well as to address common evolutionary and taxonomic problems (Arif *et al*., 2010).

The use of molecular markers at the protein or DNA level opens up new research options for several plant evolution and breeding concerns. (Nadeem *et al*., 2018). Polymorphisms in nucleotide sequences of different people can be used to investigate molecular markers, which are nucleotide sequences. These polymorphisms are caused by insertions, deletions, point mutations, duplications, and translocations, although they don't necessarily affect gene function. An ideal DNA marker would be co-dominant, distributed evenly across the genome, highly repeatable, and capable of detecting greater amounts of variation (Mondini and Noorani, 2009).

The following criteria are used to categorize molecular markers into multiple categories, according to Semagn ,*et al*., (2006):

A - Gene's mode of operation (dominant or co-dominant markers).

B- The method of detection (hybridization-based molecular markers or polymerase chain reaction-based markers) (PCR).

C- Stands for transmission method (maternal organelle inheritance ,paternal organelle inheritance, bi-parental nuclear inheritance or maternal nuclear inheritance).

RAPD is a PCR-based technology that uses a single arbitrarily short primer (8-12 nucleotides) to amplify a large number of distinct DNA fragments. The DNA segments that are amplified are chosen at random

(Nandani and Thakur,2014). In a DNA amplification-based experiment, this method finds nucleotide sequence polymorphisms. The RAPD approach has made it feasible to swiftly and efficiently test for DNA-sequence polymorphisms at a large number of loci (Arif *et al*., 2010).

many studies have been conducted to define the genetic variation of environmental patterns on plants belonging to the family Apocynaceae depending on the molecular markers, each of which showed its advantages to identify valuable signs. Where Torezan and his group (2005) explain the RAPD used to access the genetic variability in Aspisdosperma polyneuron and their results proved a decrease of genetic polymorphism of post-fragmentation cohorts in small fragments and higher genetic diversity within a population.

In 2015 Raju Pirkhezri and his group also studied the genetic variation of the *Ficus* species by using RAPD markers as a measure of genomic polymorphism so the results of combined data exhibited that *Ficus benghalensis* and the *Ficus elastica* are related cultivars with the highest similarity index ,but *Ficus benghalensis* and *Ficus benjamina* are two distant related species with a low similarity index. Also, Fadl and Ahmed (2019)

pointed for genetic documentation and studyied the taxonomic relationships, 9 species of family Euphorbiaceae, *Euphorbia tirucalli* was among them, As for Iraq, this study is the first in finding the evolutionary relationship between species (by using latex) genetically by RAPD markers.

1.9Nanotechnology:

Nanotechnology is a term used to describe a type of technology that describesthe ability to manipulate and restructure of matter at the both atomic and molecular scales in the range of 1–100 nm, and to harness the unique phenomena and properties associated with that size as opposed to single atoms, molecules, or bulk behavior in order to develop materials, devices, and systems with novel properties and functions (Roco, 2011). As nanotechnology advances, a huge number of nanomaterials with unique features emerge, bringing up a wide range of applications and research possibilities. (Sharma *et al*., 2009).

Many Greeks, Egyptians , Persians, and Romans utilized silver in some form or another to keep food supplies around 5000 years ago (Grier, 1968). During the ancient time, numerous dynasties used silverware for drinking, eating, and storing various eatable and drinkable things. This was likely owing to the awareness of antibacterial effects. (Srikar *et al*.,2016). Researchers have recently focused their attention on silver nanoparticles (AgNps) because of their exceptional protection against a wide range of bacteria, as well as the emergence of medication resistance to routinely used antibiotics (Sharma *et al*., 2009).

AgNPs have been discovered to have significant potential for usage as antibacterial, anti-parasitic, and site-specific medication, water purification systems, and other applications due to their unique features, according to a recent study (Srikar *et al*., 2016). Because of their unique qualities, AgNPs are used in a range of fields, including biomedicine (Chaloupka *et al*., 2010), medication delivery, water treatment, agriculture, and more (Srikar *et al*., 2016). They are also used in inks, adhesives, electrical devices, pastes, and other applications (Park and Lee, 2008).

Chemical reduction (Khan *et al*., 2011), microemulsion,gamma-ray radiation,microwave laser ablation, autoclave, electrochemical process, , and photochemical reduction have all been used to manufacture AgNps (Srikar *et al*., 2016). These technologies provide good results, but they have drawbacks such as the use of harmful chemicals, as well as significant operating costs and energy requirements.

Considering the disadvantages of physio-chemical approaches, microorganisms (Sharma *et al*., 2009), plant extracts (Song and Kim, 2009), and natural polymers (Huang and Yang, 2004) as reducing and capping agents are rapidly emerging as energy-efficient and a cost-effective new option for AgNP production. The combination of nanotechnology with green chemistry will open up a new world of physiologically and cytologically friendly metallic nanoparticles (Philip, 2010).

The basic physical and chemical parameters that influence AgNP synthesis are reaction temperature,reaction time, extract contents, reaction mixture pH, metal ion concentration, and agitation. The size, shape, and morphology of AgNPs are influenced by metal ion concentration, extract content and reaction time (Kora *et al*., 2010). Sadeghi and Gholamhoseinpoor (2015), as well as Khalil ,*et al*., (2014), have made similar observations. The majority of authors claim that a basic medium is ideal for the creation of AgNPs because the nanoparticles formed in the basic medium are more stable. The reaction conditions are crucial, such as stirring time and reaction temperature (El-Rafie *et al*.,2011). And one of the latest nano studies in this field is the study belonging to Chandhru *et al*., (2021) which clarified the green synthesis of AgNPs from plant latex and their antibacterial activity and photocatalytic studies.

1.10 Characteristics of Nanoparticles (NPs)

The biological action of NPs is influenced by their form. Furthermore, the biological behavior of nanoparticles is determined by their size. Nanoparticles with smaller sizes have a higher surface-to-volume ratio, which improves their reactivity (Khan *et al*., 2019). Biological and physic-chemical characterization techniques for NPs are available. Biological characterization includes antibacterial and antibiofilm activities, while physicochemical approaches are Spectrophotometry, electron microscopy and X-ray analysis.

- **Spectrophotometry** is a method that is commonly used to estimate the qualitative and quantitative properties of biomolecules. The use of UV–visible spectroscopy to analyze different nanoparticles, particularly silver nanoparticles, has shown to be quite, High big surface plasmon peaks have been seen in visible areas (400–600 nm) for several metal nanoparticles with sizes ranging from 2 to 100nm.

- **Scanning Electron Microscopy (SEM):**

This examination allows for the analysis of NPs' elementary structure, grains size, surface roughness, homogeneity, inter-metal distribution, diffusion, porosity and dimension distribution

- **X-Ray Diffraction (XRD):**

X-ray crystallography was a strong technique for studying the three-dimensional structure of crystals, such as proteins and nucleic acids. The study of three-dimensional structure can be done in a variety of ways. However, x-ray crystallography is now the most successful of these approaches (Castillo-Michel *et al.*, 2017).

- **Fourier transforms infrared spectroscopy (FTIR):**

This method is a vibrational spectroscopic technique with a wide range of applications. The formation of distinct absorption bands in the spectra as a result of molecular vibrations may aid in their detection (Hospodarova *et al*.,

2018). FTIR is used to assess the heterogeneous structure and surface behavior of materials by collecting data on the maximum values at specific lambda values in reflection spectra and absorption.

Chapter Two

Materials And Methods

2. Materials and Methods.

2.1 Collecting of latex samples

Latex samples were collected from different places in Babylon province, as shown in Table (2-1), and Figure (2-1) by breaking the plant parts and storing them in clean, sterile containers in the refrigerator until use.

Table 2-1: The plant used in the present study

Plant	Family	Place of collection	Sample collection date
Ficus carica	Moraceae	agricultural fields Al-Musayyib City in the province of Babylon.	for the period from November, 2020 to March, 2021, and As needed in each test.
Calotropis procera	Asclepiadaceae	public gardens in the province of Babylon.	
Nerium oleander	Apocynaceae	University garden and public gardens in the province of Babylon.	
Ficus elastica	Moraceae	Our University Garden , province of Babylon.	
Euphorbia tirucalli	Euphorbiaceae	Home garden , province of Babylon.	

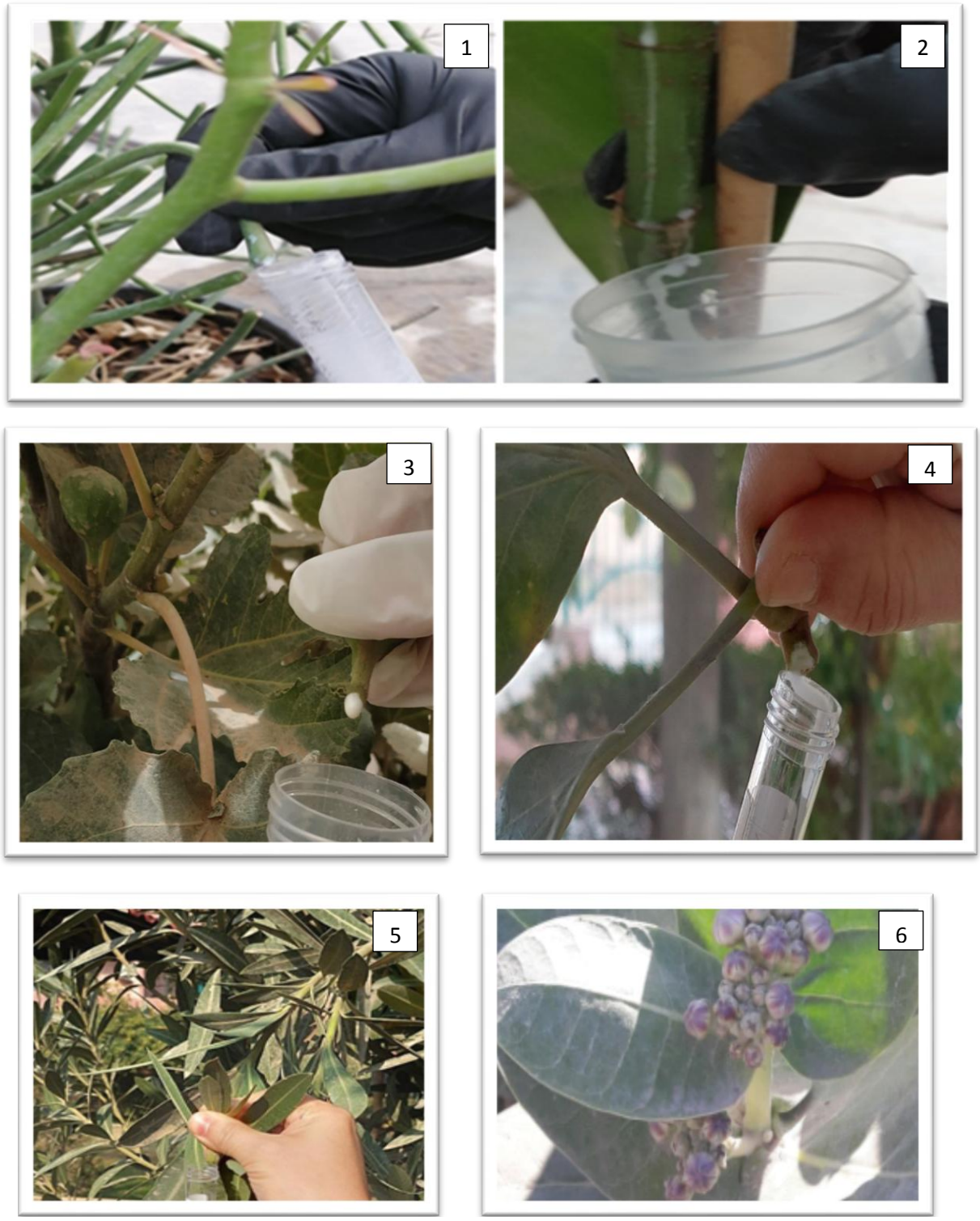


Figure (2-1): Sample collection where 1. *Euphorbia tirucalli* 2 and 4 *Ficus elastica* 3. *Ficus carica* 5. *Nerium oleander* 6. *Calotropis procera*

2.2 Chemical study: -

2.2.1 Preparation of plant latex samples to do GC- MS test:

1- 5 ml of fresh latex collected is mixed with 5 ml of absolute methanol alcohol.

2- The mixture is placed in a shaker overnight and then filtered through Whatman's filter paper, Figure (2-2). (Jadhav and Patil, 2014).

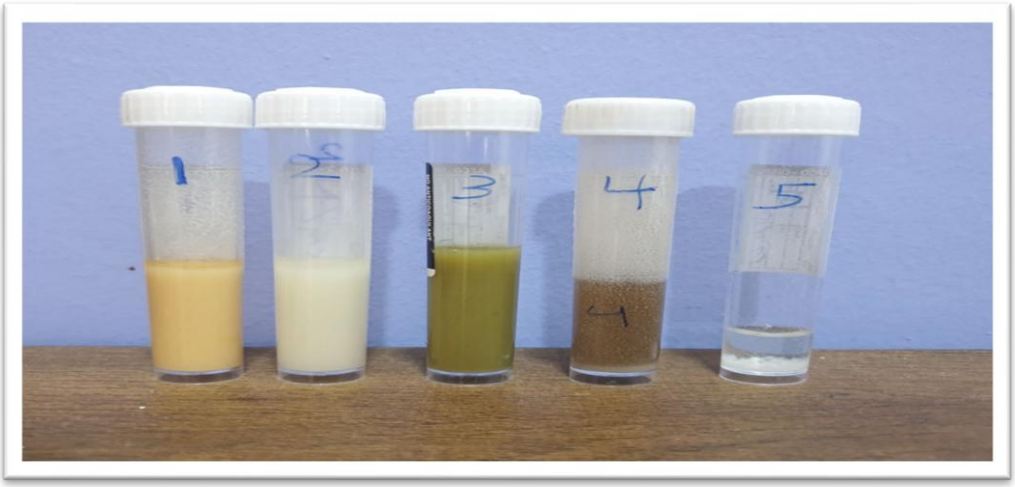


Figure 2-2: The mixture of latex with methanol after filtering where 1:*Ficus carica* , 2: *Calotropis procera* , 3: *Nerium oleander* 4: *Ficus elstica* , 5: *Euphorbia tirucalli* .

2.2.2 Conditions for analysis plant latex samples with GC– MS.

The chemical analysis of latex samples was performed at the Iraqi Ministry of Industry's Ibn Al-Bitar Research Center, utilizing an Agilent Technologies 7820A GC System as the analytical instrument. Figure (2-3).

The operating conditions of the device were as follows:

1- Separation column type HP-5ms ultra–Inert By dimensions (30m× 250µm×0.25µm)

2- At a continuous flow rate of 1.2ml/min, helium gas (99.9%) was used as a carrier gas.

3- The volume of injected fluid is 1µ, Injection Type: Splitless Injector temperature is 250 Cº. Scan Range: m/z 50-500

4- The temperature of the oven Ramp1 = 1 Cº hold to 60 Cº, Ramp2 = 60 Cº to 180 Cº (7 C°/min.), Ramp3 = 180 Cº to 280 Cº (7 C°/min.)
5- GC Inlet Line Temperature: 250 ˚C, Aux heater Temperature 310 ˚C
6- The device pressure is 11.93 psi
7- The sample analysis took 36 minutes in total.

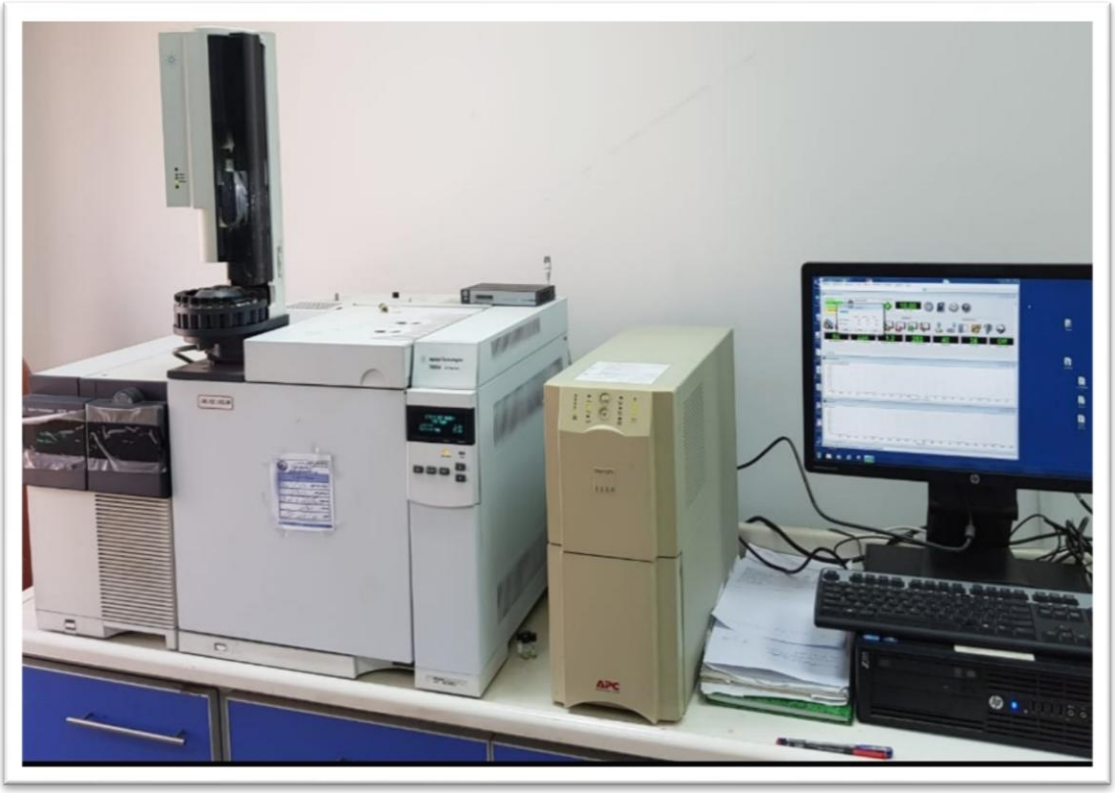


Figure (2-3): Device analysis of GC–MS.

2.3 Molecular study

The Materials and devices of their types and origin were used in the completion of the molecular study listed in Table (2-2):

Table 2-2: Materials and devices are used

No.	Devices/materials	Company / origin
1	DNA extraction kit	FAVROGEN Korea
2	DNA Ladder 10000 bp	Bioneer / Korea
3	Green master mix kit	Bioneer / Korea
4	Primers	Bioneer / Korea
5	High-speed cooling Centrifuge	Hettich/ Germany
6	Oven	Hettich/ Germany
7	Incubator	Bioneer / Korea
8	Vortex	Capp / Germany
9	Micropipettes	Germany
10	Eppendorf tubes	China
11	Water bath	Gonalab /Korea
12	Distilled water	Lab. service
13	Refrigerator	Hettich/ Germany
14	Electrophoresis	Bioneer/ Korea
15	U.V transilluminator	Analytikjena U. S
16	PCR cabinet	Bioneer/ Korea
17	Sensitive balance	Kern / Germany
18	Conventional PCR	Analytik Jena /Germany
19	Agarose	Promega / USA
20	Tris-borate – EDTA (TBE),	Bio basic / Canada
21	Loading dye	Bio basic / Canada
22	Uv-vis-spectrophotometer	Shimadzu / Japan

The genetic material DNA was isolated from the latex of the five plant species approved in this study using kits supplied by a FAVORGEN company, the components of which are shown in Table (2-3)

Table 2-3: The components of the kit used to extract DNA from latex are described.

No.	components	Quantity
1	Wash buffer	25 ml
2	R.B.C. lysis buffer	135 ml
3	FABG buffer	40 ml
4	W1 buffer	45 ml
5	Elution buffer	30 ml
6	FATG buffer	30 ml
7	Collection tube	100 pcs
8	FABG column	50 pcs

2.4.1 DNA extraction:

The Kit FAVORGEN provides a simple and fast way to obtain pure DNA, The DNA was extracted from the latex approved in the study according to the attached leaflet as follows:

- 200 µl FABG Buffer added to 50µl latex sample, vortex for five seconds and then incubated for ten minutes at 70°C (inverting the tube each three minutes).

- For the DNA elution step, pre-heat the needed elution buffer in a water bath at 70°C.
- 200µ l of 100 percent ethanol was added to the mixture and vortex for 10 seconds; the sample was well mixed if any precipitate appeared.
- In a collecting tube, place an FABG column. Transfer the sample mixture to the FABG column with caution, centrifuged for one minute at 14000 rpm, discard a collection tube, and replace the FABG column in a fresh Collection Tube
- To the FABG column, add 400 µl of W1 Buffer and centrifuge for 30 sec at 14,000 rpm.
- After discarding the flow-through, return the FABG Column to the collection tube.
- Add 600µl of wash buffer and centrifuge at 14000 rpm for 30 seconds. Return the FABG column to the collection tube after discarding the flow-through.
- Centrifuge for another 3 minutes at 14000 rpm to dry the column.
- Add100µl of Elution buffer and then incubated at 37C° for 10 min centrifuge for 1min. at 14000rpm. to elute the DNA and store it at 4C°. The total volume was 100µl.

2.4.2 Measurement of concentration and purity of DNA

The concentration and purity of the DNA were measured using a UV-Vis-Spectrophotometer. The concentration and purity of the DNA were measured after making a dilution by taking 1995 microliters of distilled water and mixing it with 5 microliters of the DNA sample of each plant species used in this study. Absorption within wavelengths of 260 nm as a standard of concentration, then the absorbance was measured at wavelength 280 nm and the ratio of absorption on both wavelengths was 260/280 nm as a standard for measuring purity

2.4.3 PCR-RAPD technique interaction with Random multiplication of DNA fragments

Reactions RAPD were carried out on samples of the DNA of five plant species studied by following steps:

A- The primers used in interaction:

Five random primers were chosen: OPB18, OPC2, BH10, BH11 and BH14 (Table 2-4) from the (Macrogen /Korea) company, were prepared in the form of dry primers prepared by dissolving in deionized distilled water, for each primer to obtain a concentration of 100 picomoles/µl as a stock solution and stored at 20 C° until use. where the used solution was prepared by adding 90 µl from deionized distilled water to 10 µl of stock solution, final volume 100 µl.

Table 2-4: Sequence Nucleotides in Primers used in the Study

No	Primers	Sequence of Primers 5' to 3'
1	OPB18	CCACAGCAGT
2	OPC2	GTGAGGCGTC
3	BH10	GAGAGAGAGAGACC
4	BH11	GTGTGTGTGTGTCC
5	BH14	CTCCTCCTCGC

B-Use of Polymerase Chain Reaction (PCR) technology to amplify DNA

The polymerase chain reaction was performed by using a PCR premix kit and equipped by the company (Promega). The polymerase chain reaction was performed in a volume of 25 microliters per sample consisting of the components shown in Table (2-5).

Table 2-5: The components of the PCR Reaction Mixture.

No.	Components	volume
1	Green master mix (Promega)	12.5 µl
2	Primer (10pmol)	2.5 µl
3	DNA template	5 µl
4	Nuclease free water	5 µl
5	Total volume	25 µl

The reaction mixture was prepared by mixing the components mentioned in the above table in a sterile tube with a capacity of 0.5 ml. The work was carried out inside the sterilization hood, taking into account the absence of contamination, where the components were mixed by micropipette and then placed in a centrifuge to complete the mixing of the reaction components.

Five sterile tubes of 0.5 ml size were prepared and marked according to the samples, then 20 µl of the reaction mixture were added to each tube and 5 µl of the DNA sample in the tube designated for it and mixed well by placing it in the centrifuge and then placed in the thermopolymer device under the conditions mentioned in Table (2-6).

Table 2-6: The conditions of PCR thermopolymer

Name of Primer	Initial denaturation		Denaturation		Annealing		Extension		Final extension	
	Temp Cº	Time (s)	Temp Cº	Time (s)	Temp Cº	Time (s)	Temp Cº	Time (s)	Temp Cº	Time (s)
OPC2	94	60	94	30	40	60	72	120	72	300
OPB18	95	300	94	60	36	60	72	120	72	240
BH14	94	220	94	60	40	60	72	120	72	300
BH10	94	220	94	60	40	60	72	120	72	300
BH11	94	220	94	60	40	60	72	120	72	300

C-The electrophoresis method was carried out as follows:

A glass plate was equipped with a strong adhesive tape around the edges and a special etching comb was attached to it at one end of the gel. Agarose gel at a concentration of 1.5% were prepared by dissolving 1.5 g of agarose in 10 ml of TBE buffer and then completing volume to 100ml in distilled water. The mixture is microwaved until the agarose powder dissolves, and the solution is taken out of the microwave before it boils and then the agarose gel were left to cool down to 50°C.

3µl of ethidium bromide dye were added after the mixture had cooled down and gently mixed, the agarose gel was poured slowly, avoiding bubbles in the solution, so that the gel did not deform and then left for 30 minutes to cool.

After 30 minutes, the comb and adhesive tape were gently lifted from the frozen agarose and the plate was fixed on its support in the horizontal electrophoresis unit and immersed in the buffer solution TBE and connected to the electricity source so that the negative electrode was with its counterpart and the positive with its counterpart taking into account the placement of the

agarose mold so that the end containing the drill was on one side. The negative pole is the far end of the drill towards the positive pole.

5 µl of DNA Ladder was added into the first hole and used to find out the size of the separated DNA segments. The electrophoresis device was closed and then the electric current was passed with a voltage of 75 volts for an hour and a half, after which the gel was removed from the relay tank and exposed to ultraviolet rays utilizing a device transmission UV and photographed.

D- 1 Kb Plus DNA Ladder:

The ladder is designed to determine the approximate size and amount of DNA on an agarose gel. This ladder contains fifteen bundles of DNA (base pairs) 100, 200, 300, 400, 500, 700, 1000, 1500, 2000, 3000, 4000, 5000, 6000, 8000, 10000, All bands appear with equal density except for bands (500, 3000) which appear in high intensity.

2.4.4 Analysis of the results of the RAPD indicators:

The results obtained from the electrophoresis process regarding the number and size of the bundles were taken and converted into numerical data depending on the presence or absence of the bundles by placing the number (1) when the bundle is present and the number (0) when the bundle is absent on the agarose gel.

The data was arranged in the form of a table that includes the results of all the primers for the samples under study. The genetic dimension coefficient between the samples was calculated according to the Jaccard coefficient of genotype similarity in the Unweighted Pair Group Technique Using An Arithmetic Average (UPGMA) method and Numerical Taxonomy System (NTSYS-PC) program using to obtain a cluster analysis diagram to build a genetic relationship tree diagram that shows the extent of the proximity and

distance of the species among them, and then the formal polymorphism, the primers efficiency and the discriminating ability were calculated using the following equations:

Polymorphism % = (Number of different bands per primer /Number of main bands per primer) *100

Primer efficiency % = (Total number of primer bands / Total number of bands all primers) *100

Discriminating ability % = (Number of different bands per primer / Sum of heterogeneous bands for all primers) *100.

2.5 Preparation of silver nanoparticles and some biological applications:

2.5.1 Laboratory Devices and Tools

The Laboratory Devices and Tools used in this study with the name of the manufacturer and the country of origin were listed in theTable (2-7)

Table 2-7: Laboratory Instruments and Tools

No.	Instruments / Tools	Company / origin
1	High-speed cooling Centrifuge	Hettich/ Germany
2	Vortex Mixer	Capp / Germany
3	Shaker Incubator	Genex / USA
4	Oven	Hisense/ China
5	Refrigerator	Hitachi / Japan
9	Incubator	Bioneer / Korea
10	ELISA Reader	Paramedical / Italian
11	Sensitive Electronic Balance	Kern / Germany
12	Micropipette (vary sizes)	Germany

2.5.2 Laboratory Chemicals and materials

The Laboratory Chemicals and Instruments and Disposable materials and Disposables used in this study are listed in Table (2-8).

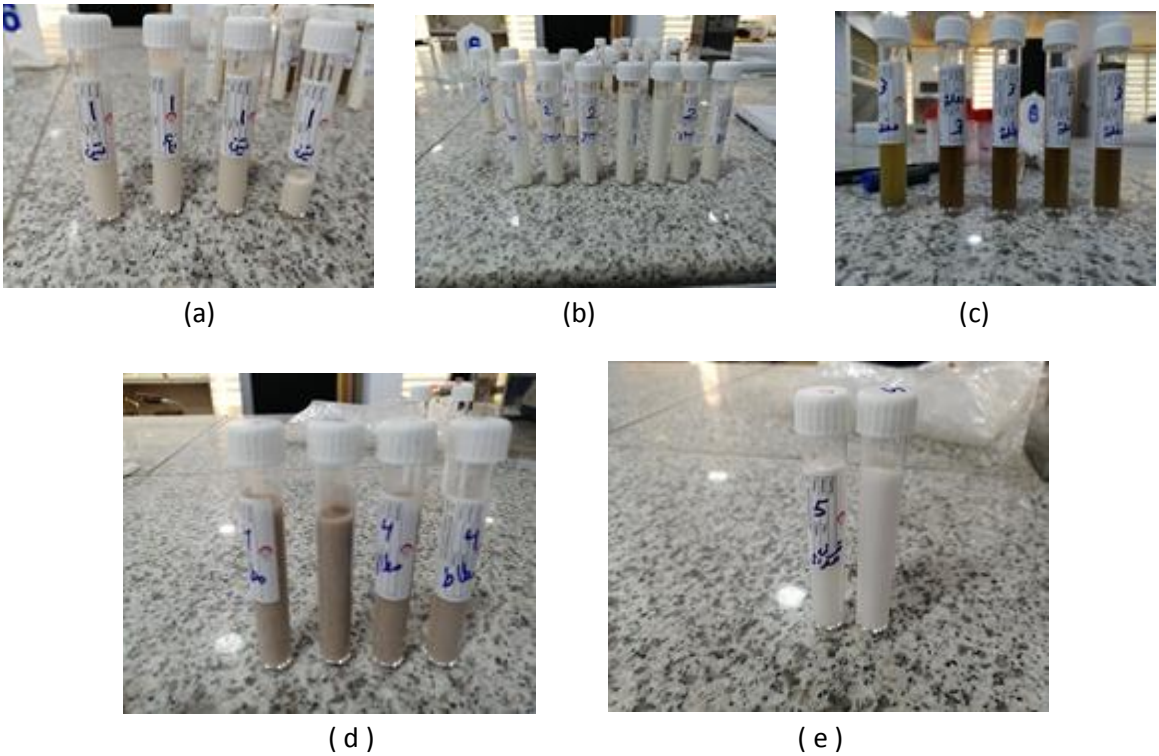
Table 2-8: Laboratory chemicals, instruments and disposable materials

No.	Chemicals and materials	Volume / Concentration
1	AgNo3	1mM
2	Filter paper	Whatman No.1
3	Phosphate buffer saline	200µl
4	Sodium acetate	200µl
5	Crystal violet dye	1%
6	Ethanol	200µl
7	2,2-diphenyl-1-picryl-hydrazyl (DPPH)	1.01 mM
8	Methanol	95%
9	Triton X-100	15 µl, 1%
10	Nutrient agar	Accumax
11	Disposable plastic Petri dishes	Afco/ Jordan
12	Medical gloves	Broche /PCR
13	Plastic test tube	Dolphine/Syria
14	Sterile swab	Lab. service / S.P. A
15	Tips	Dolphine/Syria
16	Wood sticks	Supreme / China

2.5.3 Synthesis of AgNPs

According to Chandhru *et al*. (2021), The AgNPs was synthesized by an environment-friendly method. As follow:

- Latex of studied samples was aseptically collected (Figure 2-4 a,b,c,d,e) and centrifuged at 10,000 rpm for 5 mins. Then, the solution was filtered with filter paper Whatman No.1 (Figure 2-5).
- 40 mL of latex was added into a 500 mL beaker.
- Then, 160 mL of distilled water was added and stirred for 1 min
- 16 ml of 1 mM AgNO3 was added into the above solution with constant stirring. The stirring was continued for 24 hours in an incubator shaker.
- The solution was changed in colour as shown in the figure (3-12).
- The unreacted latex was removed by the centrifugation of 10,000 rpm for 1hr.
- The solution was dried for 24 hours using an electric oven, AgNPs were collected in a 60 mL standard measuring container and stored at 4°C.



(a) (b) (c)

(d) (e)

Figure 2-4: a,b,c,d,e collecting latex samples

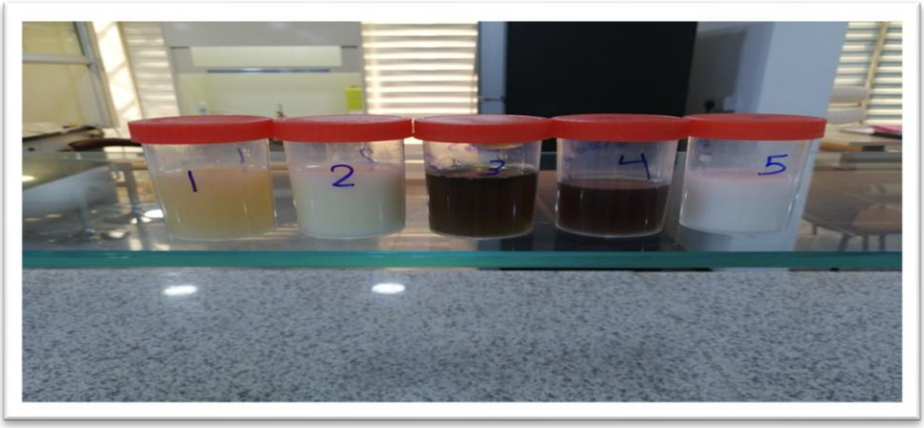


Figure 2-5: Leachate latex samples after centrifugation

2.5.4 Characterization of biosynthesis latex AgNPs.

Physical and biological characterization are the most common methods for determining the properties of nanoparticles.

Physical characterization of biosynthesis latex AgNPs

UV-Visible Spectroscopy, Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), and Fourier Transform Infrared Spectroscopy (FTIR)were used to analyze of biogenic nanoparticles which were carried out in the laboratories of the University of Tehran after sending the latex nanomaterial to each plant under study, except UV-Visible spectroscopy which carried out in Al-Ameen Center for Advanced Biotechnology and Research by apparatus Shimadzu UV-visible 1800. The objective of studying the above physical properties is as follows:

1- **Spectroscopy of UV visible (latex AgNPs)**

The latex AgNPs formation and size estimate inside the composite solution.

2- **SEM Analysis of latex Ag NPs**

SEM was used to determine the surface morphology and scale of nanoparticles in composite films. The specimen was prepared by grinding latex nanoparticles, preparing a suspension of the nanoparticles, and attaching

a droplet of the suspension to the fixing matrix. Prior to SEM characterization, the samples were further air-dried and stored in a desiccator.

3- XRD Latex AgNPs investigated

X-ray diffraction analysis (XRD) is a materials science technique for determining a material's crystallographic structure. XRD measures the intensities and scattering angles of X-rays that exit a material after they have been irradiated with incoming X-rays.

4- FTIR of latex AgNPs

To detect different functional groups.

Biological characterization of biosynthesis latex AgNPs

Antibiofilm, antioxidant and antimicrobial activity are some of the methods utilized for biological characterization.

2.6 Antibiofilm effect study

To find out the effect of silver nanoparticles prepared using latex on the ability of bacteria to form a thin biofilm, a test of the nanomaterial was carried out by making seven concentrations for each latex sample from the five samples for two types of bacterial genera which are gram-positive bacteria like *Staphylococcus aureus* and gram-negative bacteria like *E. coli*.

According to the method of Barapatre *et al* (2016), The 96-well microtiter plate technique was used to test the effectiveness of Ag-NPs in biofilm development.

- 180 µ l Mueller Hinton broth was poured into each wells of a sterile microtiter plate and 10 µl of overnight bacterial culture
- 10 µl of silver nanoparticles from each one of the concentrations (16, 32, 64, 128, 256, 512, 1024) µg/mL, were added to the mixture.
- The microtiter plates were incubated at 37 °C for 24 hours..

- After incubation, the contents of each well were carefully removed and washed three times with 200L of Phosphate Buffer Saline (PBS / pH 7.2).
- Biofilms produced in the plate wall by adherent'sessile' organisms were preserved (fixed) in sodium acetate (200 µL) and stained with crystal violet dye (0.1 percent w/v).
- The plates were lefted to dry after the excess dye was removed with sterilized D.W.
- The wells were filled with 200 µL of 95 percent (v/v) ethanol after drying.
- Using an ELISA reader, the absorbance at 620 nm was measured, and the results were utilized as the indication of bacteria attaching to the well wall's surface to create biofilms. The following equation was used to compute the percentage of biofilm inhibition:

  Biofilm inhibition percent = [1 - (OD_{620} of cells treated with Ag – NPs) / (OD_{620} of non-treated control)] *100.

2.7 Antioxidant Effect Study

The free radical scavenging capacity of the latex was determined using DPPH Test, The DPPH solution was prepared in methanol, in four concentrations (64, 128, 256, 512) µg/ml of silver nanoparticles (prepared using plant latex) were prepared for each of the five species (Goyal *et al*., 2010).

- Freshly prepared DPPH solution (100µl) was taken in microplate and AgNps (100µl) was added and followed by serial dilutions to every tube such that the final volume was 200µl, and incubated for 30 minutes in the dark to react. The room was kept dark to avoid the influence of light on DPPH, which was light sensitive.

- The final samples' absorbance was measured at 517 nm with an Elisa Reader. The following equation was used to compute the DPPH radical scavenging activity:

DPPH radical scavenging activity (%) = {$Ao- A1/Ao$) * 100}.

Where **Ao** represents the control absorbance and A1 represents the absorbance in the presence of the sample.

2.8 Haemolysis effect study

According to the method of Laloy *et al* (2014), Using one healthy donor's blood, haemolysis tests were performed:

- (15) µL of nanoparticles on the concentration (64, 128, 256, 512) µg/ml (suspended Intyrode) was added to the blood samples.
- Tyrode a negative control
  - Triton X-100 a positive control were added to the 285µL of total blood.
- The suspension is incubated in an incubator shaker for 4hr.
- After the incubation time, The suspension is centrifuged for 5 minutes at 10000.
- The % haemolysis was estimated by reading the supernatant in a 96-well plate with an Elisa Reader at 550 nm.:

haemolysis (%) = {OD_{550}nm sample - OD_{550}nm tyrode}/ {OD_{550}nm Triton X-100 1% - OD_{550}nm tyrode} *100.

2.9 Antibacterial effect study

The antibacterial activity of latex AgNP suspensions on gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and gram negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*)were investigated in this study , employed the following procedures to conduct these tests, according to (Prastiyanto *et al*., 2020).

(1) TheWell diffusion method to determination of the inhibition zone.

- Dilutions of AgNPs at 25, 50, 100 ,200 µg /ml were prepared from the 400µg/ml of a stock concentration
- For this experiment, indicators bacteria were activated for 18 hours at 37°C in nutritional broth, and the turbidity tube was 0.5. The MacFarland technique was used to determine turbidity.
- A cotton swab was used to cultivate bacteria on sterilized nutrient agar plates.
- Wells were cut out with the end of a sterilized pasture pipette after 5-10 minutes.
- Solution of AgNPs 100µl of each concentration was applied to wells and incubated for 24 hours at 37° C.
- Inhibition zones were measured in mm.

(2) Minimum inhibitory concentrations (MIC)

Bacterial inoculum of *S.aureus*, *E.faecalis*, *E.coli*, as well as the *Kleb. pneumoniae* prepared by growing a single colony overnight and adjusting the turbidity to 0.5McFarland standards. Brain Heart Infusion Broth (BHI Broth media) was supplemented with different concentrations of nanoparticles (8, 16, 32, 64, 128, 256, and 512 µl/Ml) and inoculated with bacterial suspension.

AgNPs were not added to the control tubes. After 24 hours of incubation at 37°C, the MIC was calculated by monitoring visual turbidity and measuring the optical density of these culture broths at 600 nm. (Panacek *et al*., 2006).

Statistical analysis

SPSS IBM version 20 IBM was used to examine the data. ANOVA was used to represent the results of the research groups and assays, and the significance was calculated using the one-sample T-test, with P-values of 0.05 regarded statistically significant.

2.10 DNA fragmentation

The effect of silver nanoparticles on DNA extracted from *Escherichia coli*. The following concentrations of silver nanoparticles prepared by the latex were performed for the five producing plant species (128, 256, 512µg/ml). 10µl of each concentration was mixed with 10 µl of the DNA extract and incubated for 2 hr. at 37 °C, then 7µl were taken and 2 µl loading dye added to it.

The fate of DNA was analyzed by agarose gel electrophoresis. A glass plate was equipped with a strong adhesive tape around the edges and a special etching comb was attached to it at one end of the gel. Agarose gel at a concentration of 1% were made by dissolving 0.7g of agarose in 50 ml of TBE buffer the mixture is microwaved until the agarose powder dissolves, and the solution is taken out of the microwave before it boils and then the agarose gel were left to cool down to 50°C.

2µl of ethidium bromide dye solution was added after the mixture had cooled down, and gently mixed, the agarose gel was poured slowly, avoiding bubbles in the solution, so that the gel did not deform and then left for 30 minutes to cool.

After 30 minutes, the comb and adhesive tape were gently lifted from the frozen agarose and the plate was fixed on its support in the horizontal electrophoresis unit and immersed in the buffer solution TBE and connected to the electricity source so that the negative electrode was with its counterpart

and the positive with its counterpart, taking into account the placement of the agarose mold so that the end containing the drill was on one side. The negative pole is the far end of the drill towards the positive pole. 7 µl of the

control which was DNA extract diluted with distilled water with loading dye only was added into the first hole.

The electrophoresis device was closed and then the electric current was passed with a voltage of 85 volts for 2 hr., after which the gel was removed from the relay tank and exposed to ultraviolet rays utilizing a device transmission UV and photographed.

Chapter Three

Results and Discussion

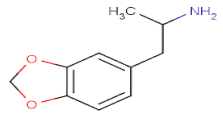
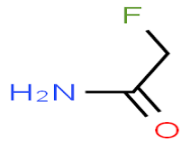
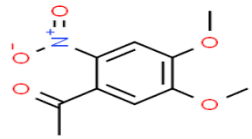
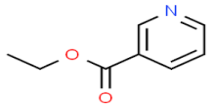
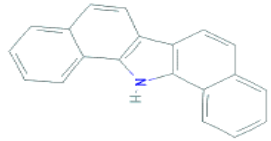
3.1: Chemical study

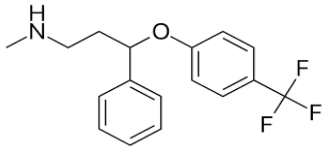
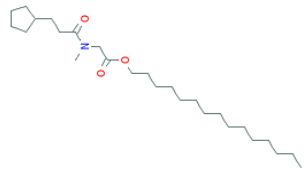
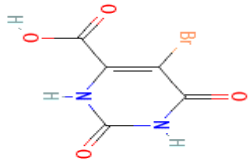
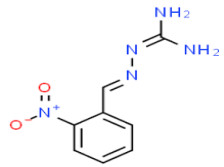
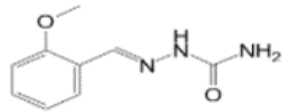
The results of the analysis of plant extracts by GC-MS technique of the methanolic latex extracts of the five present study plants showed the presence of 20 chemical compounds for each plant sample but these compounds varied among, glycosides, alkenes, fatty acids, alcohols, organic silicon compounds, Amines, and esters. The chemical profiles of the identified compounds were verified with retention time (RT), molecular formula, percentage peak area, chemical composition, and molecular weight Tables [(3-1) - (3-5)] and [Figure (3-1) - (3-5)].

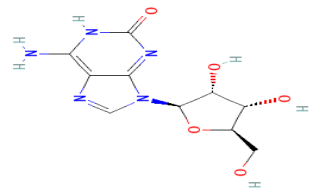
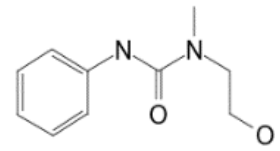
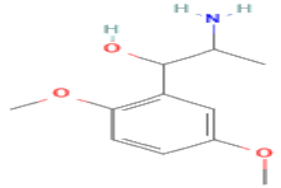
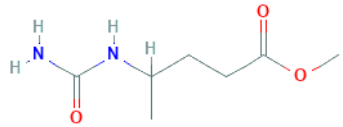
Figure 3-1: Chromatogram profile for GC-MS of *Ficus carica* latex

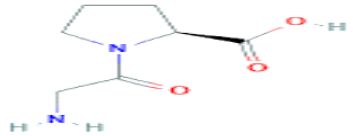
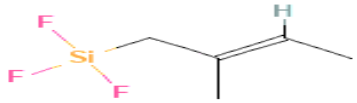
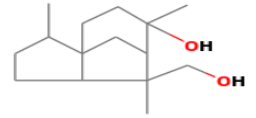
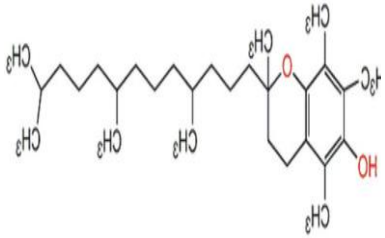
The first peak was determined in the methanolic latex extract of *Ficus carica* which was represented by Tenamfetamine with a retention time of 4.615 minutes and a peak area of 6.91 %, and the other peaks at different retention times are shown in Table (3.1), and the highest peak was dl-alpha-Tocopherol, which had a retention time of 34.246 minutes and a peak area of 23.08 % while the other less prominent peaks at different retention times shown in Table (3-1) ,Figure (3-1).

Table 3-1: GC–MS Chromatogram of the methanolic latex extract of *Ficus carica*

No.	Compound name	R.T / min	Area %	CAS	Molecular Weight g/ml	Category of compound	Formula	Structure
1	Tenamfetamine	4.615	6.91	004764-17-4	179.22	$C_{10}H_{13}NO_2$	Amines	
2	Acetamide, 2-fluoro	5.830	3.28	000640-19-7	77.06	C_2H_4FNO	Amide	
3	3-(3-Carboxy-4-hydroxyphenyl)-D-alanine	6.051	4.00	004303-95-1	225.20	$C_{10}H_{11}NO_5$	carboxylic acid, amine	
4	Oxime-, methoxy-phenyl	6.339	2.22	100022 2-86-6	151.16	$C_8H_9NO_2$	Amidoximes	
5	13H-Dibenzo[a,i]carbazole	8.786	6.34	000239-64-5	275.40	$C_{20}H_{13}N$	aromatic heterocyclic organic compound.	

No.	Compound name	R.T / min	Area %	CAS	Molecular Weight g/ml	Category of compound	Formula	Structure
6	Fluoxetine	9.168	2.03	054910-89-3	345.79	$C_{17}H_{18}F_3NO$	organic amino compound	
7	Sarcosine, N-(3-cyclopentylpropionyl)-, pentadecyl ester	11.488	2.62	100032 1-83-8	423.7	$C_{26}H_{49}NO_3$	ester	
8	5-Bromo-6-carboxy-2,4-dihydroxypyrimidine	16.848	0.98	015018-62-9	234.99	$C_5H_3BrN_2O_4$	5-Bromoorotic acid	
9	Benzaldehyde, 2-nitro-, diaminomethylidenhydrazone	17.919	1.56	102632-31-5	191.19	$C_8H_9N_5O_2$	Nitrogen compound	
10	o-Anisaldehyde, semicarbazone	18.250	1.63	005346-30-5	193.21	$C_9H_{11}N_3O_2$	Amide Amine	

No.	Compound name	R.T / min	Area %	CAS	Molecular Weight g/ml	Category of compound	Formula	Structure
11	Adenosine, 1,2-dihydro-2-oxo	19.108	1.43	001818-71-9	283.24	$C_{10}H_{13}N_5O_5$	Glycosides	
12	Urea, N-(2-hydroxyethyl)-N-methyl- N'-phenyl-	19.677	2.63	100035 1-55-9	194.23	$C_{10}H_{14}N_2O_2$	Amide Alcohol	
13	Benzenemethanol,.alpha.-(1-aminoethyl)-2.5-dimethoxy-	21.758	1.40	000390-28-3	211.2576	$C_{11}H_{17}NO_3$	Amine Alcohol ether	
14	Ureidopentanoic acid, methyl ester	23.237	7.00	100018 6-20-3	174.20	$C_7H_{14}N_2O_3$	Amide ester	
15	6,7-Dioxabicyclo [3.2.2]nonane	23.755	1.72	000283-35-2	128.17	$C_7H_{12}O_2$	Hydrocarbons, Acyclic (Alkanes)	

No.	Compound name	R.T / min	Area %	CAS	Molecular Weight g/ml	Category of compound	Formula	Structure
16	Glycyl-L-proline	25.207	5.70	000704-15-4	172.18	$C_7H_{12}N_2O_3$	Amine carboxylic acid	
17	Silane, trifluoro (2-methyl-2-butenyl)-	25.547	12.63	051676-19-8	154.20	$C_5H_9F_3Si$	ORGANOSILANE	
18	cis-10-Heptadecenoic acid, methyl ester	25.845	4.62	100033 3-62-1	282.4614	$C_{18}H_{34}O_2$	fatty acid ester	
19	Cedran-diol, (8S,14)-	32.768	8.22	062600-05-9	238.3657	$C_{15}H_{26}O_2$	Sesquiterpene alcohol	
20	dl-alpha-Tocopherol	34.246	23.08	010191-41-0	430.71	$C_{29}H_{50}O_2$	Alcohol Phenol	

In *Calotropis procera* methanolic Latex extract, the chromatogram shows 20 peaks (Figure 3-2). The retention time of the first peak was 4.360 min, which represented the compound1-Methyl-4-[nitromethyl]-4-piperidinol with a peak area of 17.13%, Octasiloxane, 1,1,3,3,5,5,7,7,9,911,11,13,13,15,15-hexadecamethyl recorded the highest peak at 31.834 min with peak area of 4.31%. While other chemical compounds recorded different retention times and with different areas, as shown in Table (3-2) and Figure (3-2).

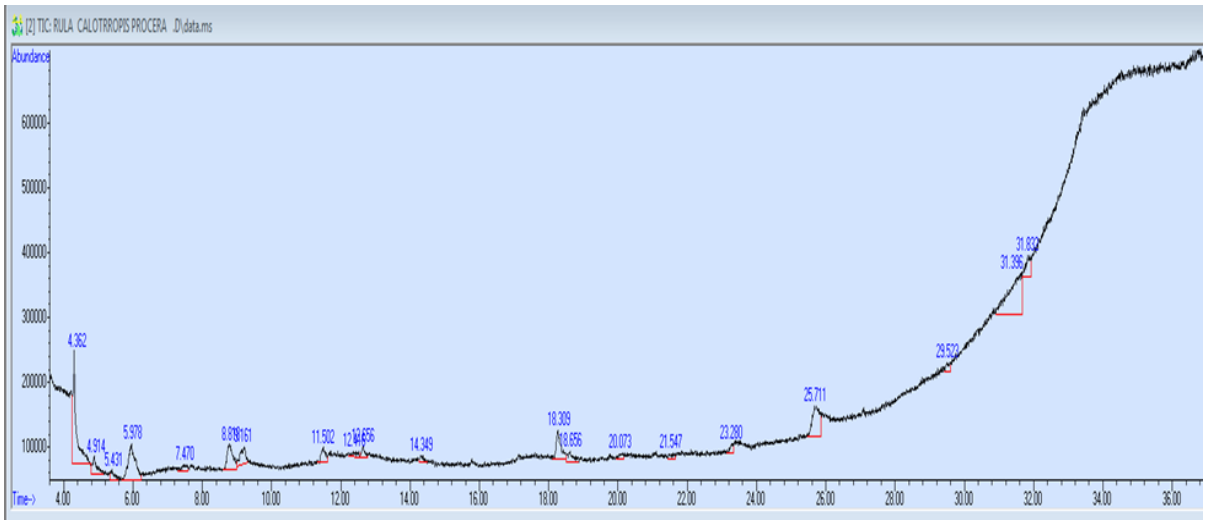
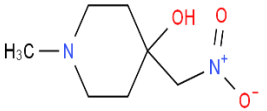
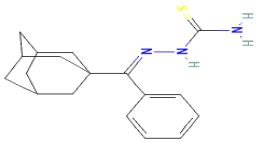
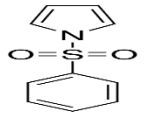
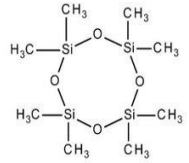
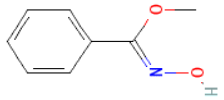
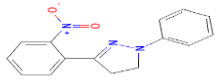
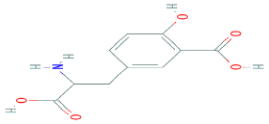
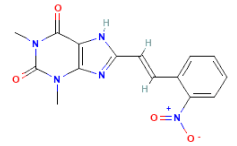
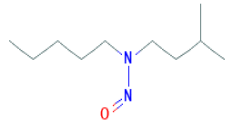
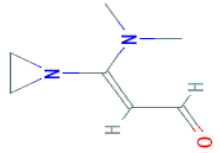
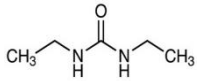
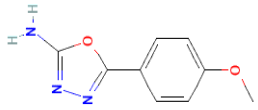
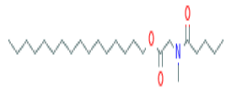
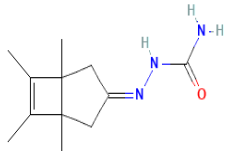
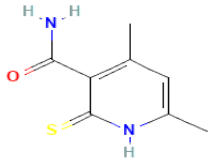
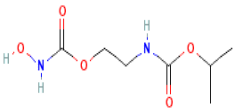
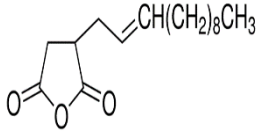


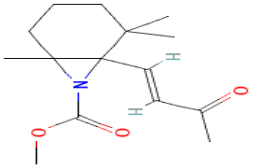
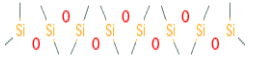
Figure 3-2: Chromatogram profile for GC-MS of *Calotropis procera* latex

Table 3-2: GC–MS Chromatogram of the methanolic latex extract of *Calotropis procera*

No.	Compound name	R.T / min	Area %	CAS	Molecular Weight g/ml	Category of compound	Formula	Structure
1	1-Methyl-4-[nitromethyl]-4-piperidinol	4.360	17.13	116250-49-8	174.2	Heterocyclic Compounds (Piperidines)	$C_7H_{14}N_2O_3$	
2	-[.alpha.-(1-Adamantyl)benzylidene]thiosemicarbazide	4.912	3.11	1000222-83-3	313.5	Semicarbazides	$C_{18}H_{23}N_3S$	
3	1-Benzenesulfonyl-1H-pyrrole	5.430	1.53	016851-82-4	207.25	Heterocyclic Compounds (Azoles)	$C_{10}H_9NO_2S$	
4	Cyclotetrasiloxane, octamethyl	5.974	10.55	000556-67-2	296.62	silicone polymer	$[-Si(CH_3)_2O-]_4$	
5	Oxime-, methoxy-phenyl	7.469	1.25	1000222-86-6	151.16	Amidoximes	$C_8H_9NO_2$	
6	1-Phenyl-3-(3-nitrophenyl)-2-pyrazoline	8.820	6.30	077242-45-6	267.28	Pyrazole	$C_{15}H_{13}N_3O_2$	

No.	Compound name	R.T / min	Area %	CAS	Molecular Weight g/ml	Category of compound	Formula	Structure
7	3-(3-Carboxy-4-hydroxyphenyl)-D-alanine	9.160	4.03	004303-95-1	225.20	Amino Acids, Aromatic	$C_{10}H_{11}NO_5$	
8	Purin-2,6-dione, 1,3-dimethyl-8-nitrophenethenyl]-	11.504	2.77	1000128-96-1	327.29	Amines	$C_{15}H_{13}N_5O_4$	
9	N-Isopentyl-N-nitroso-pentylamine	12.413	1.35	028023-80-5	186.29	Reaction of nitrite with amine compounds	$C_{10}H_{22}N_2O$	
10	2-Propenal, 3-(1-aziridinyl)-3-(di methylamino)-	12.660	1.65	049582-42-5	140.18	Amino Alcohols	$C_7H_{12}N_2O$	
11	Urea, N, N'-diethyl	14.350	0.98	000623-76-7	116.16	thiocarbonyl compound	$C_5H_{12}N_2O$	
12	1,3,4-Oxadiazol-2-amine, 5-(3,4-dimethoxyphenyl)	18.309	5.50	1000362-71-7	191.19	Heterocyclic Compounds (Oxazoles)	$C_9H_9N_3O_2$	

No.	Compound name	R.T / min	Area %	CAS	Molecular Weight g/ml	Category of compound	Formula	Structure
13	Sarcosine, N-valeryl-, hexadecyl ester	18.657	2.37	1000321-56-9	397.6	carboxylic ester	$C_{14}H_{27}NO_3$	
14	1,5,6,7-Tetramethylbicyclo[3.2.0] h ept-6-en-3-ylideneemicarbazide	20.076	1.00	120345-90-6	221.30	Bridged Bicyclo Compounds	$C_{12}H_{19}N_3O$	
15	Pyridine-3-carboxamide, 1,2-dihydro-4,6-dimethyl-2-thioxo-	21.546	1.05	079927-21-2	182.25	Amide	$C_8H_{10}N_2OS$	
16	N-Hydroxycarbamic Acid,2-(Isopropoxycarbonylamino) Ethyl Ester	23.279	1.29	112683-26-8	206.2	Ester	$C_7H_{14}N_2O5$	
17	2Dodecen-1-yl (-) succinic anhydrid	25.709	9.65	019780-11-1	266.38	Dicarboxylic Acids (Succinates)	$C_{16}H_{26}O_3$	
18	Oleic Acid	29.523	1.35	000112-80-1	282.46	Fatty Acids, Unsaturated	$C_{18}H_{34}O_2$	

No.	Compound name	R.T / min	Area %	CAS	Molecular Weight g/ml	Category of compound	Formula	Structure
19	1-Benzazirene-1-carboxylic acid,2,5a-trimethyl-1a-[3-oxo-1-buteny l] perhydro-, methyl ester	31.392	22.84	1000197-90-8	265.35	Terpenoids	$C_{15}H_{23}NO_3$	
20	Octasiloxane, 1,1,3,3,5,5,7,7,9,911,11,13,13,15,15-hexadecamethyl	31.834	4.31	019095-24-0	577.2	organosilicon compound	$C_{16}H_{48}O_7Si_8$	

In the latex of *Nerium oleander***,** the first peak belonged to a 3,4-methylenedioxy-N-methyl benzylamine compound with a retention time of 4.589 min with a peak area of 18.36%. Also, Acetamide, N-(1-methyl-3-phenylpro pyl)- had its highest peak in retention time of 28.809 and a peak area of 1.61%. While the other chemical compounds recorded different times of appearance as shown in Table (3-3), Figure (3-3).

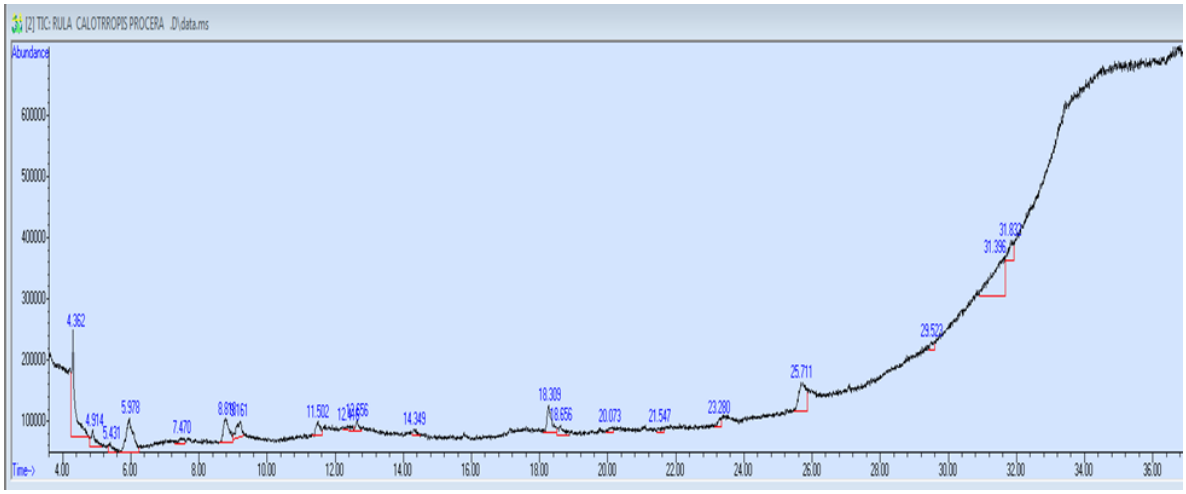
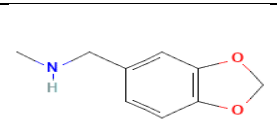
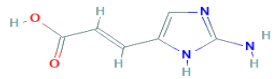
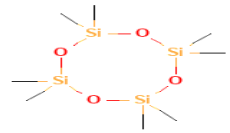
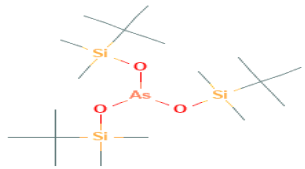
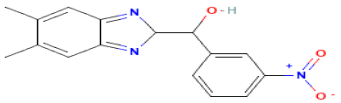
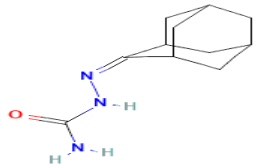
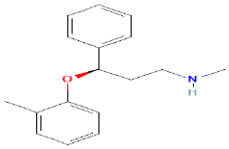
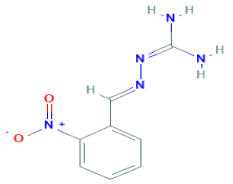
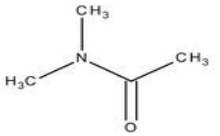
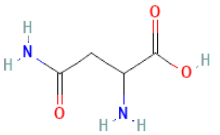
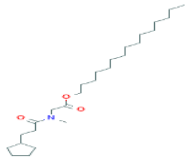
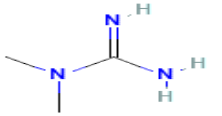
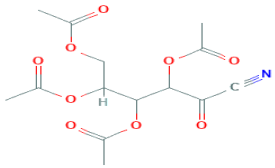
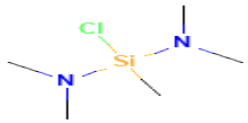
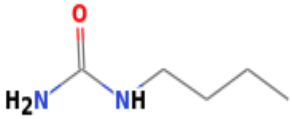
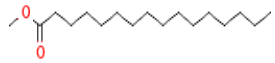
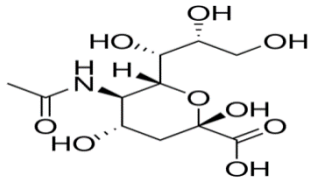
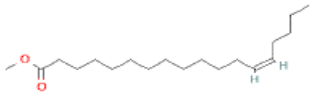


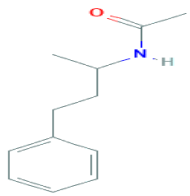
Figure 3-3: Chromatogram profile for GC-MS of *Nerium oleander* latex

Table 3-3: GC–MS Chromatogram of the methanolic latex extract *Nerium oleander*

No	Compound name	R.T / min	Area %	CAS	Molecular Weight g/ml	Formula	Category	Structure
1	3,4-methylenedioxy-N-methylbenzylamine	4.589	18.36	15205-27-3	165.19	$C_9H_{11}NO_2$	Amine	
2	Imidazole, 2-amino-5-[(2-carboxy)vinyl]	5.779	1.46	1000116-74-7	153.14	$C_6H_7N_3O_2$	phenylhydrazines	
3	Octamethylcyclotetrasiloxane	6.025	4.66	000556-67-2	296.61	$C_8H_{24}O_4Si_4$	Polymers (Siloxanes)	
4	Tris(tert-butyldimethylsilyloxy)arsane	7.435	2.88	1000366-57-5	468.7	$C_{18}H_{45}AsO_3Si_3$	ester	
5	2H-Benzimidazole-2-methanol, 5,6-dimethyl-.alpha.-(3-nitrophenyl)-	7.682	3.29	054833-62-4	297.31	$C_{16}H_{15}N_3O_3$	Heterocyclic Compounds	
6	1-(2-Adamantylidene) semicarbazide	8.174	1.66	065814-27-9	207.27	$C_{11}H_{17}N_3O$	derivatives ureas	

No	Compound name	R.T / min	Area %	CAS	Molecular Weight g/ml	Formula	Category	Structure
7	Atomoxetine	8.786	4.04	083015-26-3	255.35	$C_{17}H_{21}NO$	Amine	
8	Benzaldehyde, 2-nitro-, diaminomet hylidenhydrazone	9.168	2.87	102632-31-5	207.19	$C_8H_9N_5O_2$	Nitrogen compound	
9	N,N-Dimethylacetamide	10.876	2.30	204-826-4	87.12	C_4H_9NO	amide	
10	DL-Asparagine	11.530	2.09	003130-87-8	132.12	C4H8N2O3	alpha-amino acid	
11	Sarcosine, N-(3-cyclopentylpropion yl)-, pentadecyl ester	12.634	2.31	1000321-83-8	423.7	$C_{26}H_{49}NO_3$	Ester	
12	Guanidine, N,N-dimethyl	14.104	1.56	006145-42-2	87.12	$C_3H_9N_3$	Amidines	

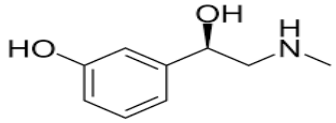
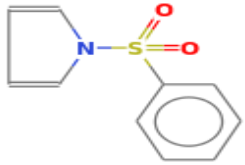
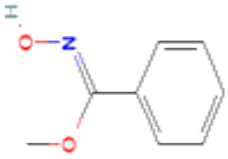
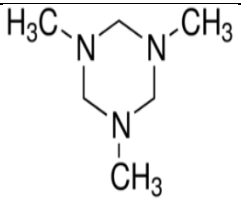
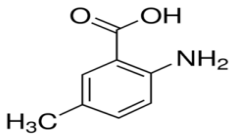
No	Compound name	R.T / min	Area %	CAS	Molecular Weight g/ml	Formula	Category	Structure
13	Tetraacetyl-d-xylonic nitrile	17.145	1.64	1000130-04-4	343.29	$C_{14}H_{17}NO_9$	Ester	
14	Silanediamine, 1-chloro-N,N,N',N'1-pentamethyl	18.275	2.72	010339-02-3	166.72	$C_5H_{15}ClN_2Si$	Amine	
15	Urea, butyl	18.641	1.83	000592-31-4	116.1616	$C_5H_{12}N_2O$	Amid	
16	Hexadecanoic acid, methyl ester	23.372	8.47	000112-39-0	270.5	$C_{17}H_{34}O_2$	Ester	
17	(-)-N-Acetylneuraminic acid,synthetic	24.774	1.69	000131-48-6	309.27	$C_{11}H_{19}NO_9$	Sialic Acids	
18	cis-13-Octadecenoic acid, methyl ester	25.751	22.92	1000333-58-3	296.4879	$C_{19}H_{36}O_2$	Fatty Acids, (Unsaturated), Ester	

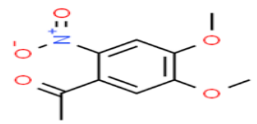
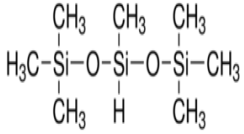
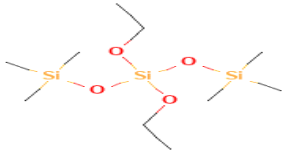
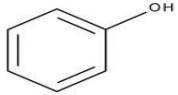
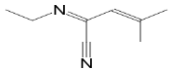
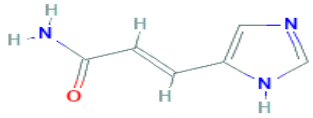
No	Compound name	R.T / min	Area %	CAS	Molecular Weight g/ml	Formula	Category	Structure
19	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans-	26.031	11.63	038520-31-9	312.5	$C_{19}H_{36}O_3$	Ester	
20	Acetamide, N-(1-methyl-3-phenylpropyl)-	28.809	1.61	128442-57-9	191.27	$C_{12}H_{17}NO$	Amid	

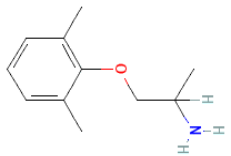
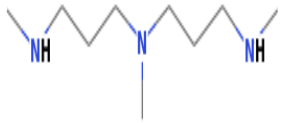
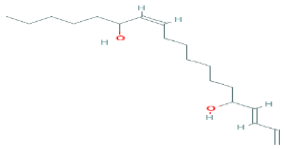
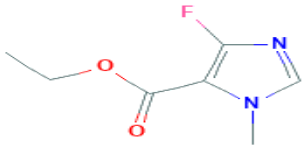
Phenylephrine compound represents the first peak in the methanolic Latex extract of *Ficus elastica* with a retention time of 4.326 min with a peak area of 2.02%. While 2,2,3,5,6,6,7-Heptamethyl [1,4,2,3,5,6,7] dioxapentasilepane compound represents the highest peak with a retention time of 31.876 min with a peak area of 1.52%. Table (3-4), Figure (3-4).

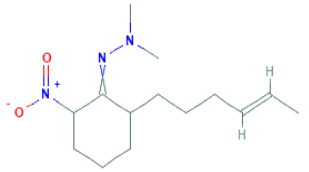
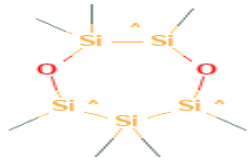
Figure 3-4: Chromatogram profile for GC-MS of *Ficus elastica* latex

Table 3-4: GC–MS Chromatogram of the methanolic latex extract of *Ficus elastica*

No	Compound name	R.T / min	Area %	CAS	Molecular Weight g/ml	Formula	Category	Structure
1	Phenylephrine	4.326	2.02	00005 9-42-7	167.21	$C_9H_{13}NO_2$	Amine alcohol	
2	1-Benzenesulfonyl-1H-pyrrole	4.623	7.16	01685 1-82-4	207.249	$C_{10}H_9NO_2S$	heterocyclic	
3	Oxime-, methoxy-phenyl	5.515	5.93	10002 22-86-6	151.16	$C_8H_9NO_2$	Amidoximes	
4	1,3,5-Triazine, hexahydro-1,3,5-trimethyl	5.949	14.27	00010 8-74-7	129.20	$C_6H_{15}N_3$	Alkanes	
5	2-Amino-5-methylbenzoic acid	7.724	19,15	00294 1-78-8	151.16	$C_8H_9NO_2$	Carboxylic acid	

No	Compound name	R.T / min	Area %	CAS	Molecular Weight g/ml	Formula	Category	Structure
6	3-(3-Carboxy-4-hydroxyphenyl)-D-alanine	8.854	13.44	00430 3-95-1	225.20	$C_{10}H_{11}NO_5$	Amino Acids, Aromatic	
7	1,1,1,3,5,5,5-Heptamethyltrisiloxane	9.109	2.20	00187 3-88-7	222.50	$C_7H_{21}O_2Si_3$	silica	
8	Silicic acid, diethyl bis (trimethylsily) ester	10.927	1.29	00355 5-45-1	296.58	$C_{10}H_{28}O_4Si_3$	ester	
9	Phenol	11.488	5.16	00010 8-95-2	94.11	C_6H_6O	phenol	
10	2-Ethylimino-4-methyl-pent-3-enenitrile	14.325	2.11	10001 89-21-6	136.19	$C_8H_{12}N_2$	N-ethyl-3-methylbut-2-enimidoyl cyanide	
11	Imidazole, 5-[2-(aminocarbonyl) vin yl]-	17.196	1.83	13520 0-64-5	137.14	$C_6H_7N_3O$	Heterocyclic Compounds (Azoles)	

No	Compound name	R.T / min	Area %	CAS	Molecular Weight g/ml	Formula	Category	Structure
12	Diethylphosphinothioic azide	19.108	1.48	05834 7-14-1	163.18	$C_4H_{10}N_3PS$	azido-diethyl-sulfanylidene-λ5-phosphane	
13	Mexiletine	21.070	2.70	03182 8-71-4	179.26	$C_{11}H_{17}NO$	Amide	
14	N,N',N''-Trimethyldiitrimethylenet riamine	22.175	1.49	00012 3-70-6	173	$C_9H_{23}N_3$	Amine	
15	Hexadecanoic acid, methyl ester	23.407	8.56	00011 2-39-0	270.4507	$C_{17}H_{34}O_2$	ester	
16	E, E,Z-1,3,12-Nonadecatriene-5,14-diol	25.624	4.97	10001 31-11-4	294.5	$C_{19}H_{34}O_2$	Alken alcohol	
17	4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester)	26.431	1.15	10001 29-56-3	172.16	$C_7H_9FN_2O_2$	ester	

No	Compound name	R.T / min	Area %	CAS	Molecular Weight g/ml	Formula	Category	Structure
18	Cyclohexane, 2-(dimethylhydrazono) -3-[4-hexenyl]-1-aci-nitro-, (E, E)	27.357	1.06	11268 3-26-8	267.37	$C_{14}H_{25}N_3O_2$	cycloalkane	
19	Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15- hexadecamethyl	30.390	2.50	01909 5-24-0	577.2	$C_{16}H_{48}O_7Si_8$	organosilicon compound	
20	2,2,3,5,6,6,7-Heptamethyl [1,4,2,3,5,6,7] dioxapentasilepane	31.876	1.52	10003 11-71-8	277.67	$C_7H_{21}O_2Si_5$	Hetero cyclic	

The first peak was determined in the methanolic latex extract of *Euphorbia tirucalli*, which was represented by (tert-Butyldimethylsilyl trifluorom ethanesulfonate) which had a retention time of 4.029 minutes and a peak area of 3.82%, and the highest peak was Acridine-9-carbaldehyde, which had a retention time of 32.649 minutes and a peak area of 3.42%, in the, while the other peaks at different retention times shown in Table (3-5), Figure (3-5).

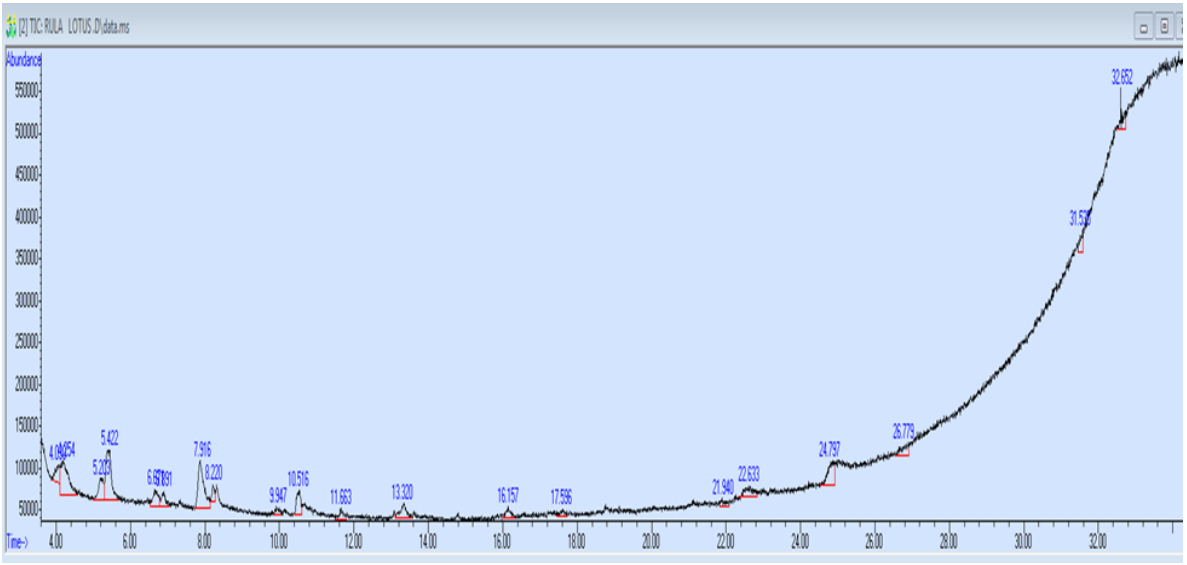
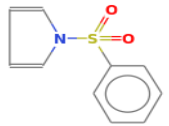
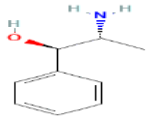
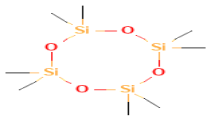
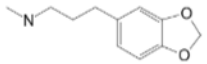
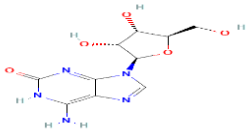
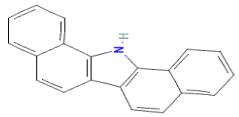
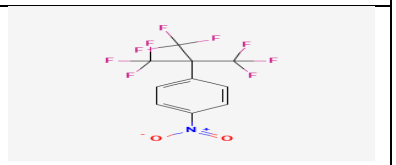
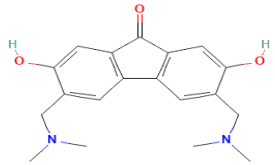
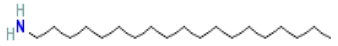
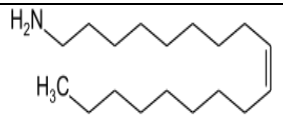
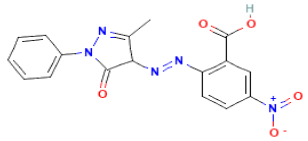
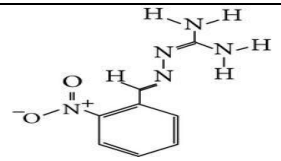
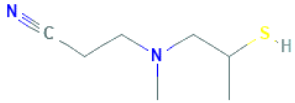
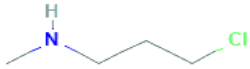
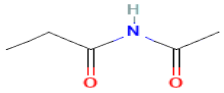
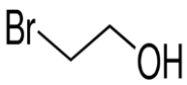
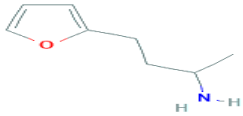


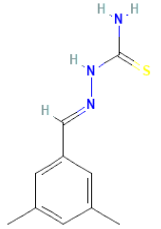
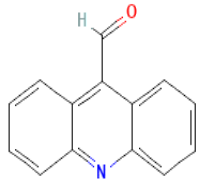
Figure 3-5: Chromatogram profile for GC-MS of *Euphorbia tirucalli* latex

Table 3-5: GC–MS Chromatogram of the methanolic latex extract of *Euphorbia tirucalli*

No	Compound name	R.T / min	Area %	CAS	Molecular Weight g/ml	Formula	Category	Structure
1	tert-Butyldimethylsilyl trifluorom ethanesulfonate	4.029	3.82	069739-34-0	264.34	Alkanesulfonic Acids	$C_7H_{15}F_3O_3SSi$	
2	1-Benzenesulfonyl-1H-pyrrole	4.258	12.79	016851-82-4	207.249	Heterocyclic Compounds (Azoles)	$C_{10}H_9NO_2S$	
3	Norpseudoephedrine	5.201	5.69	036393-56-3	151.21	Propanolamines	$C_9H_{13}NO$	
4	Cyclotetrasiloxane, octamethyl	5.422	14.13	000556-67-2	296.61	Organic Silicone compound	$C_8H_{24}O_4Si_4$	
5	N-methyl-3,4-methylenedioxyphenylpropan-3-amine	6.671	4.17	1000378-88-4	193.25	Benzodioxoles	$C_{11}H_{15}NO_2$	
6	Adenosine, 1,2-dihydro-2-oxo	6.892	3.18	001818-71-9	283.24	Purine Nucleosides	$C_{10}H_{13}N_5O_5$	

No	Compound name	R.T / min	Area %	CAS	Molecular Weight g/ml	Formula	Category	Structure
7	13H-Dibenzo[a,i]carbazole	7.920	13.69	000239-64-5	267.3	Heterocyclic Compounds, 2-Ring (Indoles)	$C_{20}H_{13}N$	
8	4-(Nonafluoro-tert-butyl) nitrobenzene	8.217	1.88	036714-78-0		Aromatic compounds	$C_{10}H_4F_9NO_2$	
9	3,6-Bis-dimethylaminomethyl-2,7-di hydroxy-fluoren-9-one	9.950	1.60	1000318-33-0	326.4	Enzyme	$C_{19}H_{22}N_2O_3$	
10	Nonadecylamine	10.519	5.15	001430-05-3	283.5	Aliphatic Compounds	$C_{19}H_{41}N$	
11	Oleylamine	11.666	2.06	000112-90-3	267.49	Amines	$C_{18}H_{37}N$	
12	2-(4,5-Dihydro-3-methyl-5-oxo-1-phenyl-4-pyrazolyl)-5-nitrobenzoic acid	13.323	3.94	020307-76-0	367.3	Nitrobenzoates	$C_{17}H_{13}N_5O_5$	

No	Compound name	R.T / min	Area %	CAS	Molecular Weight g/ml	Formula	Category	Structure
13	Benzaldehyde, 2-nitro-, diaminomet hylidenhydrazone	16.160	2.23	102632-31-5	207.18	Nitrogen compound	$C_8H_9N_5O_2$	
14	N-Methyl-N-[2-cyanoethyl]-2-mercap to propylamine	17.596	1.51	1000257-11-6	158.27	organic amino compound	$C_7H_{14}N_2S$	
15	3-Chloro-N-methylpropylamine	21.937	1.44	065232-62-4	107.58	Propylamines	$C_4H_{10}ClN$	
16	Propanamide, N-acetyl	22.633	4.45	019264-34-7	115.13	Amides	$C_5H_9NO_2$	
17	Ethanol, 2-bromo	24.800	8.21	000540-51-2	124.96	Alcohols	C_2H_5BrO	
18	propylamine,3-(furan-2-yl)-1 methyl-	26.779	3.73	1000315-88-1	139.19	organic amino compound	$C_8H_{13}NO$	

No	Compound name	R.T / min	Area %	CAS	Molecular Weight g/ml	Formula	Category	Structure
19	3,5-Dimethylbenzaldehyde thiocarba moylhydrazone	31.536	2.90	1000195-15-1	207.30	organooxygen compound	$C_{10}H_{13}N_3S$	
20	Acridine-9-carbaldehyde	32.649	3.42	1000318-45-4	207.23	Heterocyclic Compounds, 3-Ring (Acridines)	$C_{14}H_9NO$	

According to the results of the GC-MS study, all plant latex includ amine and amide while esters are found in all except the latex of *Euphorbia tirucalli*.

The results of this study revealed the presence of common chemical compounds which has a biologically important, as shown in Tables (3-6) and (3-7) in the latex of some plant species under study. Where the two plants *Ficus carica* and *Ficus elastica* which belong to the same genus and family are similar in containing the compound Oxime-, methoxy-phenyl which is used in the treatment of skin (Zareba, *et al*., 2002), but the *Ficus carica* was unique in containing the Benzaldehyde, 2-nitro-, diaminomet hylidenhydrazone, as Antimicrobial (Krishnamoorthy and Subramaniam, 2014), dl-. alpha. -Tocopherol as synthetic form of vitamin E with potent antioxidant properties (Bottje, *et al*.,1997), Cedran-diol, (8S,14)-) compounds as Antimicrobial and Anti-inflammatory (Muthukumarasamy and Mohan, 2012). Also, the *Ficus elastica* was unique in containing the Octasiloxane, 1,1,3,3,5,5,7,7,9,911,11,13,13,15,15-hexadecamethyl, that work as anti-bacterial activity according to Salem (2018).

furthermore the Hexadecanoic acid, methyl ester compounds are common in *Nerium oleander* and *Ficus elastica* which used as Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary (Krishnamoorthy and Subramaniam, 2014)

The current study also showed the presence of chemical compounds between plant species belonging to completely different families, namely: *Ficus carica* and *Euphorbia tirucalli* where they both containing (13H-Dibenzo[a,i]carbazole, Adenosine, 1,2-dihydro-2-oxo) compounds.

Calotropis procera and *Ficus elastica* both containing (Octasiloxane, 1,1,3,3,5,5,7,7,9,911,11,13,13,15,15-hexadecamethyl compound) which used as anti-bacterial activity (Salem, 2018). *Ficus carica* and *Calotropis procera* containing 3-(3-Carboxy-4-hydroxyphenyl)-D-alanine compound. *Euphorbia tirucalli* and *Calotropis procera* are similar in their containment of Cyclotetrasiloxane, octamethyl compound that act as antimicrobial, Antiseptic, Hair Conditioning Agent, Skin- Conditioning Agent-Emollient; Solvent (Mary and Giri, 2016) (Table 3-6), Table (3-7).

The presences of Oleic Acid in *Calotropis procera* latex gave it characters like Antifungal, anti-inflammatory, antioxidants, antibacterial (Awonyemi *et al.*, 2020), in addition the presence of Imidazole, 2-amino-5-[(2-carboxy) vinyl], Imidazole, 2-amino-5-[(2-carboxy) vinyl], Tris (tert-butyldimethylsilyloxy) arsane , Atomoxetine and Tetraacetyl-d-xylonic nitrile in *Nerium oleander* latex are made it as Antimicrobial and anti-inflammatory activity , Antioxidant, antibacteria1, antifungal and Anti-tumor (Kadhim, *et al.*, 2016; Salem, 2018; Michelson *et al*., 2003; Hameed *et al*., 2015).

From this study confirms the variation between the chemical constituent of latex among plant species, which also shows their different potential of therapeutic activities.

Table 3-6: The common compounds in the latex of some plant species under study

No	compounds	Species
1	Oxime-, methoxy-phenyl	*Ficus carica, Calotropis procera, Ficus elastica*
2	3-(3-Carboxy-4-hydroxyphenyl)-D-alanine	*Ficus elastic , Calotropis procera*
3	13H-Dibenzo[a,i]carbazole	*Ficus carica, Euphorbia tirucalli.*
4	Adenosine, 1,2-dihydro-2-oxo	*Ficus carica, Euphorbia tirucalli.*
5	1-Benzenesulfonyl-1H-pyrrole	*Calotropis procera, Ficus elastica, Euphorbia tirucalli*
6	Cyclotetrasiloxane, octamethyl	*Euphorbia tirucalli, Calotropis procera* and *Nerium oleander*
7	Hexadecanoic acid, methyl ester	*Ficus elastica, Nerium oleander*

Table 3-7: Biological importance compounds in the latex of some plant species under study

No	Compound name	Plant species	Biological importance	References
1	Oxime-, methoxy-phenyl	*Ficus carica, Calotropis procera, Ficus elastica*	Use in the treatment of skin	(Zareba, *et al.*,2002)
2	Benzaldehyde, 2-nitro-, diaminomet hylidenhydrazone	*Ficus carica*	Antimicrobial	(Krishnamoorthy and Subramaniam, 2014)
3	Cedran-diol, (8S,14)-	*Ficus carica*	Antimicrobial Anti-inflammatory	(Muthukumarasamy and Mohan, 2012)
4	dl-. alpha. -Tocopherol	*Ficus carica*	Synthetic form of vitamin E with potent antioxidant properties	(Bottje, *et al.*,1997)
5	Oleic Acid	*Calotropis procera*	Antifungal, anti-inflammatory, antioxidants, antibacterial	(Awonyemi, *et al.*, 2020)
6	Octasiloxane, 1,1,3,3,5,5,7,7,9,911,11,13,13,15,15-hexadecamethyl	*Calotropis procera, Ficus elastica*	Anti-bacteria1activity	(Salem, 2018)
7	Imidazole, 2-amino-5-[(2-carboxy) vinyl]	*Nerium oleander*	Antimicrobial and anti-inflammatory activity	(Kadhim, et *al.*, 2016)
8	Tris(tert-butyldimethylsilyloxy)arsane	*Nerium oleander*	Antioxidant, antibacterial, antifungal	(Salem, 2018)
9	Atomoxetine	*Nerium oleander*	Atomoxetine efficacious treatment for ADHD	(Michelson, *et al.*, 2003)
10	Benzaldehyde, 2-nitro-, diaminomet hylidenhydrazone	*Nerium oleander, Euphorbia tirucalli*	Antimicrobial	(Krishnamoorthy and Subramaniam, 2014)
11	Tetraacetyl-d-xylonic nitrile	*Nerium oleander*	Anti-tumor and anti-oxidant	(Hameed, *et al.*, 2015)
12	Hexadecanoic acid, methyl ester	*Nerium oleander, Ficus elastica*	Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary	(Krishnamoorthy and Subramaniam, 2014)
13	Cyclotetrasiloxane, octamethyl	*Euphorbia tirucalli. Calotropis procera*	Antimicrobial, Antiseptic, Hair Conditioning Agent, Skin- Conditioning Agent-Emollient; Solvent	(Mary and Giri, 2016)

3.2: Molecular study

Even though many families in Iraq's flora have been extensively studied morphologically and phytochemically (Harborne *et al.*, 1971), there are still many unanswered questions about the classification of genera within tribes and subfamilies, so molecular information has aided in classification and plant breeding. As a result, molecular methods to plant systematics are becoming more common (Soltis *et al*., 1992). The DNA was extracted from latex considering its different chemical compositions, which may have taxonomic implications and importance in understanding the evolutionary history of the groups in which it is found (Rudall, 1987). Good results have been shown for extracting using FAVORGEN Kit.

The results of the study showed the success of the extraction process from the latex of the five plant species using the FAVORGEN extraction kit, where the extracted concentrations ranged between (30 - 150) ng/µl and a purity of(1.3-2.2) , genetic variation among the five latex-producing species grown in Iraq was selected using five random primers by the RAPD which are:

3.2.1Primer OPB18

This primer shows twenty-five bands, where the molecular weights ranged between 100 - 1500 base pairs (100 base pairs in the latex of *Calotropis procera* and 1500 base pairs in the latex in both *Ficus carica* and *Ficus elastica*). Also, the number of bands were distributed among 4 bands in both of *Ficus elastica* and *Nerium oleander*. while the number of bands were 6 in both of *Ficus carica* and *Euphorbia tirucalli*, and 5 in *Calotropis procera*.

This primer has shown presence of three Unique bands the first with 100bp in *Calotropis procera*, 300 and 450 bp in *Euphorbia tirucalli*, as shown in Table (3-8) and Figure (3-6).

Table 3-8: Total number of bands of OPB18 primer

Species ladder	1	2	3	4	5
1500	1	1	0	0	0
900	1	0	0	1	1
710	1	1	1	1	0
700	1	0	0	1	0
600	0	1	0	0	1
550	1	0	1	0	1
500	1	1	1	1	0
450	0	0	0	0	1
400	0	0	1	0	1
300	0	0	0	0	1
100	0	0	1	0	0
total	6	4	5	4	6

(0) Absent band (1) Existing band, 1. *Ficus carica*; 2. *Ficus elastica*; 3. *Calotropis procera*; 4.*Nerium oleander*; 5. *Euphorbia tirucalli*.

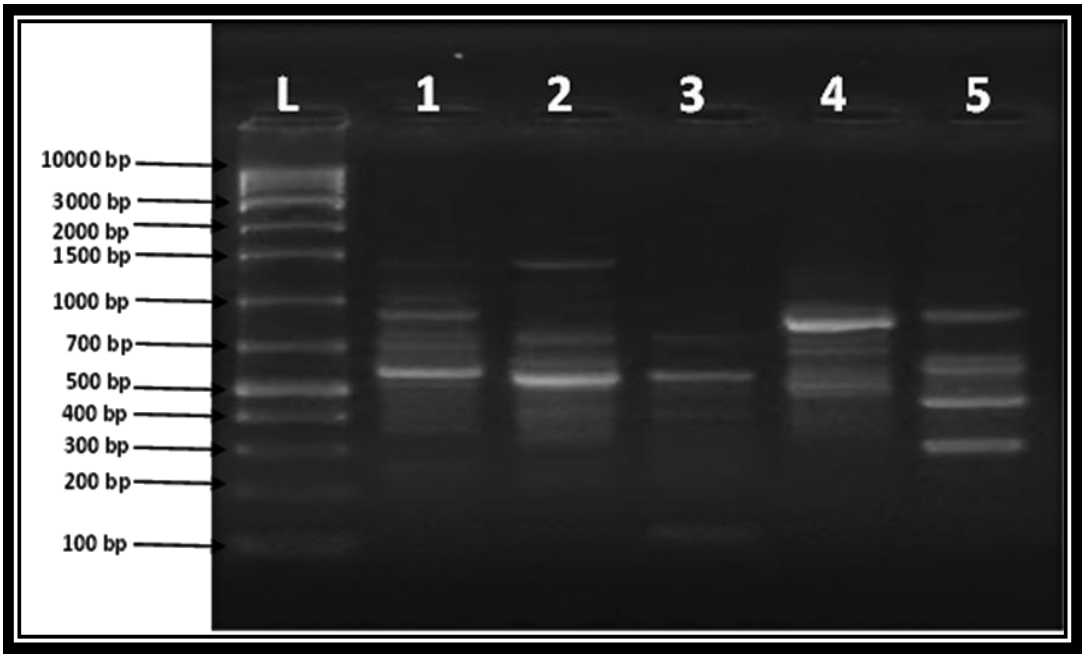


Figure 3-6: RAPD fragments' banding patterns of plant species of OPB18 primer where 1. *Ficus carica* 2. *Ficus elastica* 3. *Calotropis procera* 4. *Nerium oleander* 5. *Euphorbia tirucalli*.

3.2.2 Primer BH14

This primer shows the lowest number of bands were twenty-three, and the molecular weights ranged between 150 - 1800 base pairs, (150 base pairs in the latex of *Calotropis procera* and 1800 base pairs in the latex of *Euphorbia tirucalli*).

The number of bands was distributed among 5 bands in both *the Ficus elastica* and *Calotropis procera*), 6 bands in both *Nerium oleander* and *Euphorbia tirucalli*, and only one band in *Ficus carica*, This primer has shown the presence of two unique bands the first with (1100 and 1800)bp in *Euphorbia tirucalli*, 1700 and 150 bp in *Nerium oleander*, *Calotropis procera* respectively, as shown in Table (3-9) and Figure (3-7).

Table 3-9: Total number of bands of BH14 primer

Species	1	2	3	4	5
ladder	0	0	0	0	1
1700	0	0	0	1	0
1500	0	0	0	1	1
1100	0	0	0	0	1
1000	0	1	0	1	0
900	0	1	1	1	1
800	0	1	1	0	0
700	0	1	1	0	1
600	0	1	0	0	1
550	1	0	0	1	0
200	0	0	1	1	0
150	0	0	1	0	0
total	1	5	5	6	6

(0) Absent band (1) Existing band, 1. *Ficus carica;* 2. *Ficus elastic;* 3. *Calotropis procera;* 4. *Nerium oleander;* 5. *Euphorbia tirucalli*.

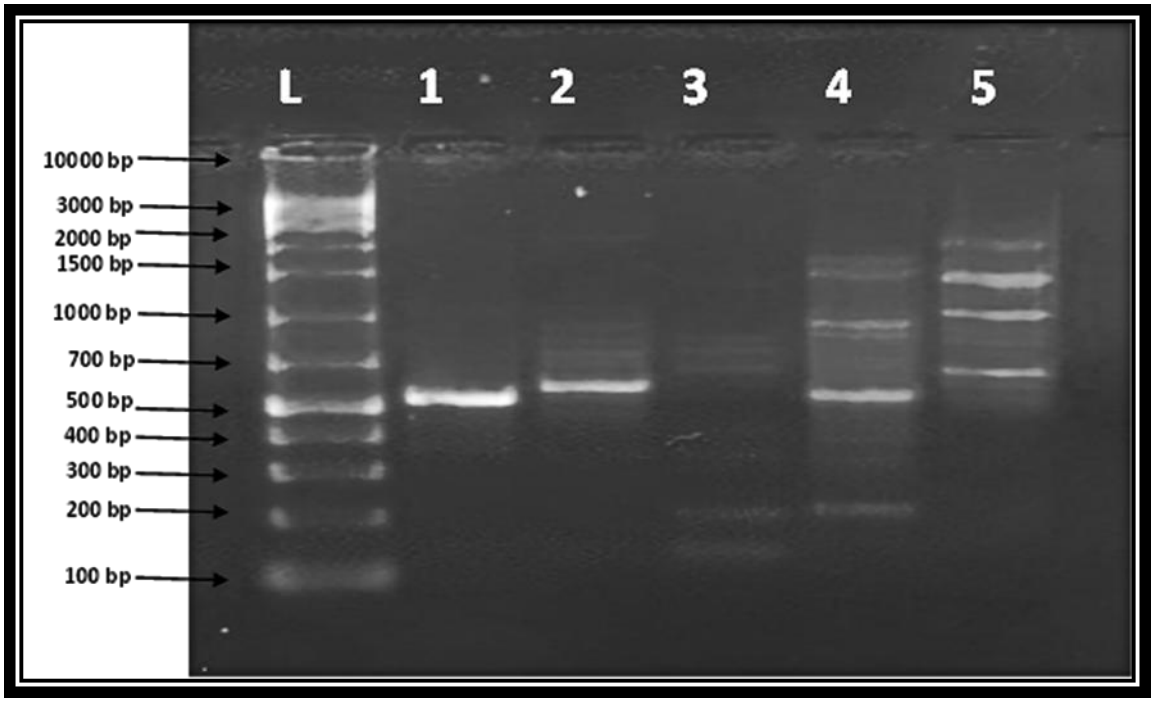


Figure 3-7: RAPD fragments' banding patterns of plant species of BH14 primer where 1. *Ficus carica* 2. *Ficus elastica* 3. *Calotropis procera* 4. *Nerium oleander* 5. *Euphorbia tirucalli*.

3.2.3 Primer BH11

The electrophoresis of the primer products showed the presence of twenty-five bands, the molecular weights ranged between 290 - 1500 base pairs, (290 base pairs in the latex of *Ficus elastica* and 1500 base pairs in the latex of *Nerium oleander*).

The number of bands was distributed among 5 bands in each of the *Ficus elastica*, *Nerium oleander* and *Euphorbia tirucalli*, 6 bands in *Ficus carica* and *Euphorbia tirucalli*, and 4 bands in *Calotropis procera.*

This primer has shown presence of four unique bands, the first with 1500bp in *Nerium oleander*, 800 bp *Calotropis procera*, 400bp in *Ficus carica,*and 290 in *Ficus elastica* , as shown in Table (3-10) and Figure (3-8).

Table 3-10: Total number of bands of BH11 primer

Species	1	2	3	4	5
ladder	0	0	0	1	0
1400	0	0	1	1	1
1000	1	0	0	1	1
900	1	0	1	0	1
800	0	0	1	0	0
600	1	1	1	1	0
500	1	1	0	0	1
450	0	1	0	1	0
400	1	0	0	0	0
350	1	1	0	0	1
290	0	1	0	0	0
total	6	5	4	5	5

(0) Absent band, (1) Existing band , 1. *Ficus carica;* 2. *Ficus elastic;* 3. *Calotropis procera;* 4. *Nerium oleander;* 5. *Euphorbia tirucalli*.

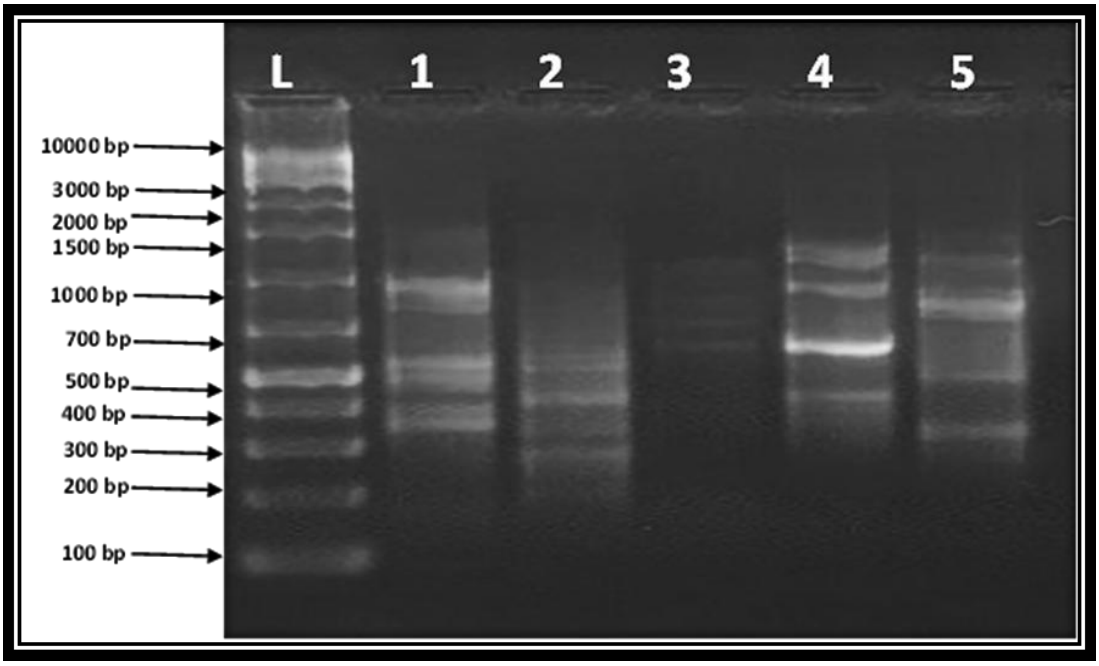


Figure 3-8: RAPD fragments' banding patterns of plant species of BH11 primer where 1. *Ficus carica* 2. *Ficus elastica* 3. *Calotropis procera* 4. *Nerium oleander* 5. *Euphorbia tirucalli*.

3.2.4 Primer BH10

The current study showed the results of the BH10 primer is the highest number of bands which represented by thirty-six bands, the molecular weights ranged between 290 - 3000 base pairs, 290 base pairs in the latex of *Ficus elastica* and 3000 base pairs in the latex in both of *Ficus carica* and *Ficus elastica*.

The number of bands was distributed among 8 bands in both of the *Ficus carica* and *Ficus elastica*, 9 bands in *Calotropis procera*, 7 in *Euphorbia tirucalli*, and 4 band in *Nerium oleander*.

This primer has shown presence of three unique bands, the first with 290bp in *Ficus elastica*, 570 bp *Euphorbia tirucalli*, and 1800 in *Calotropis procera*, as shown in Table (3-11) and Figure (3-9)

Table 3-11: Total number of bands of BH10 primer

Species	1	2	3	4	5
ladder	1	1	0	0	0
2500	1	1	1	0	0
1800	0	0	1	0	0
1500	0	0	1	1	1
1000	1	1	1	0	0
850	1	1	0	0	1
700	0	0	1	1	1
600	1	1	1	1	0
570	0	0	0	0	1
550	0	0	1	1	1
500	1	1	1	0	0
400	1	1	1	0	1
300	1	0	0	0	1
290	0	1	0	0	0
total	8	8	9	4	7

(0) Absent band, (1) Existing band , 1. *Ficus carica* 2. *Ficus elastica* 3. *Calotropis procera* 4. *Nerium oleander* 5. *Euphorbia tirucalli*.

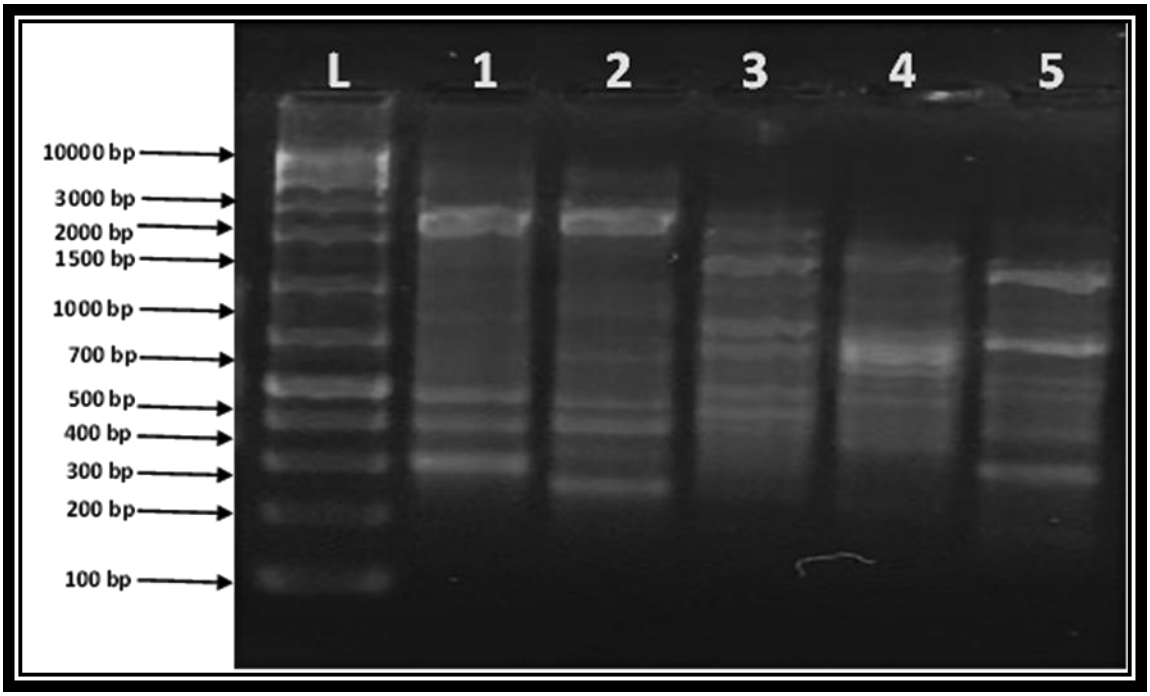


Figure 3-9: RAPD fragments' banding patterns of plant species of BH10 primer where 1. *Ficus carica* 2. *Ficus elastica* 3. *Calotropis procera* 4. *Nerium oleander* 5. *Euphorbia tirucalli*.

3.2.5 Primer OPC2

This primer shows twenty-eight bands, the molecular weights ranged between 150 - 3000 base pairs, (150 base pairs in the latex of *Euphorbia tirucalli* and 3000 base pairs in the latex in both of *Ficus carica* and *Ficus elastica*, and also in *Nerium oleander*).

The number of bands was distributed among 7 bands in both of the *Nerium oleander* and *Calotropis procera*), 2 bands in *Ficus carica* and 3 in *Ficus elastica*, and 9 bands in *Euphorbia tirucalli*

This primer has shown presence of three unique bands with (150, 450, 700) bp all in *Euphorbia tirucalli*, as shown in Table (3-12) and Figure (3-10).

Table 3-12: Total number of bands of OPC2 primer

Species	1	2	3	4	5
ladder	1	1	0	1	0
1500	1	1	0	1	0
1300	0	1	0	0	1
1000	0	0	1	1	1
850	0	0	1	1	1
750	0	0	1	1	0
700	0	0	0	0	1
500	0	0	1	1	0
450	0	0	0	0	1
380	0	0	1	0	1
300	0	0	1	1	1
200	0	0	1	0	1
150	0	0	0	0	1
total	2	3	7	7	9

(0) Absent band, (1) Existing band, 1. *Ficus carica;* 2. *Ficus elastic;* 3. *Calotropis procera;* 4. *Nerium oleander;* 5. *Euphorbia tirucalli*.

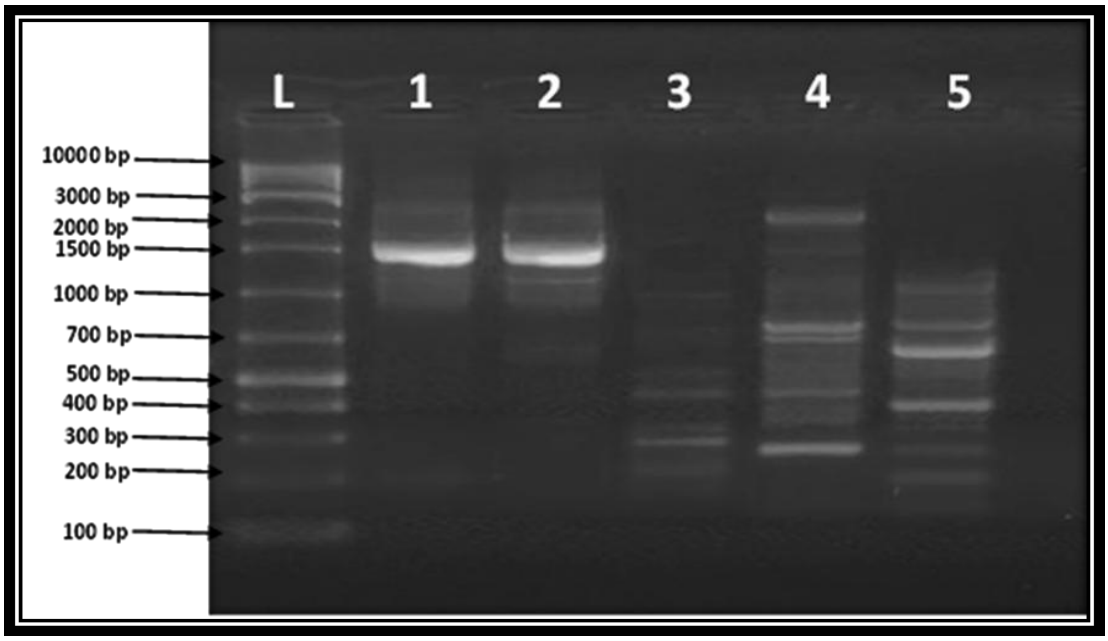


Figure 3-10: RAPD fragments' banding patterns of plant species of OPC2 primer where 1. *Ficus carica* 2. *Ficus elastica* 3. *Calotropis procera* 4. *Nerium oleander* 5. *Euphorbia tirucalli*.

In this study, genetic variation among five latex-producing species grown in Iraq was selected using five random primers by the RAPD method due to a lack of DNA sequence information regarding these species. The results of random multiplication showed the distribution of one hundred and thirty-seven amplified products spread in these species.

The highest numerical value was thirty-six bands at the BH10, while the lowest number of bands at the BH14 was twenty-three. The number of polymorphic fragments ranged from eleven in OPB18 and BH11 to fourteen in BH10 ,Where a total were sixty-one,This resulted in 45% polymorphism, with the lower value of polymorphism fragments in BH10 being 38.89% and higher value is 52.17% in BH14, Thus the efficiency of the primers used was primer BH10 the highest equal 26.27% while in the primers OPB18 and BH11, were 18.24% and 16.781% respectively, 18.52% in primer BH14, and 20.43% in the OPC2.

The results agreed with what was shown by Al-Tameme (2018) and Altameme and Ibraheam (2019) that all or some families have a specific size (base pair) and differ in their sizes from other bands of the same primer, where the number of bands and their molecular sizes differ from other bands.

From the Table (3-13), it is clear the total sum of the unique bands is seventeen, the presence of unique bands is important in determining the genetic signature of each of the studied species, and the primer whose interaction results lead to the presence of several different bands has a greater chance of finding unique bands of genetic structure, while remaining opportunities for primers that produce a small number of dissimilar bands (Williams *et al*., 1990).

Table 3-13: Number of bands produced in each random primer.

primers	Total number of bands	The number of different bands	poly morphism %	primer efficiency	Discriminating ability	Plant species	Molecular weight	Unique bands
OPB18	25	11	44	18.24	18.032	*Euphorbia tirucalli*	450,300	3
						Calotropis procera	100	
BH14	23	12	52.17	16.78	19.67	*Euphorbia tirucalli*	1800,1100,	4
						Nerium oleander	1700	
						Calotropis procera	150	
BH11	25	11	44	18.24	18.032	*Nerium oleander*	1500	4
						Calotropis procera	800	
						Ficus carica	400	
						Ficus elastica	290	
BH10	36	14	38.89	26.27	22.95	*Calotropis procera*	1800	3
						Euphorbia tirucalli	570	
						Ficus elastica	290	
OPC2	28	13	46.43	20.43	21.31	*Euphorbia tirucalli*	700,450,150	3
total num.	137	61	45	100	100			17

As for the results of the discriminating ability, the primer can reflect the difference in the genome of the species according to the total number of divergent bands, and therefore it was noted that the primer wich can identify is the primer that has the highest number of polymorphic bands according to the total number of differences

The results showed that the discrimination power of each primer varied from 18.032% in OPB18 and BH11 to 26.47% in BH10 and overlapped between them in BH14 and OPC2 at 19.67% and 21.31 % respectively, Table (3-13).

As for the genetic distance, it ranged between 0.191 - 0.455, and the lowest similarity index was 0.191 between the species *Ficus carica* and *Euphorbia*

tirucalli which belonging to different plant families, while the genetic distance between the two species *Calotropis procera* and *Nerium oleander* were 0.366 and this is consistent with the APG II (2003) classification system, The Asclepiadaceae family is a former plant family that is currently classified as a subfamily Asclepiadoideae inside the Apocynaceae family (Kumar, 2020). The highest similarity was 0.455 between the two species, *Ficus carica* and *Ficus elastica* Table (3-14).

The tree of genetic analysis of latex-producing plant species Figure (3-13) included two main groups: A and B groups, include both species *Ficus carica* and *Ficus elastica* that belong to the same family Moraceae. Group B involved two subgroups, B1 and B2, where B1 is unique to the species *Euphorbia tirucalli* which belong to the family Euphorbiaceae**.** As for group B2, it included the two species *Calotropis procera* and *Nerium oleander in* Apocynaceae when considered Asclepiadoideae as a subfamily from Apocynaceae (Kumar, 2020).

Plant genotypes have traditionally been distinguished by morphological descriptors such as plant height, flower color, fruit length and orientation, and seed characteristics (Sitthiwong *et al*., 2005), chromosome morphology (Pickersgill, 1971) and protein/enzyme profiling (Posch *et al*., 1994; Kumar *et al*., 2010) study genetic diversity within genus. However, these methodologies have a lot of weaknesses, such as the effect of the environment on trait phenotypic, cognitive interactions, and multi-directional impacts, to name a few. As a result, the current study outlined how genetic indicators (molecular markers) might be more effective than phenotypic or biochemical markers.

The RAPD patterns discovered in this work can be used to supplement the traditional approach of species identification, which focuses primarily on morphological characteristics also this work highlights the remarkable potential of molecular methods to differentiate species based on their RAPD patterns. Therefore, The RAPD approach has been utilized in a variety of taxonomic and genetic diversity investigations with great success.

Table 3-14: Similarity Matrix computed with Jaccard coefficient

Species	1	2	3	4	5
1	0	0.455	0.233	0.256	0.191
2		0	0.250	0.214	0.184
3			0	0.366	0.312
4				0	0.229
5					0

1. *Ficus carica* 2. *Ficus elastica* 3. *Calotropis procera* 4. *Nerium oleander* 5. *Euphorbia tirucalli*.

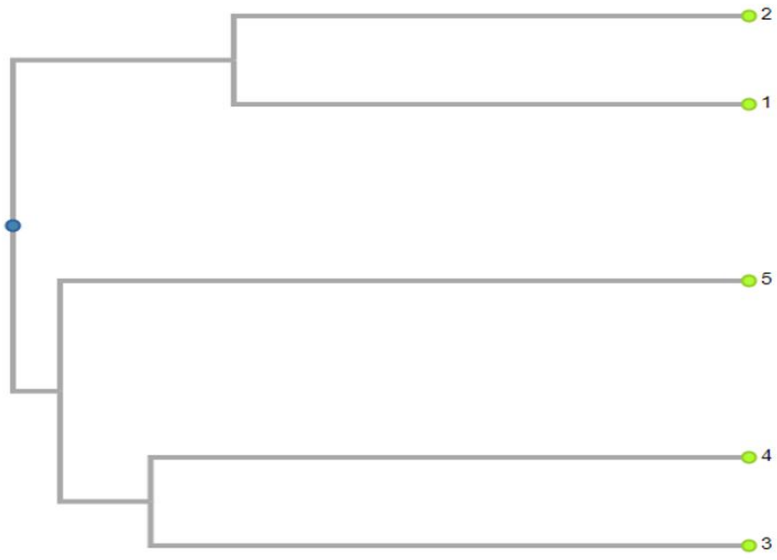


Figure 3-11: UPGMA dendrogram based on RAPD shows the genetic relationship among latex-producing species.1: *Ficus carica* 2: *Ficus elastica*, 3: *Calotropis procera*, 4: *Nerium oleander*, 5: *Euphorbia tirucalli.*

3.3: Preparation of silver nanoparticles and some biological applications:

Several studies have been reported on the preparation of silver nanoparticles from plant latex, and the characterization of the resulting nanoparticles, Chandhru *et al*. (2021) pointed to the green synthesis of silver nanoparticles from *Calotropis procera* which were well characterized by many methods such as the formation of surface plasmon resonance (SPR) band was monitored by UV-Vis spectroscopy, FT-IR spectra, XRD Pattern, SEM, Biofilm assay and Anti-bacterial study. Also, Kalaiselvi *et al*. (2019) conducted the study Biosynthesis of Silver Nanoparticles utilizing Latex from Euphorbian Plants also their antimicrobial activity.

3.3.1: The Characterization of a Biosynthesis silver nanoparticle from plant latex

3.3.1.1 Change color

Each type of latex of the five plant species showed its ability to biosynthesis silver nanoparticles after adding silver nitrate (AgNO3) at a concentration of 1 mM to the latex, The color change in the reaction mixture after 24 hours of incubation at 37 °C in a vibration incubator that reflected clear evidence of the formation of silver nanoparticles as a result of ionic reduction (Ag^{+}) to elemental silver (Ag^{0}) through a group of reducing agents in plant latex is eco-friendly and this result was confirmed by (Mohammed *et al*., 2014).

Figure (3-12) shows the clear color change that occurs in each type of latex under study, as the color of the mixture in the *Ficus carica* changed color from light brown to light orange, in the *Calotropis procera* latex mixture the color changed from milky to pink, while the color changed from green and

brown to dark green and dark brown in *Nerium oleander* and *Ficus elastica* respectively, but changed from white to brown color in *Euphorbia tirucalli.*

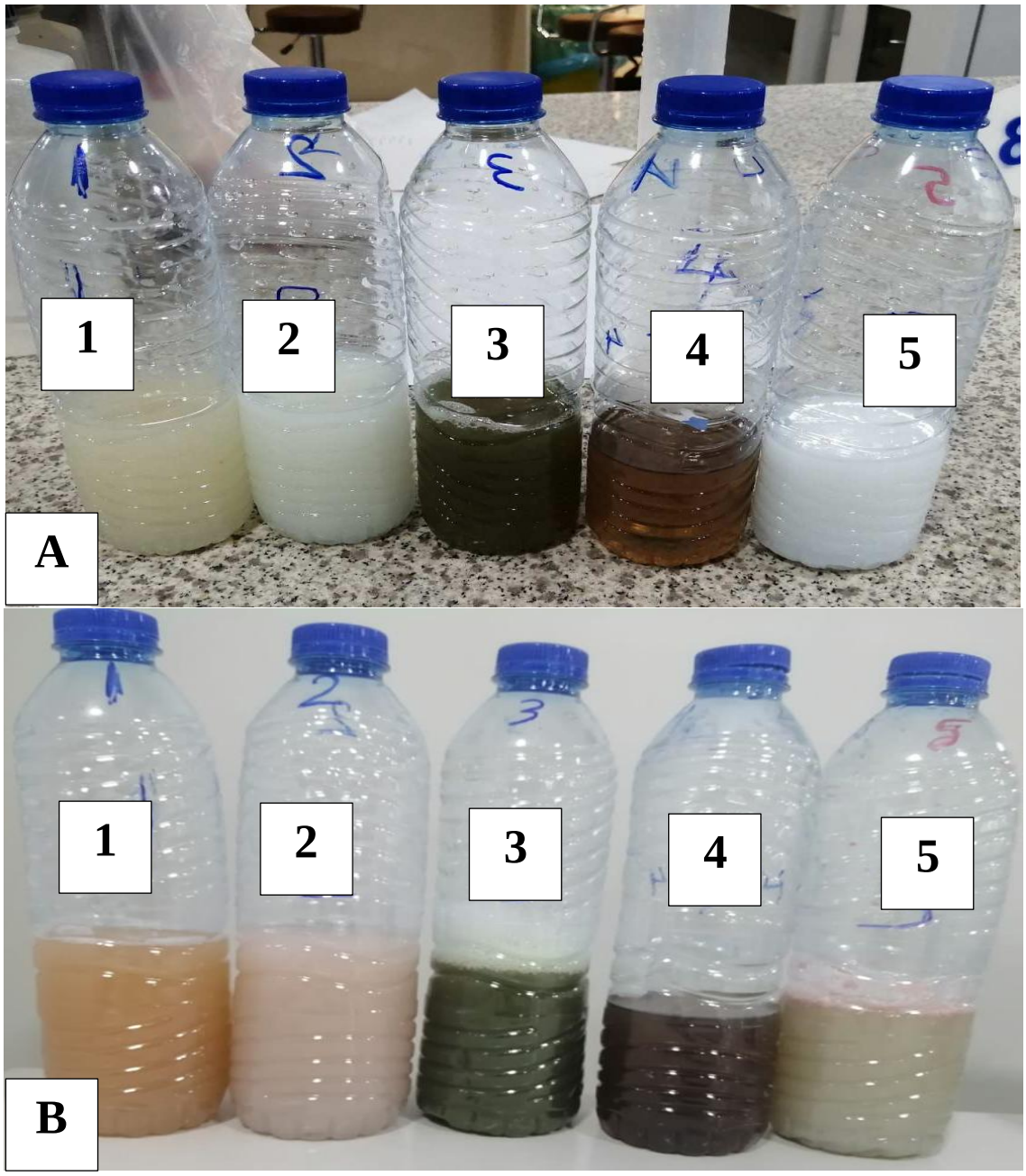


Figure 3-12: A: Biosynthesis of silver nanoparticles using plant latex before color change B: After color change1: *Ficus carica* 2: *Calotropis procera*, 3: *Nerium oleander*, 4: *Ficus elastica* 5: *Euphorbia tirucalli*

3.3.1.2 UV visible Spectroscopy

UV-visible spectrophotometric analysis of nanoparticles is a well-confirmed method, Color change was seen in the reaction mixture after 24 hours of incubation, suggesting that nanoparticles are formed in the reaction mixture Figure (3-12). The biogenesis of nanoparticles may be verified by visual inspection and detection of the surface plasmon resonance (SPR) band using UV-vis spectroscopy. The absorption spectra of nanoparticles generated in the reaction mixture reveal an absorption peak at 400 nm for AgNPs, as shown in Figure (3-13)The existence of surface plasmon resonance (SPR) of nanoparticles was demonstrated using latex from all five plant species studied.

In a study conducted by Chandhru,*et al*.,(2021) it was reported that The absorption peak were at 420 nm for AgNPs made by latex of *calotropis procera* , and 401nm by *Achras sapota*(Mondal,*et al* .,2011)

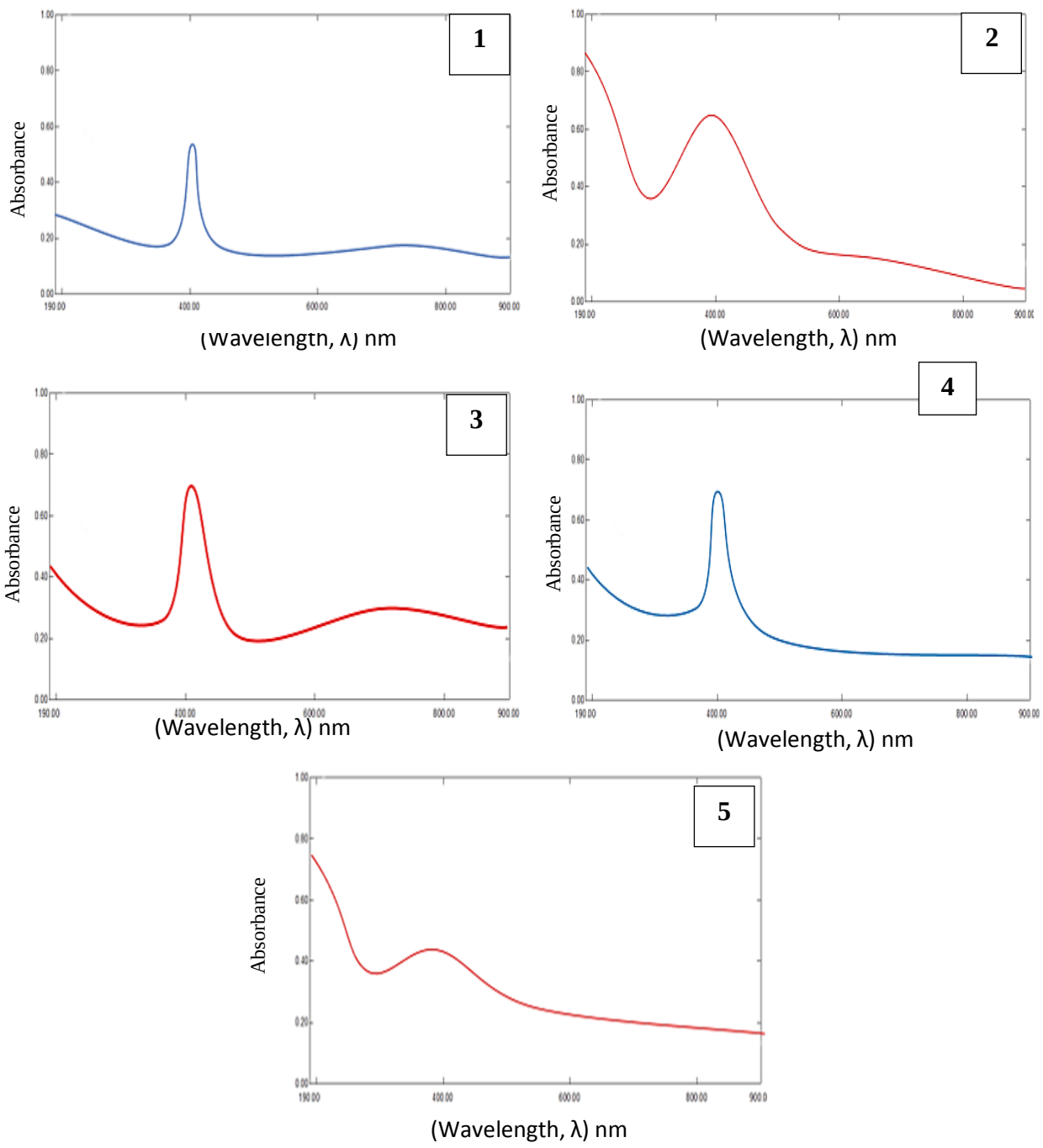


Figure 3-13: UV–visible absorption spectrum of AgNPs synthesized by 1- *Ficus carica*, 2- *Calotropis procera*, 3- *Nerium oleander*, 4- *Ficus elastica*, 5- *Euphorbia tirucalli*

3.3.1.3 Scanning Electron Microscopy (SEM) Analysis.

The size and shape of the latex AgNPs were confirmed using SEM. The results of scanning electron microscopy investigation revealed that different plant species' latex had different biogenic AgNP characteristics. According to the results of the SEM characterisation of nanoparticles, each plant species' capacity to biosynthesise silver nanoparticles differs.

It is clear in the Figure (3-14) which represents the silver nanoparticles of *Ficus carica*, *Calotropis procera*, and *Ficus elastica* latex extract, those clear homogeneous atoms with small nanoscale dimensions range (46.4, 45.4 and 47.8) nm respectively, also the clear circular shape was noticed which shows that the latex particles completely surrounded the silver nitrate nanoparticles and gained this circular shape, Table (3-13).

Table (3-15) and Figure (3-15) represented silver nanoparticles prepared by latex of *Nerium oleander* and *Euphorbia tirucalli* respectively show the heterogeneity with nanoscale dimensions of about (50.5 and 51.6) nm respectively and the clear agglomeration of the atoms, which can be attributed to the fact that the increase in the mixing time period has an important role by giving the atoms a clear assortment and the absence of inter-clumping of the atoms, as the increase of silver nitrate to the extract leads to an increase in the rate of surface roughness, which indicates the increase in the size of the grains and the decrease in the grain boundaries, and this is consistent with the results obtained from the X-ray diffraction examination.

The sizes of nanoparticles were ranged from 14 to 45.6 nm in a study conducted by Khattak,*et al*.,(2019) by *Euphorbia dracunculoides.*

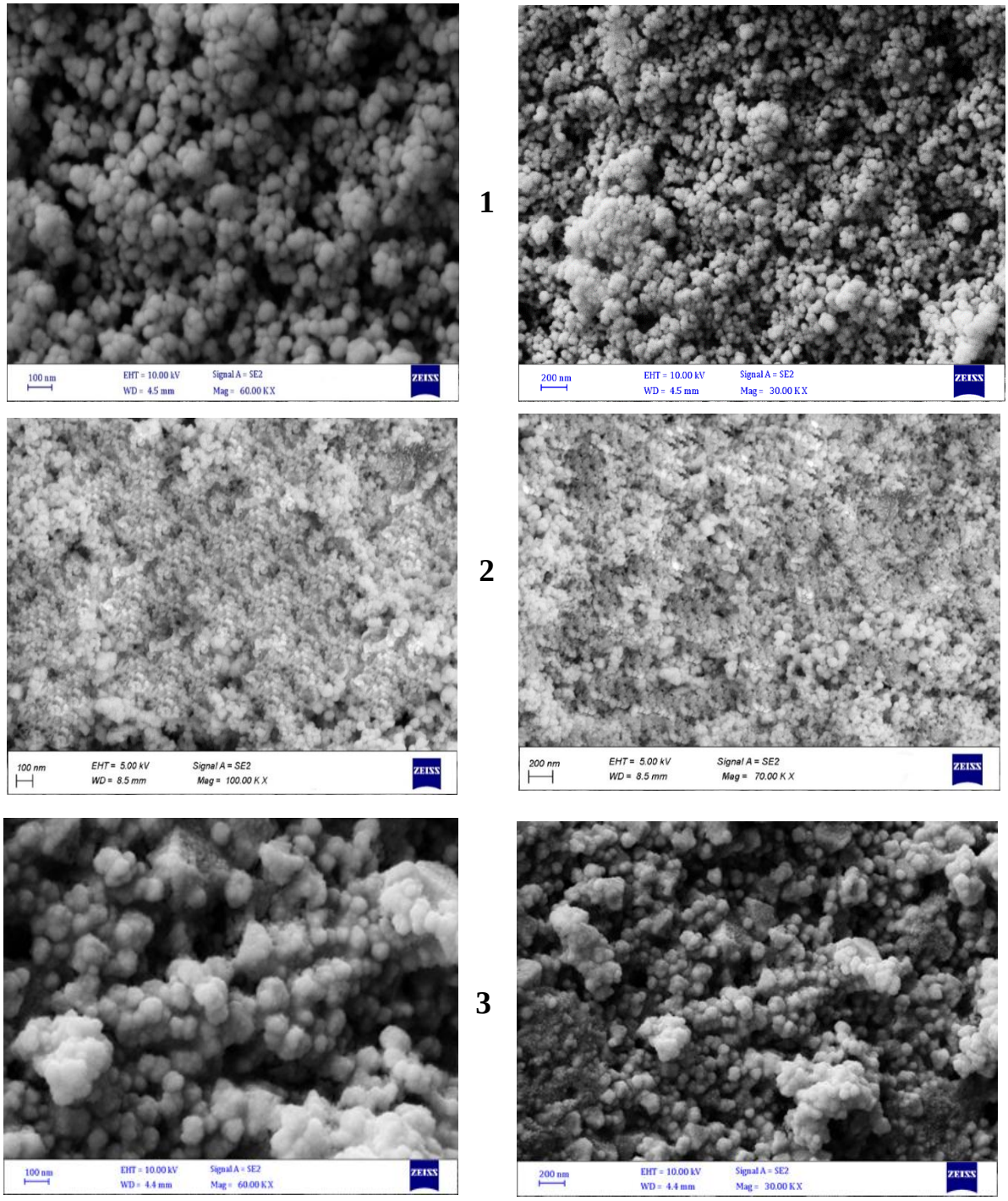


Figure 3-14: SEM of Silver nanoparticles prepared by 1- *Ficus carica 2- Calotropis procera 3- Ficus elastica*

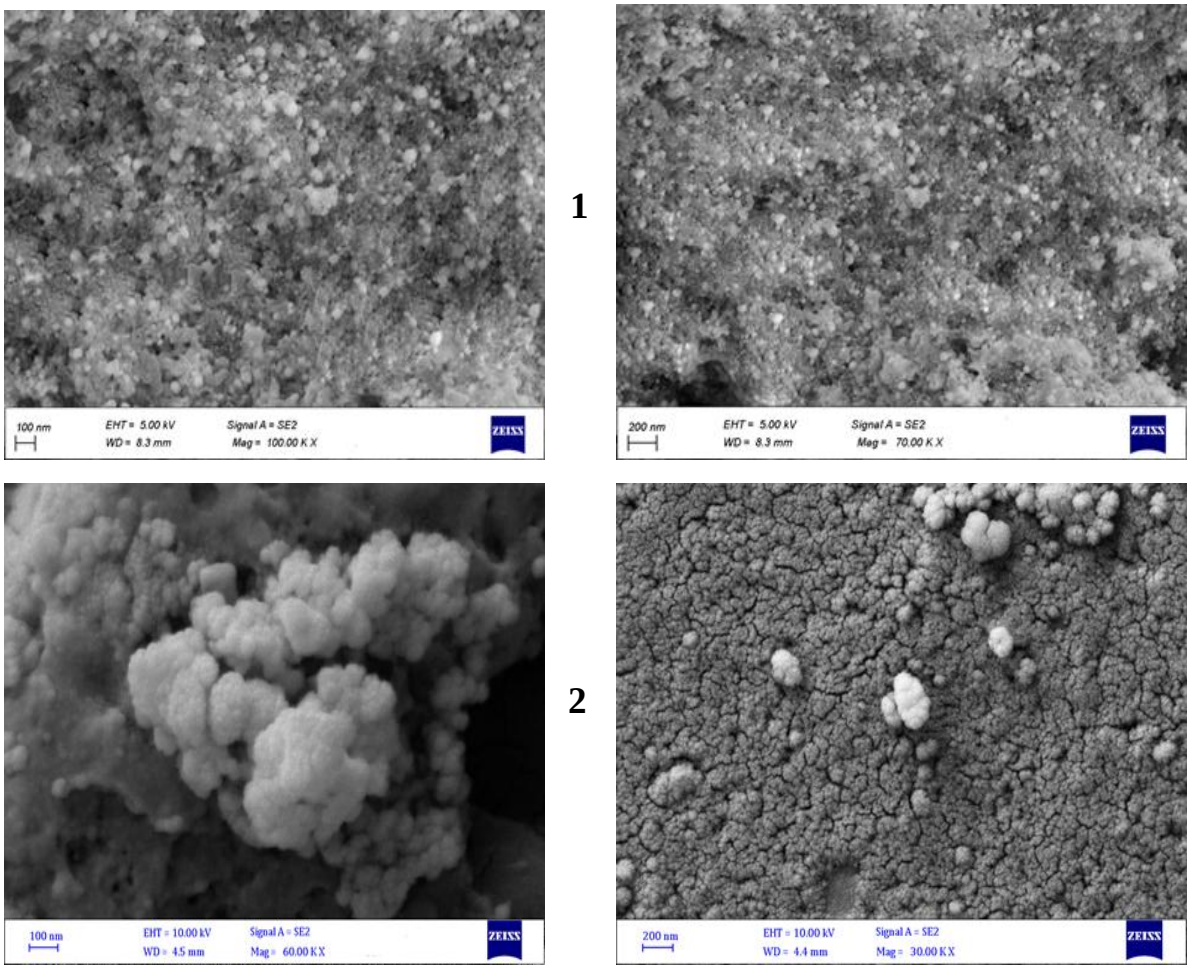


Figure 3-15: SEM of Silver nanoparticles prepared by 1- *Nerium oleander* 2- *Euphorbia tirucalli*

Table 3-15: Range of AgNPs size particles.

No	species	Size (nm)	average
1	*Ficus carica*	19-75	46.4
2	*Calotropis procera*	17- 77	45.4
3	*Nerium oleander*	32- 80	50.5
4	*Ficus elastica*	22- 64	47.8
5	*Euphorbia tirucalli*	40- 68	51.6

3.3.1.4 X-Ray Diffraction (XRD)

Through the results of the examination of X-ray diffraction, it was noted that all the materials that were prepared from silver nitrate with latex were a polycrystalline structure, where Figure (3-16) show the presence of several peaks, all of which belong to the composition of the material being worked on with a hexagonal structure, and when comparing the results that were reached from the surface area and the diffraction angles (2θ) corresponding to the locations of the characteristic peaks of the membrane models prepared by the ionic rotation method with the values mentioned in the standard card of the American Institute for Testing Materials, before mixing and that this displacement tends towards a larger value (2θ) and that the explanation for this displacement is due to the small ionic radius of the silver nitrate nanoparticle compared to the ionic radius of the milk material. The size of the crystal due to the increase in the distance between the crystal levels and then a decrease in the diffraction angle, i.e. the displacement of the characteristic peaks to the right in the diffraction pattern because it is related to an inverse relationship.

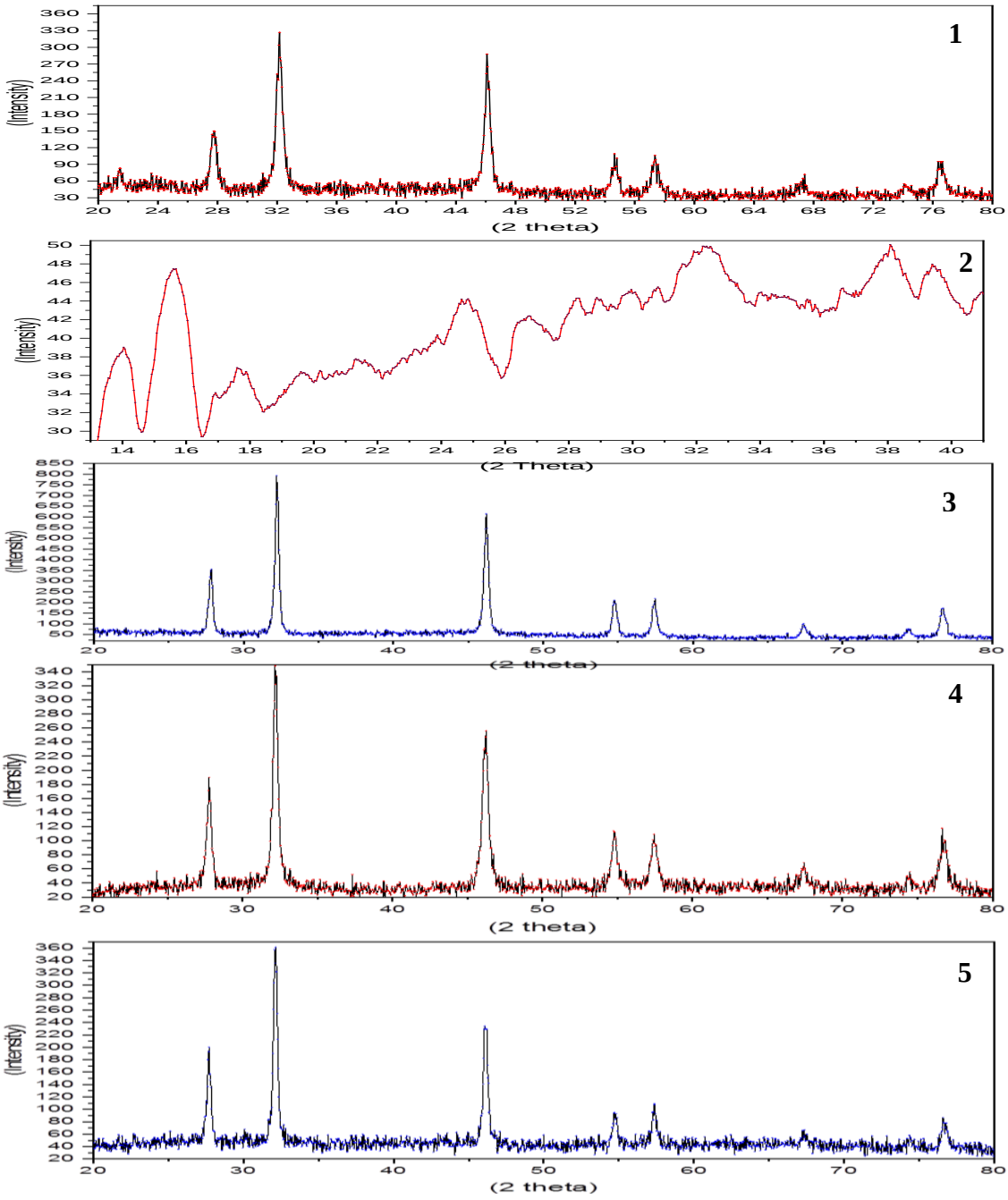


Figure 3-16: XRD analysis of Biosynthesized nanoparticles by 1-*Ficus carica*, *2-Calotropis procera, 3- Nerium oleander*, *4- Ficus elastica,* 5-*Euphorbia tirucalli*

3.3.1.5 Fourier Transform Infrared Spectroscopy (FT-IR)

The infrared study is one of the most important methods for determining the absorption bands resulting from the active groups present in the compound formula under study. The difference in the intensity of the main absorption band sites is an indicator of the occurrence of a chemical reaction and the formation of the desired result, as the absence of a difference in the intensity of the main absorption band, indicates that a chemical reaction did not occur. To identify these bands and track changes in them, the spectrum regions belonging to these compounds were divided into four main regions:

1- The first region ranges between (500-1500 cm^{-1}), which is often called the fingerprint region, where there is a location of the bonds for the original substance only (except for some aromatic compounds that can show locations in this region). This region contains most of the absorption bands for the active groups of organic compounds, so this region is very important in defining the absorption bands belonging to the aliphatic (C-C), aromatic (C-N), (C-O) ketone and aldehyde bands, and the locations of these bands according to the groups associated with this group, there are strong, medium-strength, and weak-strength ties.

2- The area ranged between (1650-1780 cm^{-1}). This area contains an absorption band for the active groups of different organic compounds, especially the amine domain (C=C) and appears in it may be sharp and strong, meaning that the absorbance is high compared to the permeability and it is called peak (strong) as for the number (1600-1680), the bond is (C=O) and the peak is not (strong).

3- The third region extends from (2000-3000 cm^{-1}) in which the triple chemical bond appears.

4- The fourth zone extends from (3000-4000 cm^{-1}).

Figures (3-17) to (3-21) of the FTIR spectrum showed that all the bonds are in the same place with the difference in the mixing period between silver nitrate and plant latex, where it was observed in the fingerprint region that the absorbance was low compared to the transmittance and this region extends from (500-1470 cm^{-1}) the clear similarity is noted in this area for all figures and it was called the fingerprint because only the original materials used appear in it, which always has a single bond.

This area, which goes beyond the footprint area, is one of the very important areas, where the high absorbance intensity was observed, where the bond is sharp and has little permeability, and in which it is bilateral (Carbon-Carbon) (C = C). As for what was observed in the region extending for all shapes from (1700-2900 cm^{-1}), usually the bonds are very weak in most compounds, and as shown in all shapes after this region, a wide beak appeared with high absorption and low permeability, which is in an area (3000-3500 cm^{-1}) and the peak in it was called broad (OH) or with a thorn.

In this region, the peak is wide and medium in absorption and is specific to alcohol and phenol, but if it is broad and high in absorption, It is called (Very broad) and is specific to (Acid), which is related to carbon, and this is also observed in all plant species Figures (3-17) to (3-21) that appeared during the examination process and determined the type of bond, its strength and wavenumber. The results of FTIR are in agreement with the results of the GC–MS Chromatogram study in terms of the presence of nitrocompound, Amide, amine, phenol, alkane, and ester, Table (3-16) to (3-20).

Table 3-16: The peak values, Functional group and bond group of *Ficus carica* latex AgPNs

No	Peak values	Functional group	Group
1	3550.17	Alcohol and hydroxy compound	O-H stretching
2	3475.37	Alcohol and hydroxy compound	O-H stretching
3	3413.97	Alcohol and hydroxy compound	O-H stretching
4	3237.61	Alcohol and hydroxy compound	O-H stretching
5	2930.42	alkene/alkyl)/Methylene (>CH2)	C-H stretching
6	2031.92	Carbonyl compound /Transition metal carbonyls	N=C=S stretching
7	1735.37	Carbonyl compound /Aldehyde	C=O stretching
8	1638.92	Carbonyl compound /Amide	C=C stretching
9	1617.9	Carbonyl compound /Quinone or conjugated ketone	C=C stretching
10	1385.29	Saturated Aliphatic (alkene/alkyl)/Methyl (−CH3)	C-H bending
11	1243.41	Ether and oxy compound /Aromatic ethers, aryl -O stretch	C-N stretching
12	1171.63	Simple hetero-oxy compounds / Organic sulfates	C=O stretching
13	1079.77	Simple hetero-oxy compounds/Silicon-oxy compounds / silicone (Si-O-Si)	(Si-O-Si)
14	1040.06	Ether and oxy compound / Primary amino / Primary amine, CN stretch	CN stretch
15	881.01	Ether and oxy compound / Peroxides, C-O-O-stretch	C-O-O- stretch
16	481.67	Thiols and thio-substituted compounds / Polysulfides (S-S stretch)	(S-S stretch)

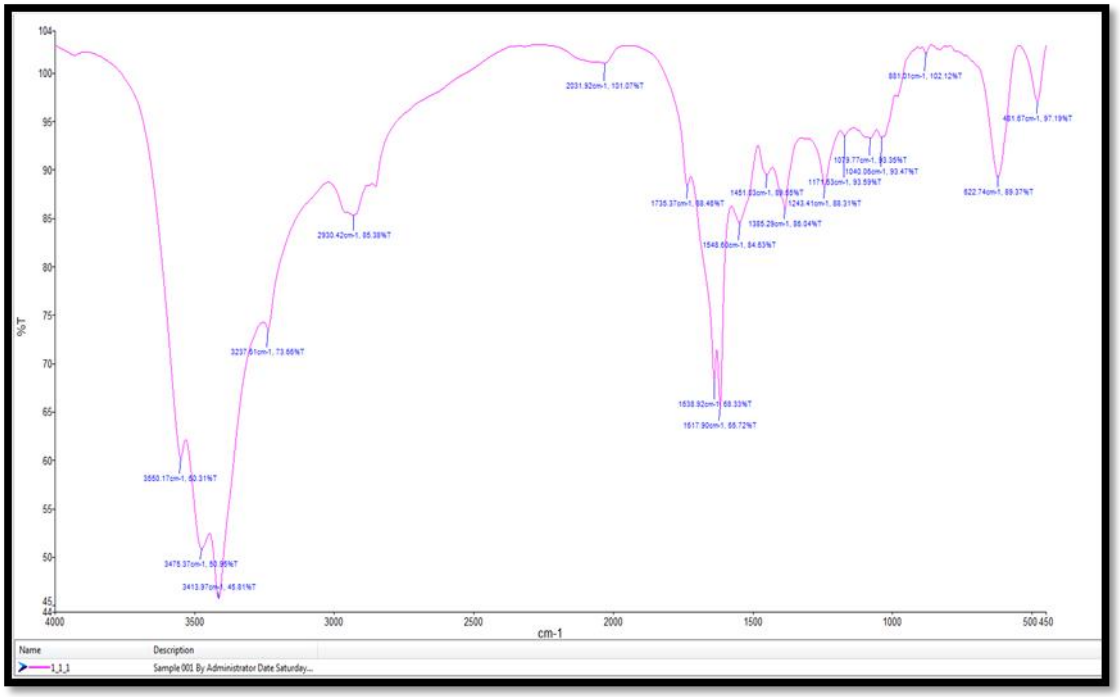


Figure 3-17: Fourier Transform Infrared Spectroscopy of Biosynthesized nanoparticles by *Ficus carica*

Table 3-17: The peak values, Functional group and bond type of *Calotropis procera* latex AgPNs

No	peak values	Functional group	Group
1	3549.46	Alcohol and hydroxy compound	O-H stretching
2	3474.53	Alcohol and hydroxy compound	O-H stretching
3	3414.06	Alcohol and hydroxy compound	O-H stretching
4	3237.99	Alcohol and hydroxy compound	O-H stretching
5	2031.72	Carbonyl compound /Transition metal carbonyls	N=C=S stretching
6	1732.26	Carbonyl compound / Aldehyde	C=O stretching
7	1638.93	Carbonyl compound /Amide	C=C stretching
8	1618.71	Carbonyl compound /Quinone or conjugated ketone	C=C stretching

9	1384.27	Saturated Aliphatic (alkene/alkyl)/gem-Dimethyl or ‘‘iso’’- (doublet)	S=O stretching
10	1246	Ether and oxy compound/	C-N stretching
11	1092.58	Aromatic ethers, aryl -O stretch	C-O stretching
12	1029.44	Aromatic ethers, aryl -O stretch	aryl -O stretch
13	622.05	Thiols and thio-substituted compounds/Disulfides (S-S stretch)	(S-S stretch)
14	482.65	Thiols and thio-substituted compounds/Aryl disulfides (S-S stretch)	(S-S stretch)

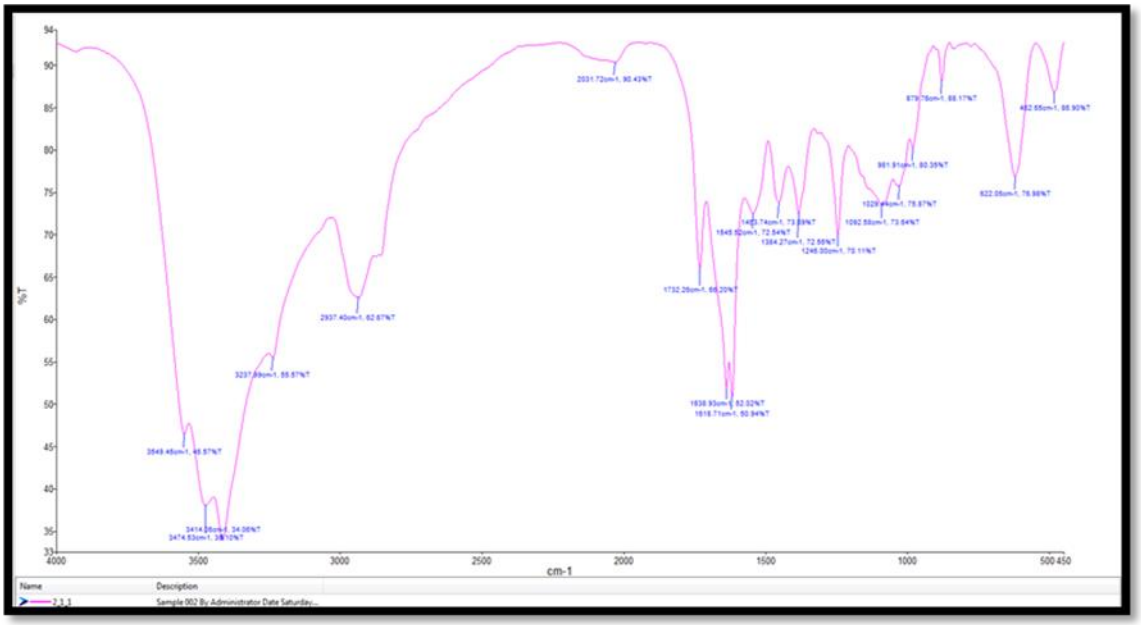


Figure 3-18: Fourier Transform Infrared Spectroscopy of Biosynthesized nanoparticles by *Calotropis procera*

Table (3-18): The peak values, Functional group and bond type of *Nerium oleander* latex AgPNs

No	**peak values**	**Functional group**	**group**
1	3413.29	alcohol	O-H stretching
2	2934.69	amine salt	N-H stretching
3	2876.14	amine salt	N-H stretching
4	1782.36	aromatic compound	C-H bending
5	1739.35	aromatic compound	C-H bending
6	1640.32	conjugated alkene	C=C stretching
7	1544.04	nitro compound	N-O stretching
8	1447.15	unknown	
9	1379.76	alcohol	O-H bending
10	1319.68	phenol	O-H bending
11	1261.44	alkyl aryl ether	C-O stretching
12	1201.98	fluoro compound	C-F stretching
13	1167.99	tertiary alcohol	C-O stretching
14	1098.26	aliphatic ether	C-O stretching
15	1065.04	primary alcohol	C-O stretching
16	1030.11	sulfoxide	C-O stretching
17	894.18	alkene	C=C bending
18	699.35	alkene	C=C bending
19	617.46	halo compound	C-I stretching

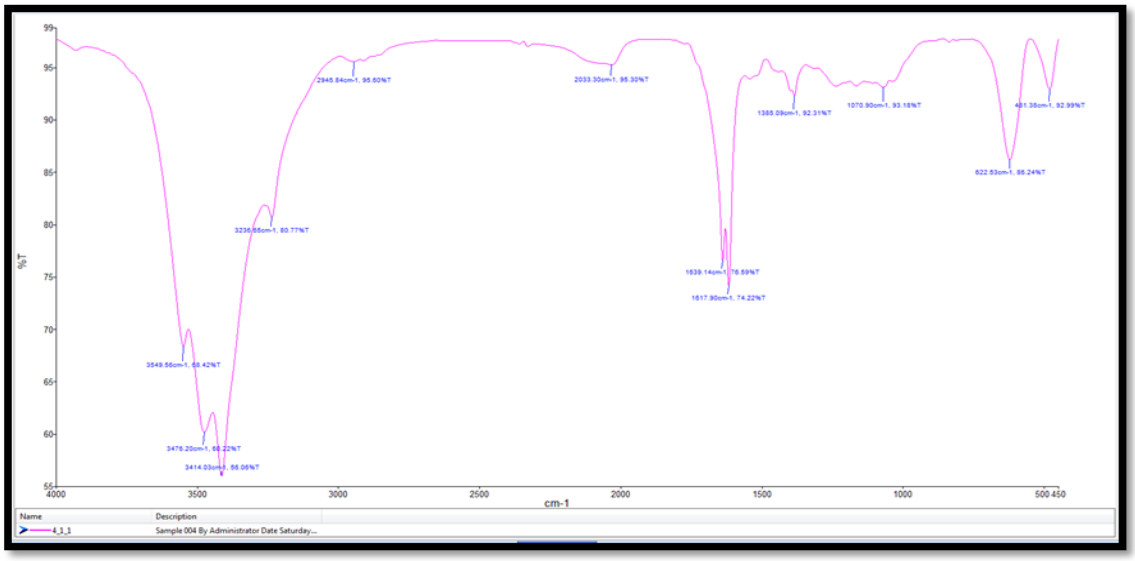


Figure 3-19: Fourier Transform Infrared Spectroscopy of Biosynthesized nanoparticles by *Nerium oleander*

Table 3-19: The peak values, Functional group and bond type of *Ficus elastica* latex AgPNs

No	Peak values	Functional group	Group
1	3549.56	alcohol	O-H stretching
2	3476.2	alcohol	O-H stretching
3	3414.03	alcohol	O-H stretching
4	3236.65	alcohol	O-H stretching
5	2945.84	amine salt	N-H stretching
6	2033.3	isothiocyanate	N=C=S stretching
7	1639.14	conjugated alkene	C=C stretching
8	1617.9	conjugated alkene	C=C stretching
9	1385.09	sulfonyl chloride	S=O stretching
10	1070.9	primary alcohol	C-O stretching
11	622.53	C-I stretching	halo compound
12	481.38	Aryl disulfides(S-S stretch)	Thiols and thio-substituted compounds

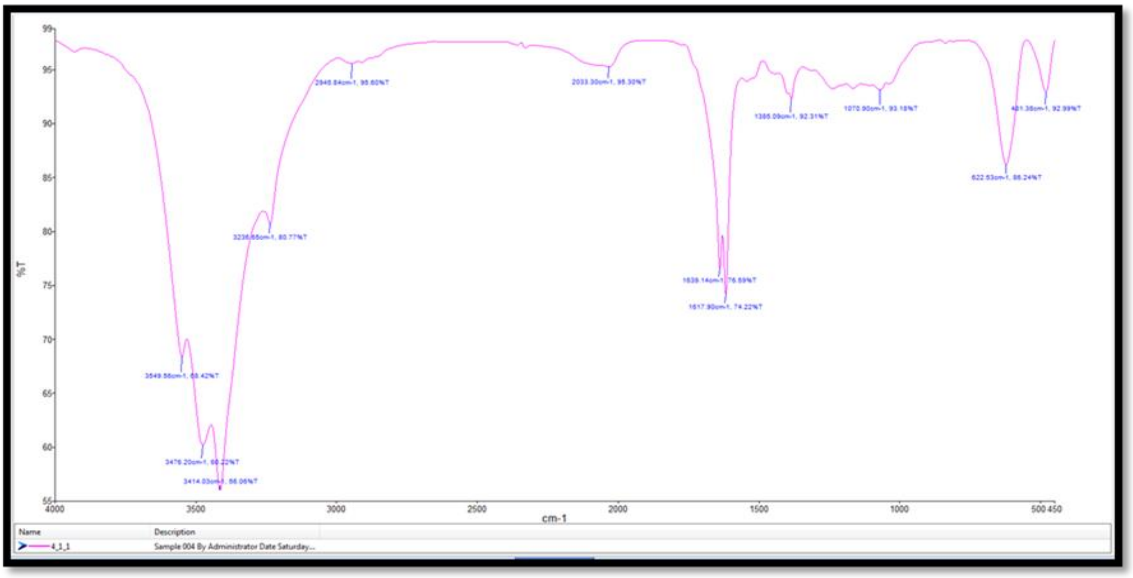


Figure 3-20: Fourier Transform Infrared Spectroscopy of Biosynthesized nanoparticles by *Ficus elastica*

Table 3-20: The peak values, Functional group and bond type of *Euphorbia tirucalli* latex AgPNs

No	peak values	Functional group	Group
1	3549.67	alcohol	O-H stretching
2	3475.12	alcohol	O-H stretching
3	3414.11	alcohol	O-H stretching
4	3238.81	alcohol	O-H stretching
5	2962.87	alcohol	O-H stretching
6	2875.11	alcohol	O-H stretching
7	2032.92	aldehyde	C-H stretching
8	1639.19	alkane	C=C stretching
9	1618.26	conjugated alkene	C=C stretching
10	1547.16	nitro compound	N-O stretching
11	1376.36	phenol	O-H bending
12	1250	aromatic ester	C-O stretching
13	1155.28	tertiary alcohol	C-O stretching

14	1094.79	aliphatic ether	C-O stretching
15	1027.34	amine	C-N stretching
16	837.94	halo compound	C-Cl stretching
17	622.55	halo compound	C-I stretching
18	481.11	Aryl disulfides (S-S stretch)	Thiols and thio-substituted compounds

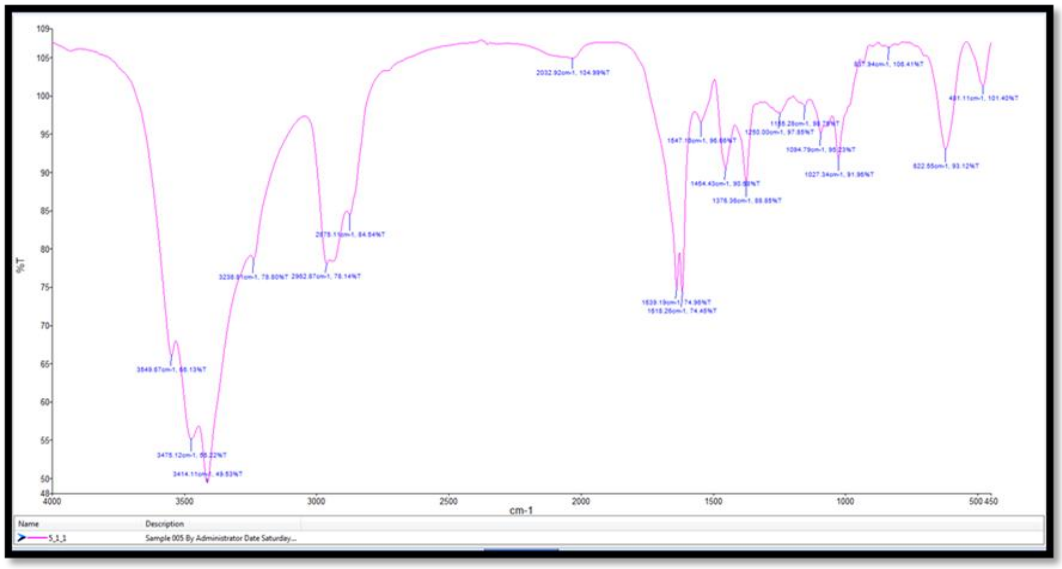


Figure 3-21: Fourier Transform Infrared Spectroscopy of Biosynthesized nanoparticles by *Euphorbia tirucalli*

3.3.2: Biological applications of Ag silver nanoparticles:

3.3.2.1 Antibiofilm effect

Biofilm is the most important feature of bacteria that enhances the binding of bacteria to the surfaces of instruments. 96-well microtiter plate method was applied method, Given *E. coli* and *S.aureus*.capacity to form a biofilm, biosynthesized Ag-NPs were investigated for biofilm inhibition capability against its (Barapatre et al., 2016).

The production of biofilms was significantly reduced by 40–99%. Increases in Ag-NPs concentration reduced the quantity of biofilm development in all situations. The Ag-NPs (at the concentration of 1024μg/ml) decreased the production of biofilms in both gram negative and gram positive bacteria, up to 99% in *Ficus carica*, *Nerium oleander* and *Euphorbia tirucalli* while for silver nanoparticles made by a *Calotropis procera* plant it was 99% against gram positive bacteria , 98% against the gram negative bacteria, but *Ficus elastica* was reached to 98% against both of bacterial species (gram negative and gram positive) as shown in Figures (3-22) to (3-26).

Whereas the lowest concentrations of silver nanoparticles showed a clear discrepancy in the ability to inhibit the formation of biofilm between gram positive and negative bacteria. The inhibition rate at concentration of 16 μg/ml silver nanoparticles prepared by the *Ficus carica* plant against *S.aureus* was 87 %, while it was 40 % for the same concentration and the same plant used to prepare silver nanoparticles, but against *E.coli* bacteria.

So is the case in *Calotropis procera* the inhibit biofilm formation against *S.aureus* was 61% with the concentration of 16 μg/ml while against *E.coli* were 52%, in *Nerium oleander* the percent of inhibition was 53% against *S.aureus*, 40% against of *E. coli*.

The inhibition rate at concentration of 16 µg/ml silver nanoparticles prepared by the *Ficus elastica* against Gram positive and negative bacteria were relatively close where it is 48% against *S.aureus* and 46% against *E.coli*, but it was the complete opposite in *Euphorbia tirucalli* the inhibition rate was highest against gram negative bacterial species and lowest against gram positive bacterial species, where it reached 65% against *E. Coli* and 50% against *S.aureus*. As for the percentage of inhibition of biofilm formation at concentrations (512,256,128,64,32) µg/ml for the same plant species, it ranged from 93 to 99 %, as shown in Figures (3-22) to(3-26).

Thus, the results indicate that silver nanoparticles prepared from the latex of *Ficus carica*, *Calotropis procera* and *Nerium oleander* had a higher effect against gram positive bacteria, while the ratio was highly converged against both gram positive and negative bacteria in *Ficus elastica* which was belong to the same family of *Ficus carica*, Moraceae. In the *Euphorbia tirucalli*, the highest effect was against gram-negative bacteria, unlike what happened in other plant species.

The differences in inhibitory action between the five Ag-NPs can be attributed to a variety of variables, including antibacterial effectiveness and physical properties such nanoparticle size,which affects restricted penetration, and other chemical properties such as material-biofilm affinity (Park et al., 2013).

Goswami et al. (2015) discovered that AgNPs mediated biofilm eradication inhibited *S. aureus* by 89 % and *E. coli* by 75 % At 15 mg/ mL. The results of this work show that silver nanoparticles may efficiently and quickly detach biofilms generated by *E. coli* and S. aureus at clinically feasible silver nanoparticle concentrations. that means these AgNPs could be used as biofilm disruptors.

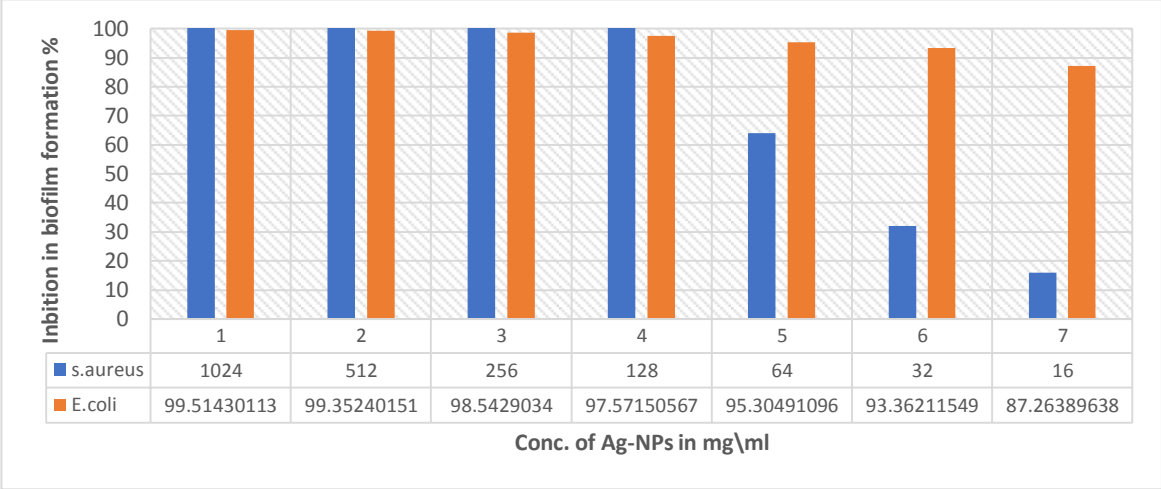


Figure 3-22: Determination of percent antibiofilm inhibition of *Ficus carica* synthesized silver nanoparticles (Ag-NPs) on *S. aureus*, and *E. coli*, by microtiter plate method

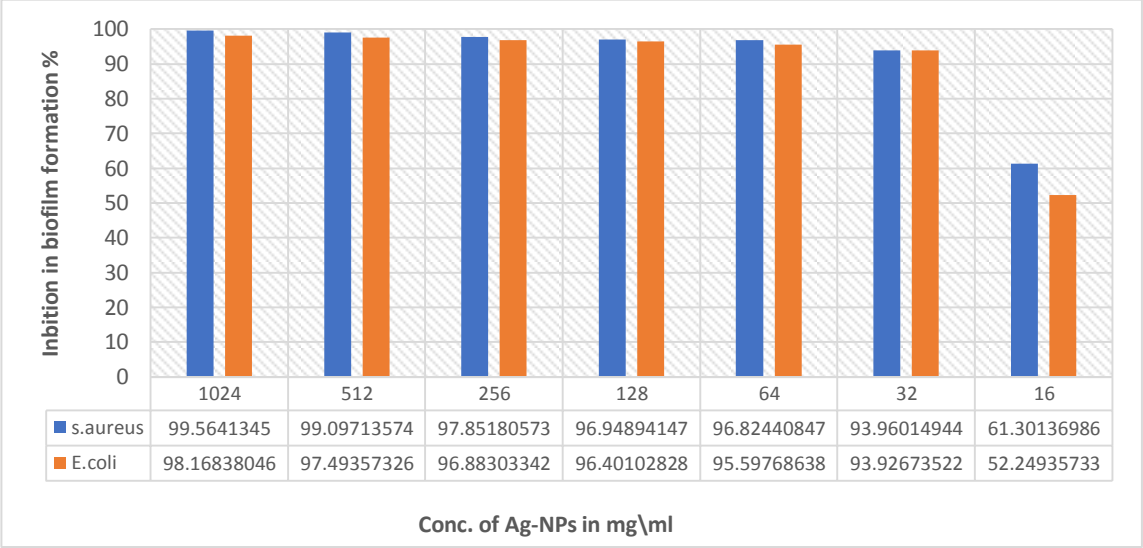


Figure 3-23: Determination of percent antibiofilm inhibition of *Calotropis procera* synthesized silver nanoparticles (Ag-NPs) on *S.aureus*, and *E.coli*, by microtiter plate method.

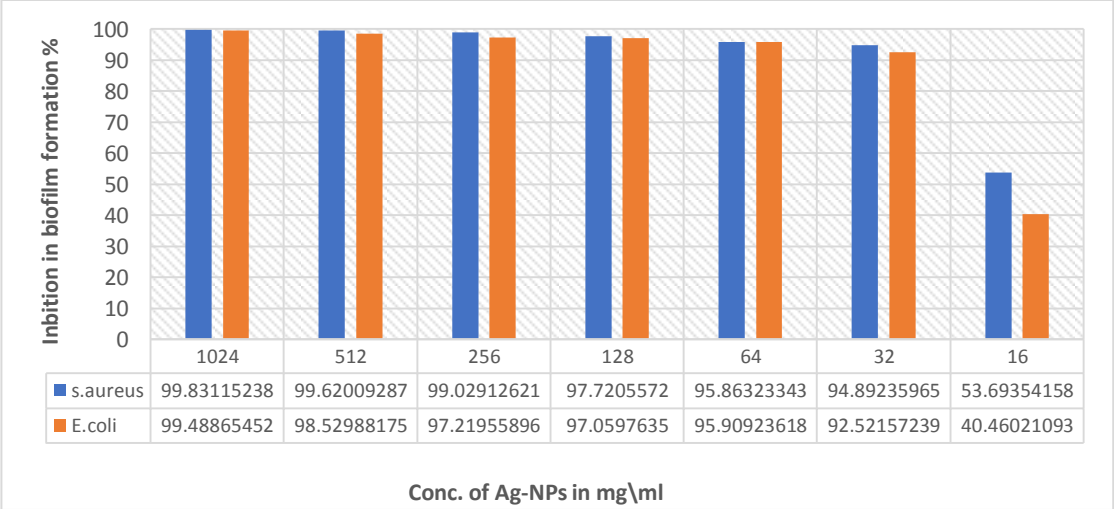


Figure 3-24: Determination of percent antibiofilm inhibition of *Nerium oleander* synthesized silver nanoparticles (Ag-NPs) on *S.aureus*, and *E.coli*, by microtiter plate method

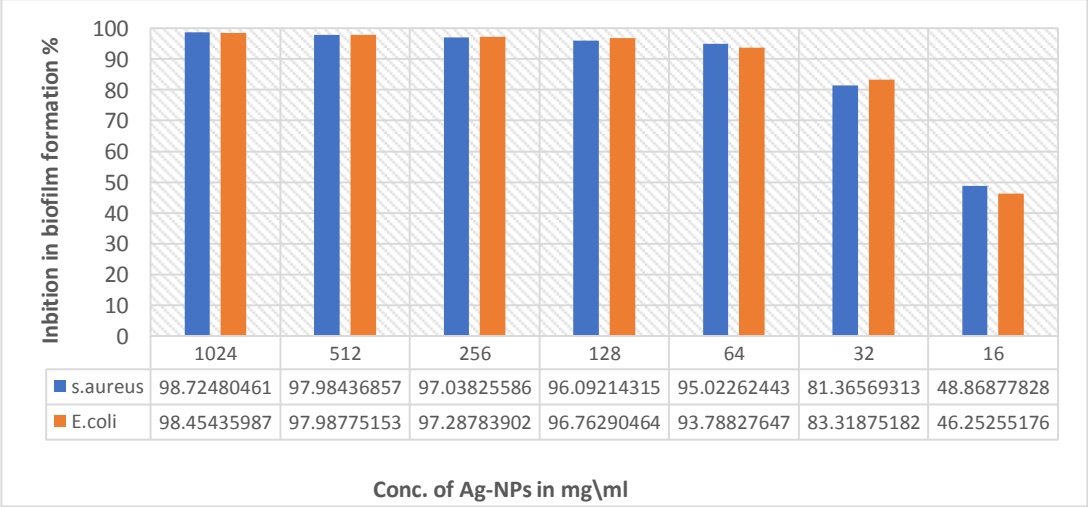


Figure 3-25: Determination of percent antibiofilm inhibition of *Ficus elastica* synthesized silver nanoparticles (Ag-NPs) on *S.aureus*, and *E.coli*, by microtiter plate method.

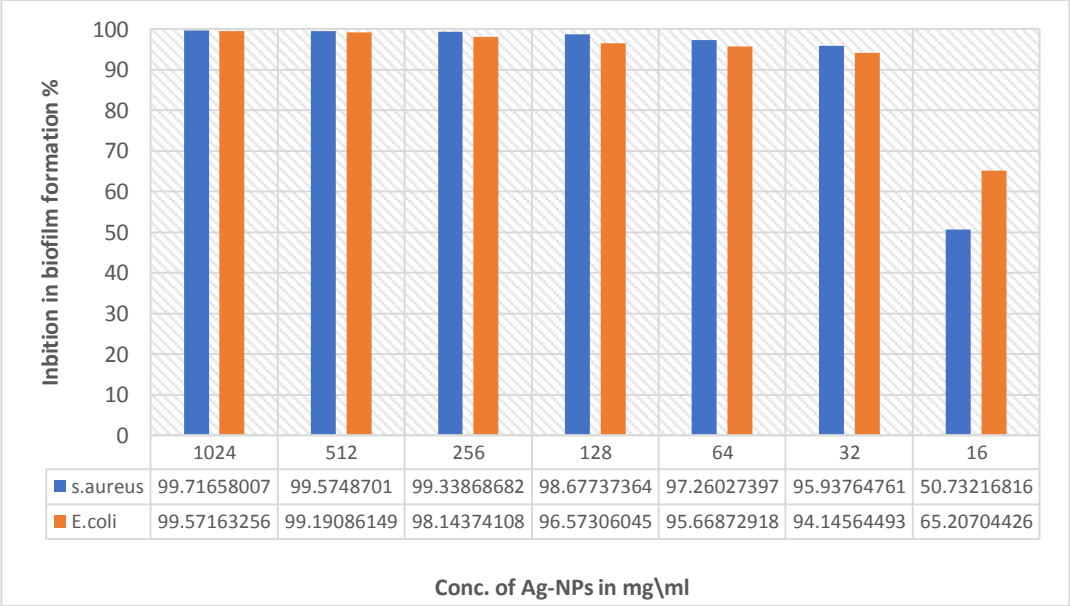
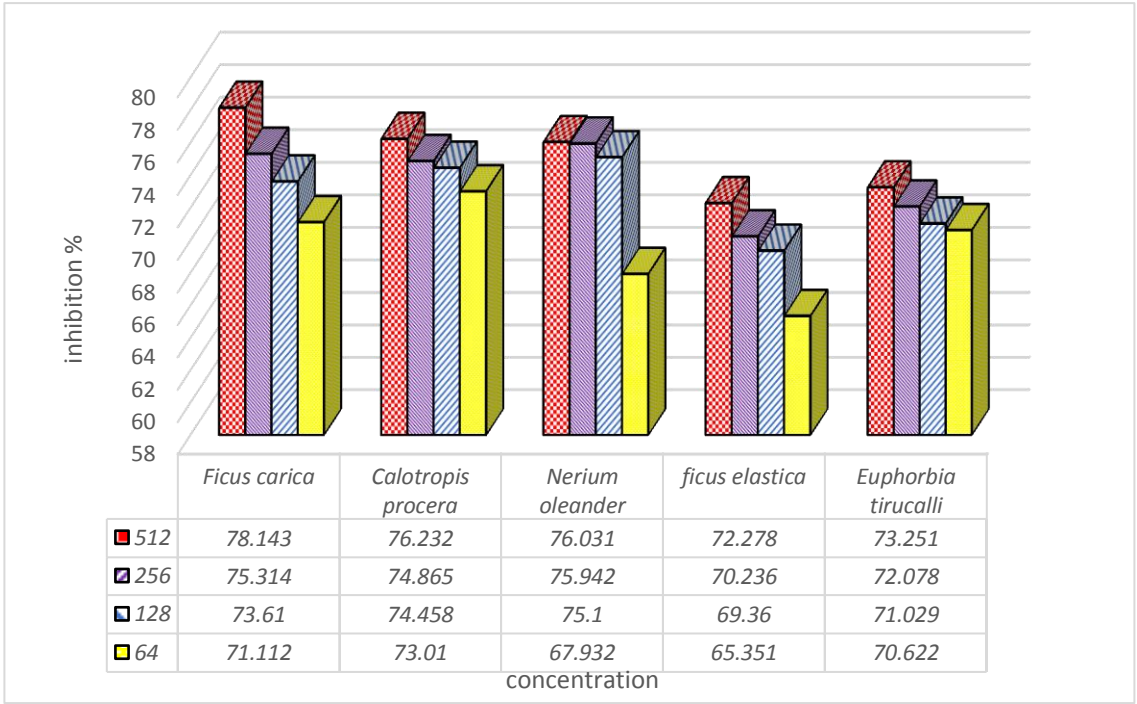


Figure 3-26: Determination of percent antibiofilm inhibition of *Euphorbia tirucalli* synthesized silver nanoparticles (Ag-NPs) on *S.aureus*, and *E.coli*, by microtiter plate method.

3.3.2.2 Antioxidant Effect

The antioxidant activity of the AgNPs biosynthesized from latex was determined in vitro by decreasing DPPH free radicals using the (1-Diphenyl-2-picrylhydrazyl DPPH) free radicals scavenging assay. After 30 minutes of introducing nanoparticle concentrations of 64, 128, 256, and 512 g/ml to The absorbance of a DPPH solution was measured at 517 nm.

The results revealed that nanoparticles have the potential to scavenge DPPH free radicals, as seen by the color change. The ability of nanoparticles to reduce DPPH improved as the concentration of biogenic latex AgNPs increased, and it is ranged between 65% which is the lowest percentage in 64 µg/ml in *Ficus elastica* and 78% which is the highest percentage in 512µg/ml in *Ficus carica*, While the other percentage of nanoparticles to reduce DPPH were distributed as shown in Figure (3 -27).



	Ficus carica	Calotropis procera	Nerium oleander	ficus elastica	Euphorbia tirucalli
512	78.143	76.232	76.031	72.278	73.251
256	75.314	74.865	75.942	70.236	72.078
128	73.61	74.458	75.1	69.36	71.029
64	71.112	73.01	67.932	65.351	70.622

Figure 3-27: Percentage of nanoparticles to reduce DPPH

Due to higher concentrations of phytochemical substances, all five species of latex-producing plants that were used in this study have antioxidant potential. Due to the presence of a variety of bioactive phytochemical substances, they play an important function in medicine such as polyphenols, phenolic acids, flavonoids, anthocyanins, glycosides, carotenoids, and several water-soluble vitamins are the main phytochemicals found in these species Table (1-1, 2, 3, 4 and 5). In addition to the results shown in the analysis of the five plant extracts of the species in this study using GC-MS technology, which showed the presence of glycoside (Table 3-1), phenol and oleic acid (Table 3-2), and some other compounds with antioxidant properties shown in Table (3-7).

Because of the presence of these phytochemicals, these species are considered therapeutic plants with the wide ranges of many biological functions, including antioxidant activity. Latex from these plants can be utilized to regulate oxidative stress and cure a variety of ailments because of its high antioxidant capacity.

3.3.2.3 Hemolysis Effect

Because all materials that enter the bloodstream come into touch with RBCs, it's critical to assess the biomaterials' hemolytic capacity, less than 5% hemolysis is considered normal, according to the American Society for Testing and Materials (ASTM) (Luna-Vázquez, *et al*., 2021). In this study, hemolysis was detected by using Triton X-100 as an indicator of positive control. A sterile solution of phosphate buffer saline was used as a negative control that could store the stock solution at room temperature.

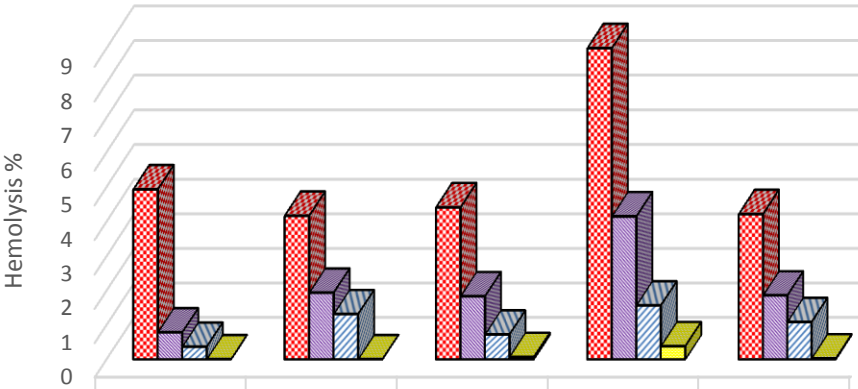
Latex AgNPs with all concentrations (64, 128, 256, 512 μg/ml) show a hemolysis rate ranging from (0-4.9) for the whole blood tested, which is

considered null, except for the first concentration in all plant species, which is the highest concentration which is the value of 512 μg/ml, the percentage of hemolysis, as it was very high in *Ficus elastic* equal to 9 % and less than the *Ficus carica* equal to 4.9%, while it was 4.1% in *Calotropis procera*, 4.4% in *Nerium oleander* and 4.2% in *Euphorbia tirucalli.*

In the second concentration 256 μg/ml the rate of hemolysis was 4.1% in *Ficus carica* were 0.78%, in *Ficus carica* and *Ficus elastica*, 1.9% in *Calotropis procera, Nerium oleander* and *Euphorbia tirucalli*.

The third concentration 128 μg/ml appeared at the rate 0.36% in *Ficus carica, Calotropis procera* 1.3%, *Nerium oleander* 0.7%, *Ficus elastica* 1.5% and *Euphorbia tirucalli* 1%, while it was 0% in all species at the lowest concentration 64 μg/ml as shown in Figure (3-28).

This result shows that there is a very high convergence of the plant species in their percentage of hemolysis, except for *Ficus elastica* where the rates of hemolysis were high as show in Figure (3-28).



	Ficus carica	Calotropis procera	Nerium oleander	Ficus elastica	Euphorbia tirucalli
512	4.927014441	4.165290621	4.409473356	9.019698191	4.213461085
256	0.782686964	1.936096433	1.834683702	4.146987674	1.857378465
128	0.363949438	1.312747688	0.72062948	1.566639788	1.083798005
64	0	0	0.066220006	0.380140537	0.023560826

Figure 3-28: Percent of hemolysis of the latex AgNPs

In terms of hemolysis susceptibility, the rate of hemolysis of *Ficus carica* is 4.9, which is very close to 5% which is considered hemolytic. *Ficus elastica* has a very high hemolysis rate (9%) and it is definitely hemolytic Also For concentrations (256, 128, and 64) µg/ml there is a difference between the two species of plants belonging to the same family they are *Ficus carica* and *Ficus elastica*, which belong to the family Moraceae, where the percentage of hemolysis was extreme as a minimum in *Ficus carica* and extreme as the upper limit in *Ficus elastica*.

It was observed that the average sizes of nanoparticles prepared from both plant species in this study were fairly similar, with the average particle size in *Ficus carica* being 46.6 and 47.8 in *Ficus elastica* table (3-13). These similarities and differences may be attributed to the different chemical compounds or chemical content of each latex of both plant types, as is clear from the results of the GC - MS analysis in this study Tables (3-1) and (3-4).

3.3.2.4Antibacterial Effect

Nanotechnology has a wide range of health-related uses,biomedical sciences, medication and gene delivery, cosmetics, and food since it is a multidisciplinary study field (Ojha and Bora, 2017). From this point of view, the anti-activity of positive and negative bacteria against nanoparticles from the latex-producing plants' understudy was applied. This study similar to previous studies that were conducted using nanoparticles composed of latex for plant species, including, *Hevea brasiliensis* Müll.Arg. (Santos *et al.,* 2018), *Euphorbia antiquorum* L. (Rajkuberan *et al.*, 2017) and *Calotropis gigantea* L. (Rajkuberan *et al.,* 2015). The results of the antibacterial activity of silver nanoparticles of four concentrations (100, 50, 25, 10 µg/ml) against the four bacterial species used in this study (gram negative bacteria *Escherichia coli* and *Klebsiella pneumoniae)* and (gram positive bacteria *Staphylococcus aureus* and *Enterococcus faecalis)* revealed activity against all bacterial species used in this study, with different inhibition zone diameters, This activity is also attributed to the presence of chemical compounds with anti-bacterial properties that were detected in the latex of the five plant species under study when conducting GC-MS analysis as show in Tables (3-1, 2, 3, 4 and 5).

The result show the greatest inhibition zone was seen against gram-positive bacteria specifically *E.faecalis* by using *Ficus carica* AgNPs with size 46.6nm and 100 µg/ml concentration where it was 22mm Figure (3-29, A), Table (3-21). Thus, silver nanoparticles prepared by latex of *Ficus carica* have the highest effect, followed by silver nanoparticles prepared by latex of *Calotropis procera* and against the same type of bacteria, which is, *E.faecalis*

where diameters of inhibition zone were (20 mm) by using *Calotropis procera* AgNPs with size 45nm and100 µg/ml concentration.

In gram negative bacteria (*E.coli* and *K.pneumoniae*) the greatest diameters of inhibition zone were equal (20mm) by using *Calotropis procera* AgNPs with 100 µg/ml concentration, Table (3-21), Figure (3-30, A, C), but the smallest diameters of the inhibition zone were (10 mm) in both of gram positive and negative bacteria, and also there was different measurements ranging between the highest and lowest diameter of the inhibition zone shown in Table (3-21), Figure (3-29) to (3-33).

It is clear from the results that the effect of silver particles prepared by *Ficus carica* latex was strong against gram positive bacteria, while *Calotropis procera* showed stronger activity against gram negative bacteria. These results agreed with related studies of synthesizing nanoparticles using latex extracts (Mohamed *et al*., 2014) which showed that AgNPs exhibited strong antibacterial activity against gram negative bacteria.

Moreover, de Souza *et al*. (2005) pointed to promising results of the antimicrobial activities of coumarins (which is present in the latex of *Ficus carica*), as reported to have several studies conducted to study the antimicrobial activity of *Ficus carica* latex extracts against multiple kinds of bacteria, such as *E.faecalis* (Al-Sabawi, 2010) and *Staph. aureus* (Rashid *et al*., 2014). According to many theories Ag-NPs may adhere to the surface of cell membrane, altering the cells permeability and respiration functions, and also the significant antibacterial activity of nanoparticles is due to their large surface area, which provides more surface area for contact with organisms

than big particles. Furthermore, Ag-NPs may interact not only with the membrane's surface but also with the bacteria inside (Sahayaraj, 2011).

Different types of nanoparticles have been utilized using various techniques to combat microbial resistance, with the produced silver nanoparticles demonstrating efficacy against a wide range of clinical bacteria strains (Saleh, 2020).

Finally,It is clear from the above that there may be no correlation between the effect of silver nanoparticles with the characteristics of some bacterial species such as the positive and negative nature of Gram stain due to differences in the cell wall such as its thickness due to the peptidoglycan layer as found by other researchers such as (Kim, et al., 2007)and (Sondi and Salopek ,2004).From this it was believed that the activity of nanoparticles against bacteria results from an analogous as a point of a specific enzyme or protein or the nature of the plasma membranes, and this belief was confirmed by Patil,et al.,(2012).

Table 3-21: Diameters of inhibition zone for each type of latex AgNPs against four species of bacteria

Plant species	Bacterial species	Concentration / diameter of inhibition zone (mm)			
		100 µg/ml	50 µg/ml	25 µg/ml	10 µg/ml
Ficus carica	*E.coli*	18	12	10	0
	K.pneumoniae	18	13	10	0
	Staph.aureus	16	10	0	0
	E.faecalis	22	17	13	0
Calotropis procera	*E.coli*	20	15	10	0
	K.pneumoniae	20	13	0	0
	Staph.aureus	18	12	0	0
	E.faecalis	16	14	10	0
Nerium oleander	*E.coli*	18	13	10	0
	K.pneumoniae	17	12	0	0
	Staph.aureus	16	10	0	0
	E.faecalis	20	14	10	0
Ficus elastica	*E.coli*	18	14	10	0
	K.pneumoniae	14	12	0	0
	Staph.aureus	15	10	0	0
	E.faecalis	18	14	0	0
Euphorbia tirucalli	*E.coli*	15	0	0	0
	K.pneumoniae	17	12	0	0
	Staph.aureus	16	0	0	0
	E.faecalis	18	12	10	0

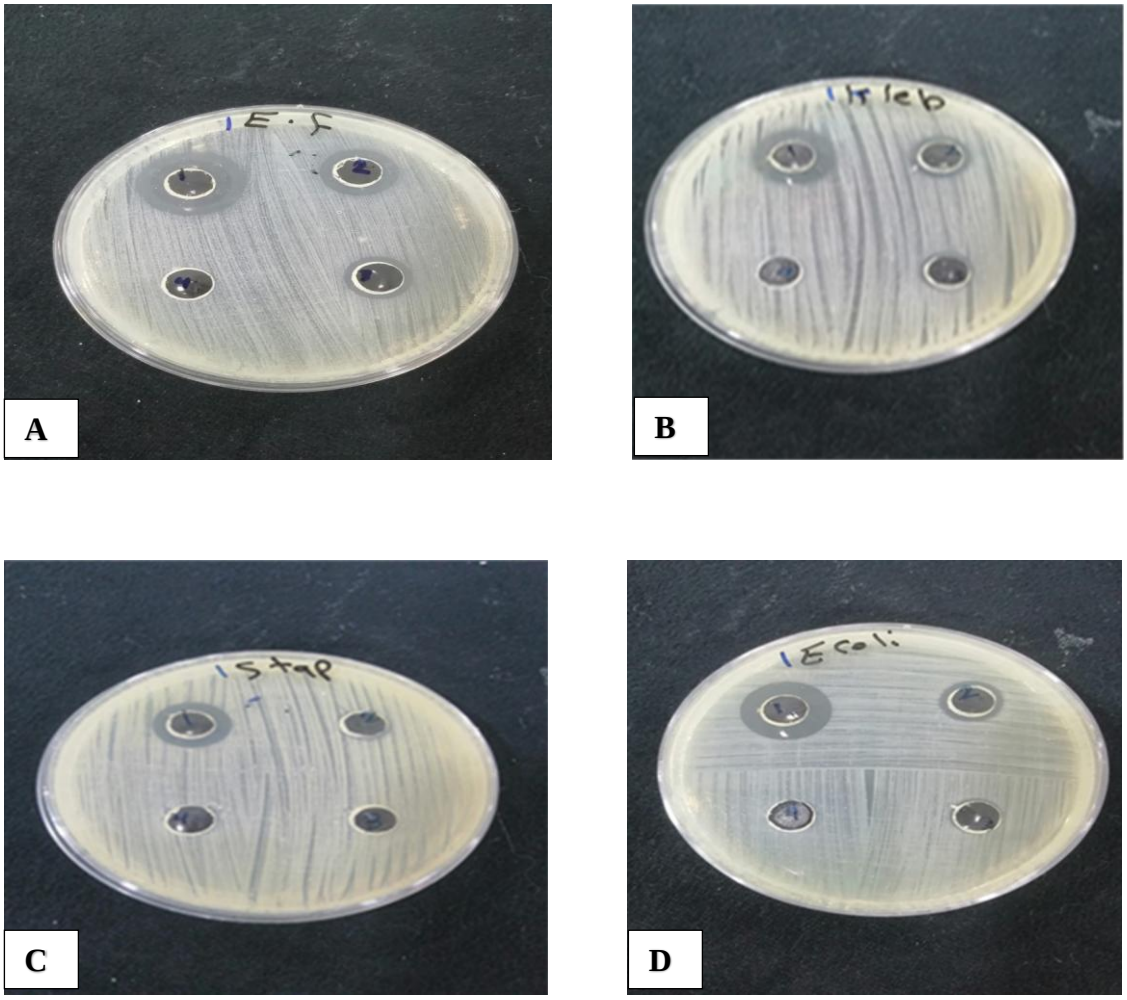


Figure 3-29: The Antibacterial activity of AgNPS produced from the latex of *Ficus carica* (with four concentrations 1.100 , 2.50 , 3.25 , 4.10) µg/ml against A- *Enterococcus faecalis*, B- *Klebsiella pneumoniae*, C- *Staphylococcus aureus*, D- *Escherichia coli*

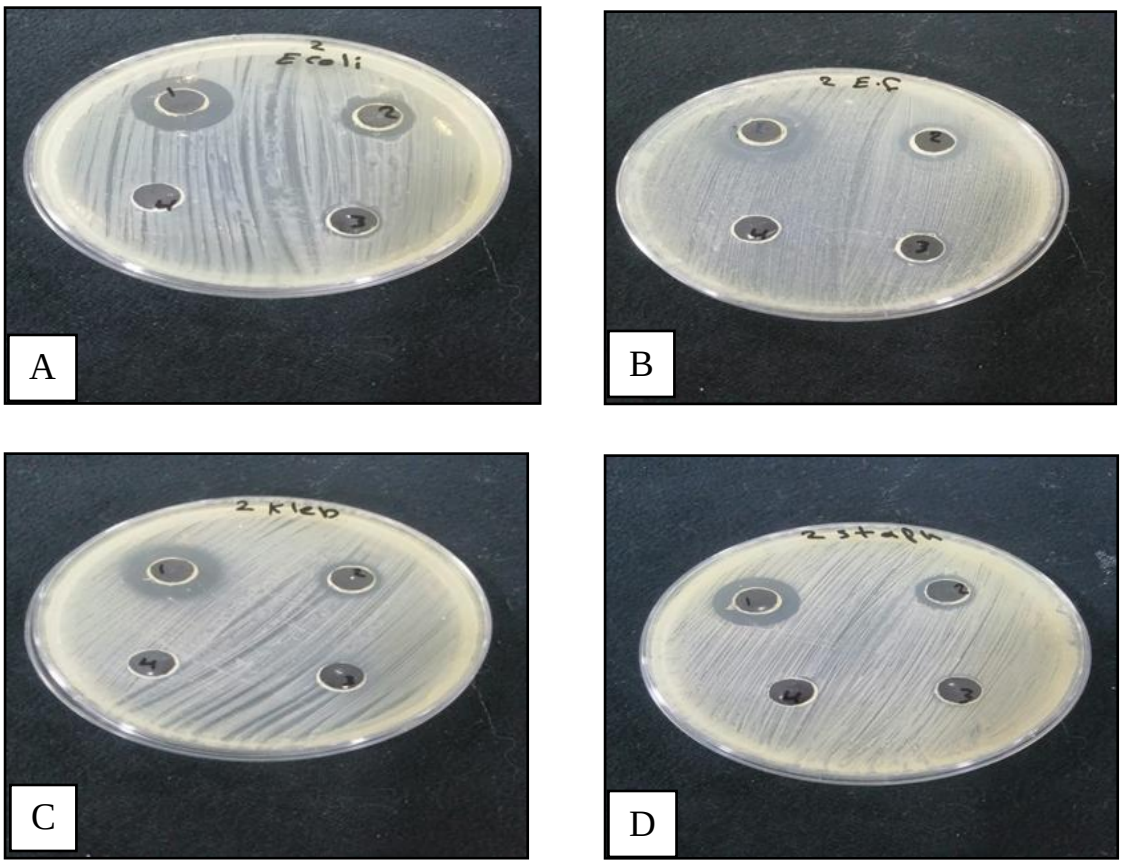


Figure 3-30: The Antibacterial activity of AgNPS produced from the latex of *Calotropis procera* (with four concentrations 1.100 , 2.50 , 3.25 , 4.10) µg/ml against A- *Escherichia coli*, B- *Enterococcus faecalis*, C- *Klebsiella pneumoniae*, D- *Staphylococcus aureus*.

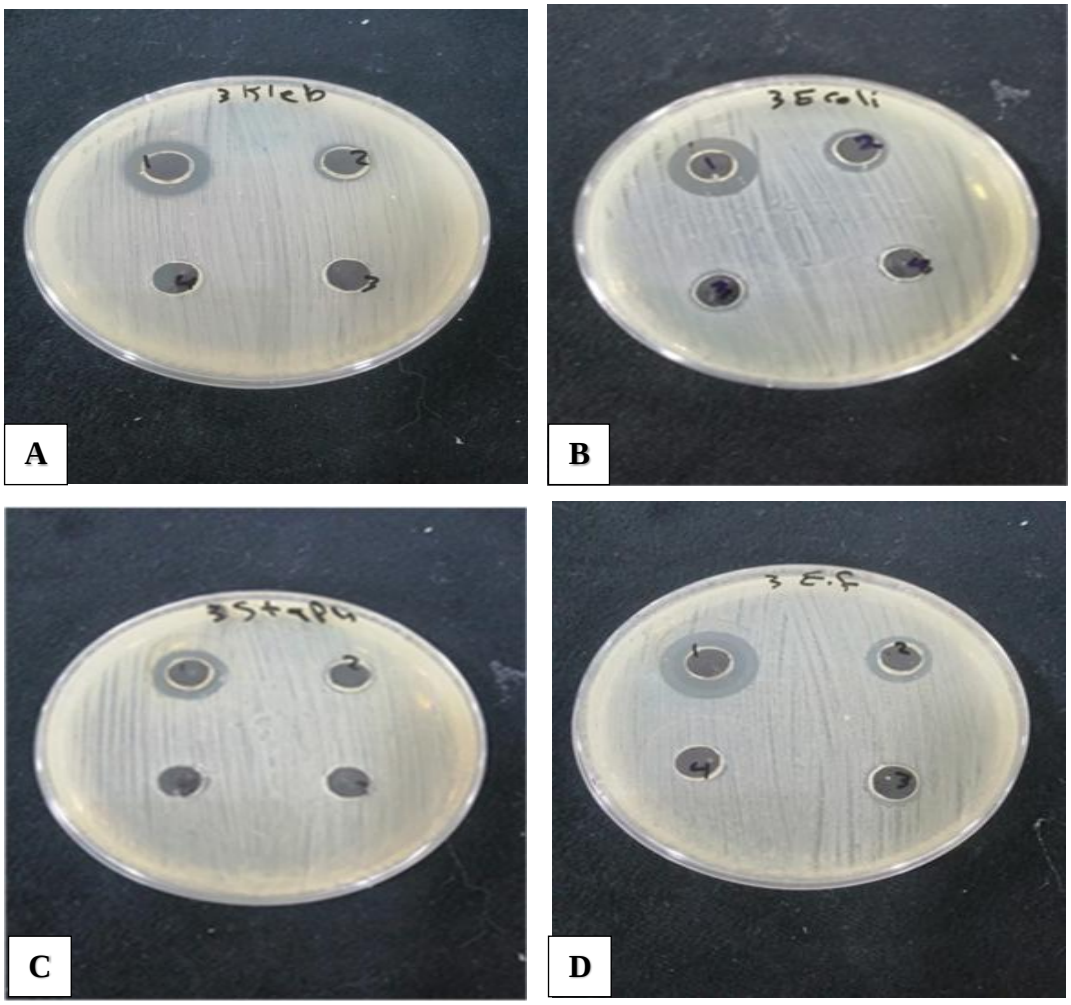


Figure 3-31: The Antibacterial activity of AgNPS produced from the latex of *Nerium oleander* (with four concentrations 1.100 , 2.50 , 3.25 , 4.10) µg/ml against A- *Klebsiella Pneumoniae,* B- *Escherichia coli,* C- *Staphylococcus aureus,* D- *Enterococcus faecalis*

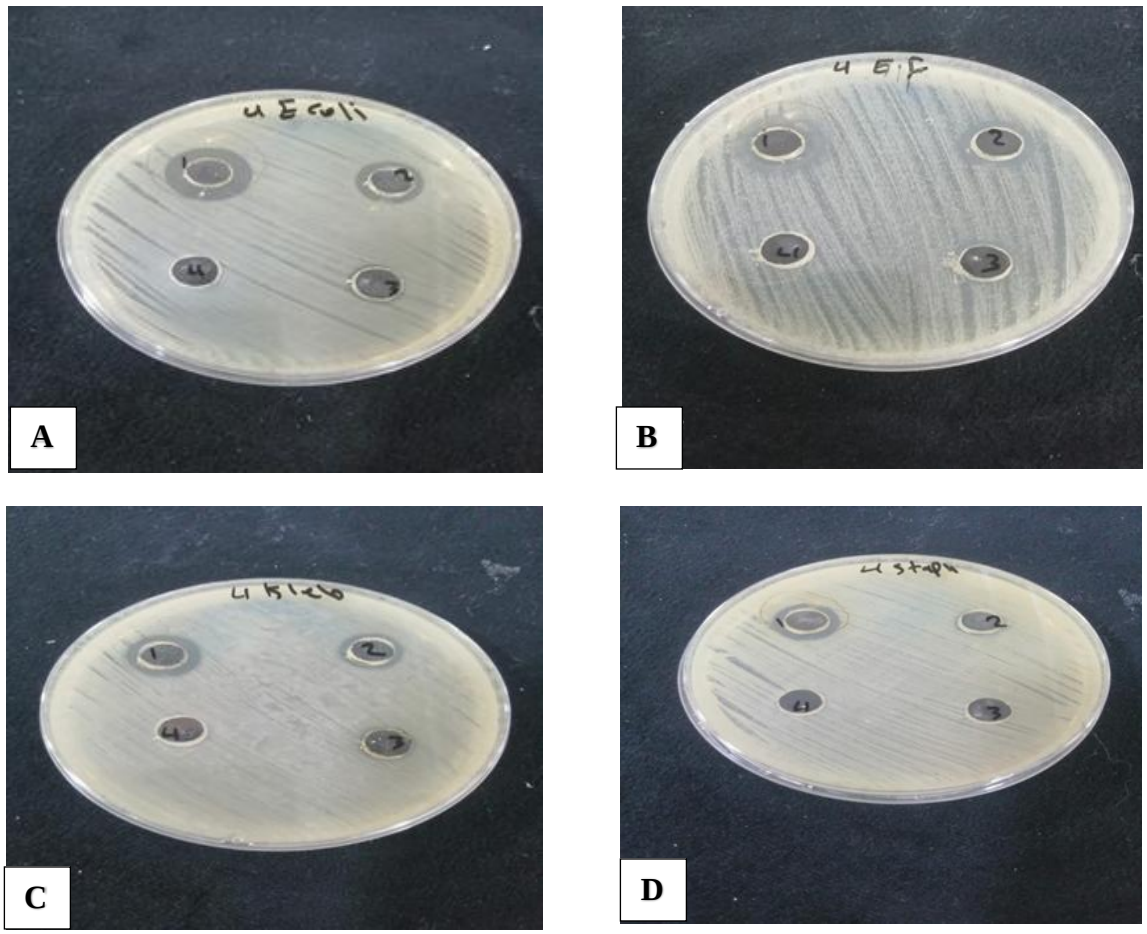


Figure 3-32: The Antibacterial activity of AgNPS produced from the latex of *Ficus elastica* (with four concentrations 1.100 , 2.50 , 3.25 , 4.10) μg/ml against A- *Escherichia coli,* B- *Enterococcus faecalis*, C- *Klebsiella Pneumoniae*, D- *Staphylococcus aureus*

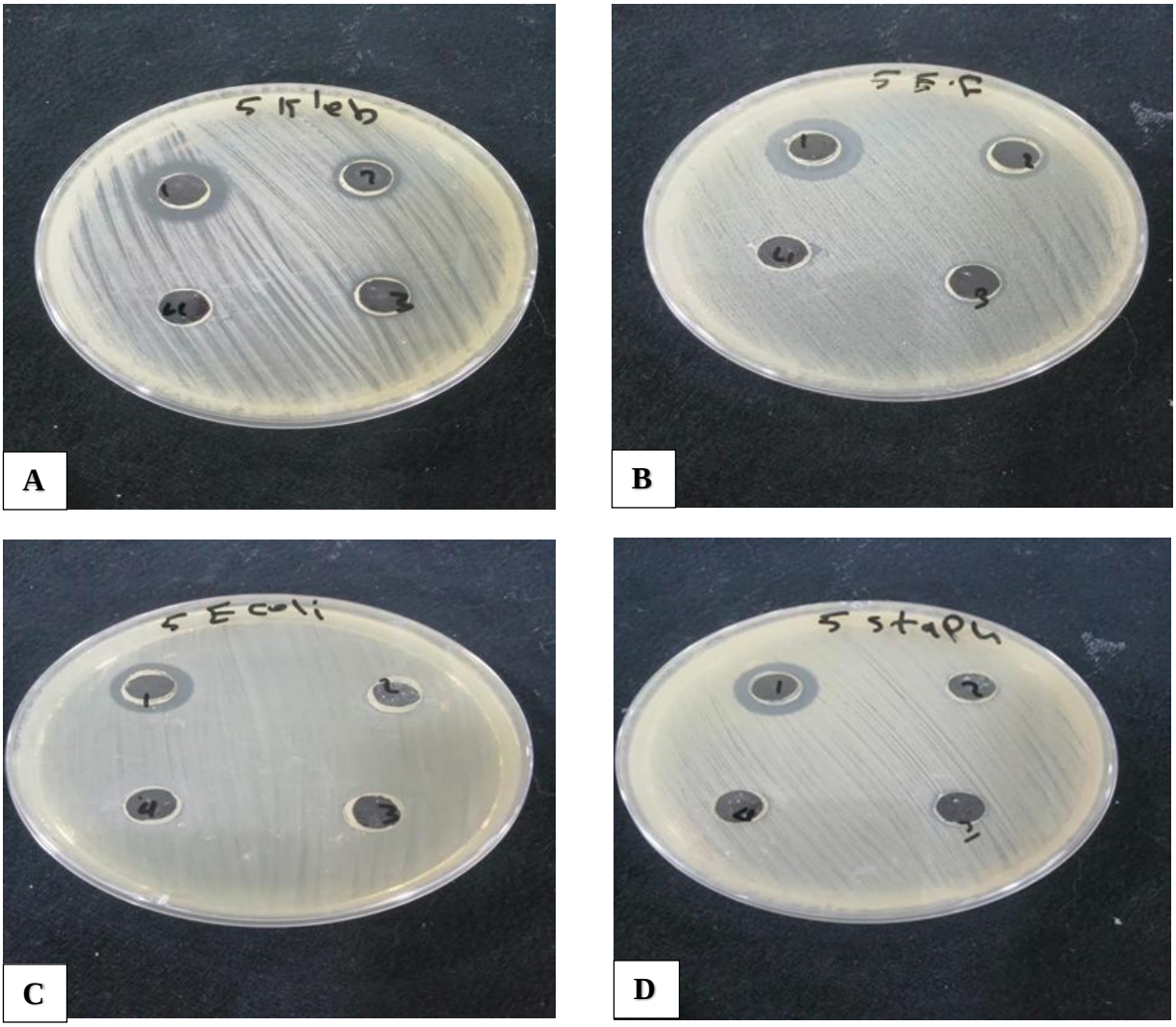


Figure 3-33: The Antibacterial activity of AgNPS produced from the latex of *Euphorbia tirucalli* (with four concentrations 1.100 , 2.50 , 3.25 , 4.10) µg/ml against A- *Klebsiella pneumoniae,* B- *Enterococcus faecalis*, C- *Escherichia coli*, D- *Staphylococcus aureus*

3.3.2.5 Minimum Inhibitory Concentration (MIC):

The preliminary investigation in screening the antibacterial activity of latex AgNPs used the Well diffusion technique to determine the inhibition zone; consequently, a further assessment in identifying the antibacterial activity of AgNPs utilizing MIC value was required.

The MIC of latex AgNPs was quantified by measuring the O.D at 600 nm and calculating the MIC values of all the latex AgNPs (with seven concentrations of 512, 256, 128, 64, 32, 16, 8 µg/ml) for each of the five plant species under study tested against four bacterial species (*Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus*, *Enterococcus faecalis*).

The results are shown in Table (3-22) and Figure (3-34) represent the distribution of the average concentration of AgNPs *Ficus carica*, where the first concentration was 512µg/ml the strongest inhibitory effect, while the lowest inhibitory concentration was 16 µg/ml, where the P-value is 0.001 which represented high significant for all concentrations except the concentration of 8 µg/ml was very close to results of the control. This was confirmed when the statistical study was conducted, as there was no significant difference (P-value = 0.173) between the value of the last concentration and the control.

Table 3-22: Value of absorbance in latex AgNPs of *Ficus carica*

Conc. / **species**	**512 µg/ml**	**256 µg/ml**	**128 µg/ml**	**64 µg/ml**	**32 µg/ml**	**16 µg/ml**	**8 µg/ml**	**control**
S. aureus	0.0028	0.0059	0.0094	0.0123	0.0345	0.1432	0.2243	0.2312
E. faecalis	0.0032	0.0067	0.0078	0.0089	0.0132	0.0432	0.2943	0.3051
E. coli	0.0021	0.0047	0.0069	0.0092	0.0122	0.0642	0.1932	0.1932
K. pneumoniae	0.0012	0.0039	0.0074	0.0098	0.0153	0.0472	0.2413	0.2742

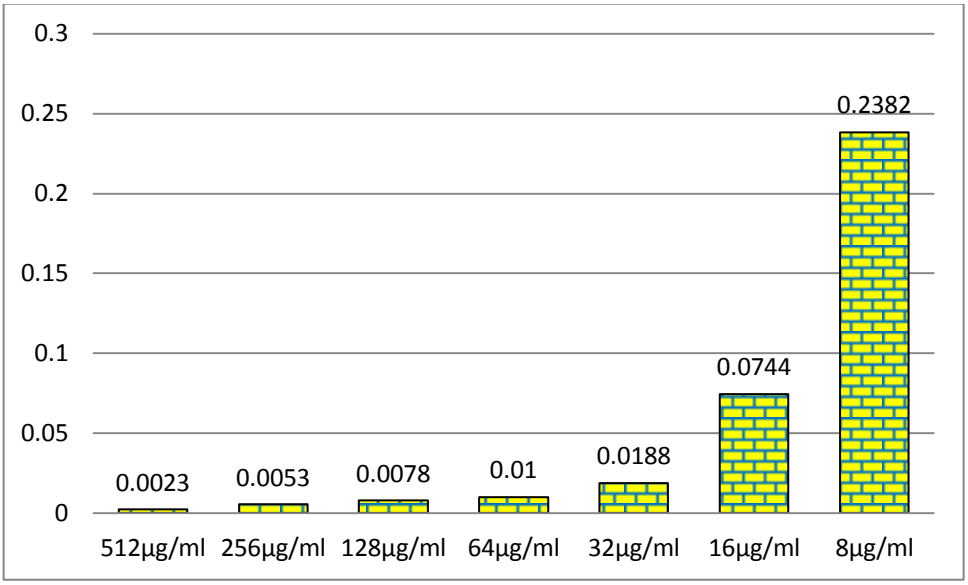


Figure 3-34: Distribution of mean of concentration of latex AgNPs *Ficus carica*

As shown in Figure (3-35) data represents the distribution of the mean concentration according to the kind of bacteria treated with latex AgNPs of *Ficus carica*, where the strongest effect of silver nanoparticles prepared against *E. coli*, followed by *K.pneumoniae* with a rate of 0.0376 and 0.0465 respectively, then *E. faecalis* at a rate of 0.0539 and finally *S. aureus* with a

rate of 0.0617. But when conducting the statistical study, there were no significant differences, as the P-value was equal to 0.953.

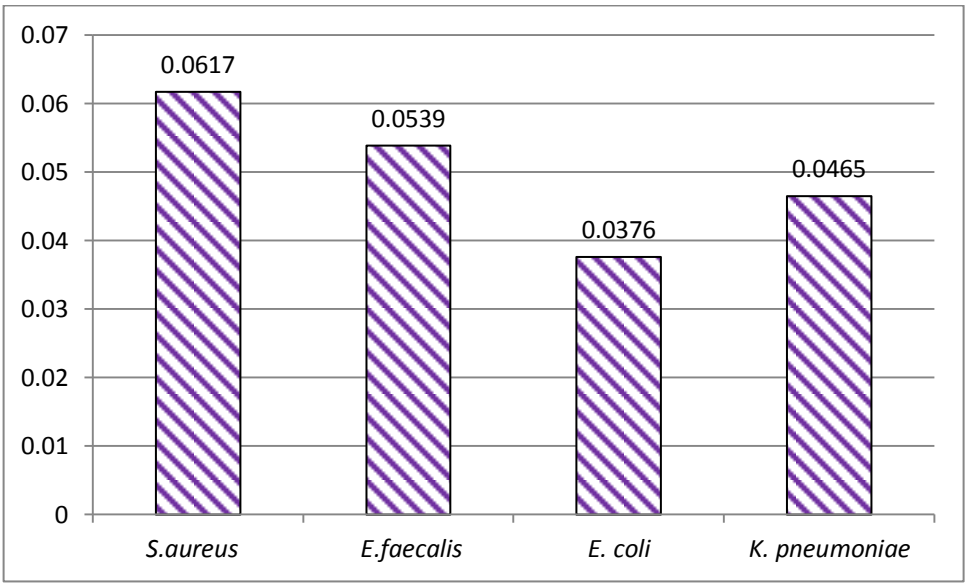


Figure 3-35: distribution of the mean concentration according to bacteria treated with latex AgNPs of *Ficus carica*

Table (3-23) show significant and highly significant differences between the control group and all concentrations except a concentration of 8µg/ml were not found a significant difference at a P-value ≤ 0.05. Therefore, the differences between concentrations within *Ficus carica* show a highly significant difference but differences among bacteria within *Ficus carica* show non-significant differences

Table 3-23: Distribution of different concentrations according to bacteria treated with latex AgNPs of *Ficus carica*.

Conc. / **species**	**Control**	**512 µg/ml**	**256 µg/ml**	**128 µg/ml**	**64 µg/ml**	**32 µg/ml**	**16 µg/ml**	**8 µg/ml**	**Mean**	**SD**	**P-value (ANOVA)**
S. aureus	0.2312	0.0028	0.0059	0.0094	0.0123	0.0345	0.1432	0.2243	0.0617	0.08715	0.953 (N.S)
E. faecalis	0.3051	0.0032	0.0067	0.0078	0.0089	0.0132	0.0432	0.2943	0.0539	0.10685	
E.coli	0.1932	0.0021	0.0047	0.0069	0.0092	0.0122	0.0642	0.1932	0.0376	0.05985	
K. pneumoniae	0.2742	0.0012	0.0039	0.0074	0.0098	0.0153	0.0472	0.2413	0.0465	0.08723	
Mean	0.250	0.0023	0.0053	0.0078	0.0100	0.0188	0.0744	0.2382			
SD	0.0489	0.0008	0.0012	0.00108	0.00154	0.01054	0.04672	0.04232			
P-value (t-test)	-	0.002 (H.S)	0.002 (H.S)	0.002 (H.S)	0.002 (H.S)	0.003 (H.S)	0.023 (Sig.)	0.173 (N.S)			
P-value (ANOVA)	-	0.001 (H.S)									

Sig = significance, N.S = non significance, H.S= highly significance P value ≤ 0.05

The results Table (3-24) are given from the nanoparticles prepared using latex of C*alotropis procera*, in Figure (3-36) which demonstrates the variation of average AgNPs *Calotropis procera* concentrations, with the first concentration having the largest inhibitory impact at 512 µg/ml and the lowest inhibitory concentration at 16 µg/ml.

As indicated in Table (3-25), the P-value for the six concentrations (512, 256, 128, 64, 32, 16) µg/ml is 0.001 that means high significance when compared with control. The findings on 8µg/ml value were remarkably close to those of the control. When a statistical analysis was done utilizing (t-test),

no significant difference (P-value = 0.148) was found between the value of the last concentration and the value of the utilized control.

Table 3-24: Value of absorbance in AgNPs *Calotropis procera*

Conc. / species	512 µg/ml	256 µg/ml	128 µg/ml	64 µg/ml	32 µg/ml	16 µg/ml	8 µg/ml	control
S. aureus	0.0038	0.0067	0.0123	0.0235	0.0376	0.1542	0.2432	0.2632
E. faecalis	0.0032	0.0067	0.0078	0.0089	0.0132	0.0432	0.2943	0.3051
E. coli	0.0028	0.0047	0.0075	0.0102	0.0193	0.0542	0.1921	0.1932
K. pneumoniae	0.0023	0.0056	0.0098	0.0132	0.0253	0.0872	0.2353	0.2375

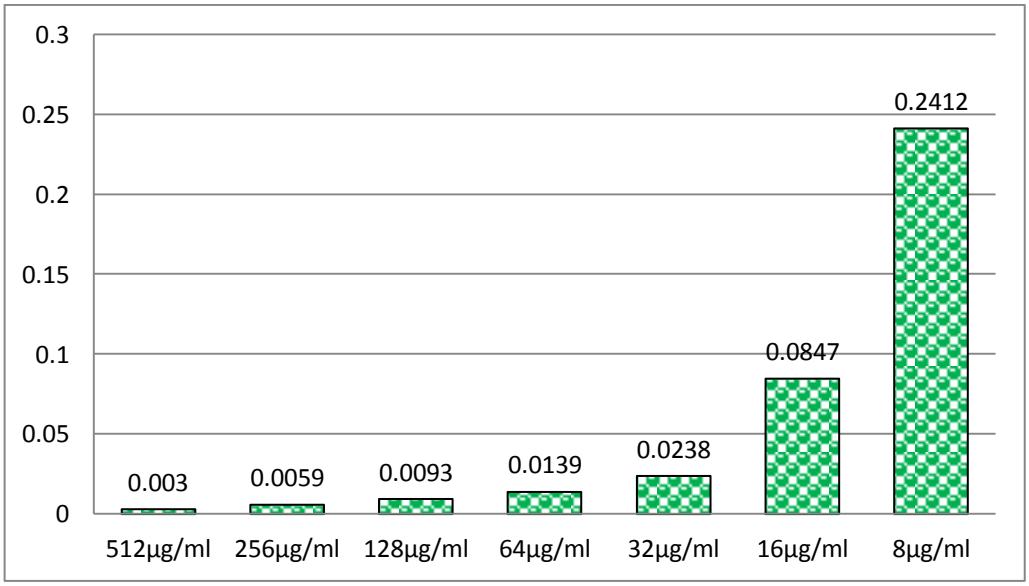


Figure 3-36: Distribution of mean concentration of latex AgNPs *Calotropis procera*

The distribution of mean concentration according to bacteria treated with latex AgNPs of *Calotropis procera* is shown in Figure (3-37). The strongest effect of silver nanoparticles prepared by *Calotropis procera* latex against *E. coli*, followed by *E.faecalis* with a rate of 0.0415 and 0.0539, respectively.

subsequently, *K.pneumoniae* has a rate of 0.0541, followed by *S. aureus*, which has a rate of 0.0687. However, there were no significant differences in the statistical study, as the P-value was equivalent to 0.940, as indicated in Table (3-25).

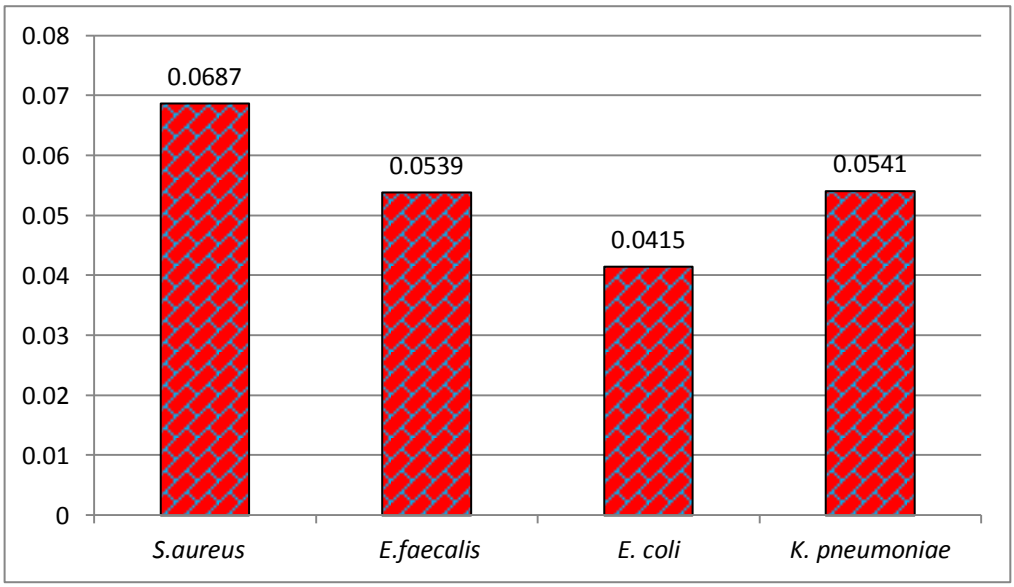


Figure 3-37: distribution of the mean concentration according to bacteria treated with latex AgNPs of *Calotropis procera*

Except for the 8 µg/ml concentration, where there is a non-significant difference at a p-value of 0.05, Table (3-23) shows substantial and extremely significant differences between the control group and all concentrations. Differences in concentrations within *Calotropis procera* are highly significant, having a p-value of less than 0.05. *Calotropis procera* also shows non-significant variations among bacteria within plants, with a P-value of less than 0.05.

Table 3-25: Distribution of different concentrations in *Calotropis procera*

Conc. / **species**	**Control**	**512 µg/ml**	**256 µg/ml**	**128 µg/ml**	**64 µg/ml**	**32 µg/ml**	**16 µg/ml**	**8 µg/ml**	**Mean**	**SD**	**P-value (ANOVA)**
S. aureus	0.2632	0.0038	0.0067	0.0123	0.0235	0.0376	0.1542	0.2432	0.0687	0.09310	0.940 (N.S)
E. faecalis	0.3051	0.0032	0.0067	0.0078	0.0089	0.0132	0.0432	0.2943	0.0539	0.10685	
E.coli	0.1932	0.0028	0.0047	0.0075	0.0102	0.0193	0.0542	0.1921	0.0415	0.06870	
K. pneumoniae	0.2375	0.0023	0.0056	0.0098	0.0132	0.0253	0.0872	0.2353	0.0541	0.08507	
Mean	0.24975	0.0030	0.0059	0.0093	0.0139	0.0238	0.0847	0.2412			
SD	0.0468	.00006	.000096	0.0022	0.0066	0.0104	0.0499	0.0419			
P-value (t-test)	-	0.002 (H.S)	0.002 (H.S)	0.002 (H.S)	0.002 (H.S)	0.003 (H.S)	0.016 (Sig.)	0.148 (N.S)			
P-value (ANOVA)	-	0.001 (H.S)									

Sig = significance, N.S = non significance, H.S= highly significance P value ≤ 0.05

In silver nanoparticles prepared with *Nerium oleander* latex, the results are shown in Table (3-26). The maximum inhibitory ratio was 512 µg/ml, while the lowest inhibitory ratio was 32 µg/ml, with a substantial and extremely significant difference between the control group and all concentrations except 16 and 8 µg/ml, where there is a non-significant difference at P-value 0.05. The results also showed that there were significant differences between the concentrations among them where P-value was 0.001, Table (3-27), Figure (3-38).

Table 3-26: Value of absorbance in AgNPs *Nerium oleander* latex

Conc. / **species**	**512 µg/ml**	**256 µg/ml**	**128 µg/ml**	**64 µg/ml**	**32 µg/ml**	**16 µg/ml**	**8 µg/ml**	**control**
S. aureus	0.0012	0.0024	0.0813	0.0121	0.0251	0.0632	0.1743	0.1832
E. faecalis	0.0031	0.0071	0.0092	0.0142	0.0453	0.0832	0.1754	0.1798
E. coli	0.0018	0.0032	0.0087	0.0121	0.0893	0.1834	0.1912	0.1976
K. pneumoniae	0.0069	0.0099	0.0193	0.0776	0.1965	0.2072	0.2212	0.2292

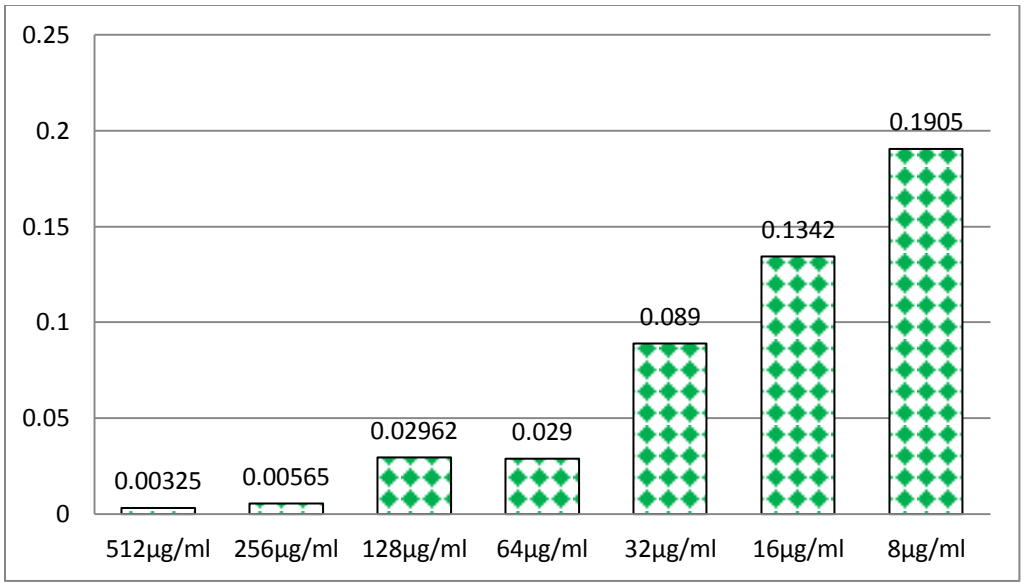


Figure 3-38: Distribution of mean concentration of latex AgNPs *Nerium oleander*

Despite the obvious inhibitory effect of silver nanoparticles prepared with Nerium oleander latex against gram positive bacteria (*E.faecalis* and *S.aureus*) with values (0.0482 and 0.0513 respectively), and at a higher percentage than their effect on gram negative bacteria (*E.coli* and *K.pneumoniae*) with values (0.0699 and 0.1055 respectively), when statistical studies were conducted, there were no significant differences when P-value =0.55, Figure (3-39).

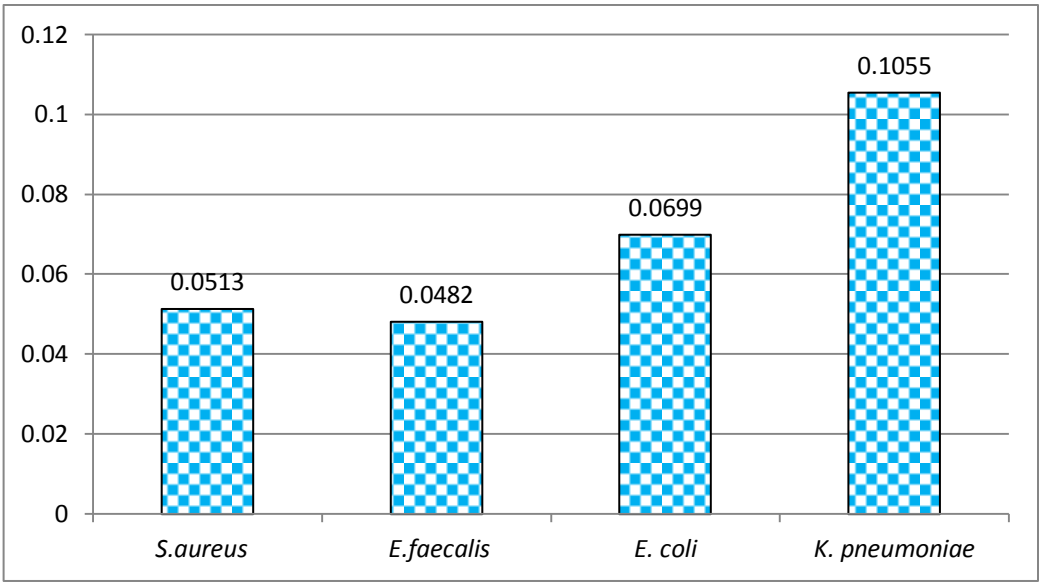


Figure 3-39: distribution of the mean concentration according to bacteria treated with latex AgNPs of *Nerium oleander*

Table (3-27) show significant and highly significant differences between the control group and all concentrations except with 16 and 8 µg/ml concentrations where is a non-significant difference at p-value ≤ 0.05. As a result, changes in concentrations within *Nerium oleander* are highly significant, whereas differences between bacteria are non-significant at a P-value of 0.05.

Table 3-27: Distribution of different concentrations in *Nerium oleander*.

Conc. / **species**	**Control**	**512 µg/ml**	**256 µg/ml**	**128 µg/ml**	**64 µg/ml**	**32 µg/ml**	**16 µg/ml**	**8 µg/ml**	**Mean**	**SD**	**P-value (ANOVA)**
S. aureus	0.1832	0.0012	0.0024	0.0813	0.0121	0.0251	0.0632	0.1743	0.0513	0.06230	0.55 (N.S)
E. faecalis	0.1798	0.0031	0.0071	0.0092	0.0142	0.0453	0.0832	0.1754	0.0482	0.06299	
E.coli	0.1976	0.0018	0.0032	0.0087	0.0121	0.0893	0.1834	0.1912	0.0699	0.08577	
K. pneumoniae	0.2292	0.0069	0.0099	0.0193	0.0776	0.1965	0.2072	0.2212	0.1055	0.09923	
Mean	0.1974	0.00325	0.00565	0.02962	0.029	0.089	0.1342	0.1905			
SD	0.0225	0.0025	0.0034	0.0347	0.0324	0.0764	0.0716	0.0218			
P-value (t-test)	-	0.001 (H.S)	0.001 (H.S)	0.006 (H.S)	0.001 (H.S)	0.028 (Sig.)	0.097 (N.S)	0.06 (N.S)			
P-value (ANOVA)	-	0.001 (H.S)									

Sig = significance, N.S = non significance, H.S= highly significance P value ≤ 0.05

The results of MIC of AgNPs *Ficus elastica* against the bacterial species used in this study are shown in Figure (3-40) which represents the distribution of the average concentration of AgNPs *Ficus elastica*, where the first concentration was 512 µg / ml the strongest inhibitory effect, while the lowest inhibitory concentration was a value of 16 µg/ml. As shown in Table (3-28). where the P-value is 0.001 as a high significant for the six concentrations (512, 256, 128, 64, 32, 16) µg/ml. As for the seventh concentration, which has a value of 8 µg/m, its results were very close to the results of the control, group which were 0.1686 and 0.1692, respectively. This was corroborated by a statistical investigation, which found no significant difference (P-value

=0.774) between the value of the seventh concentration and the value of the user control, which was a bacterial growth devoid of silver nanoparticles.

Table 3-28: Value of absorbance (OD at 600 nm) in AgNPs *Ficus elastica*

Conc. / **species**	**512 µg/ml**	**256 µg/ml**	**128 µg/ml**	**64 µg/ml**	**32 µg/ml**	**16 µg/ml**	**8 µg/ml**	**control**
S. aureus	0.0019	0.0049	0.0083	0.0165	0.0487	0.0923	0.1736	0.1853
E. faecalis	0.0028	0.0046	0.0089	0.0169	0.0689	0.0954	0.2253	0.2303
E. coli	0.0034	0.0069	0.0097	0.0102	0.0433	0.0794	0.1643	0.1712
K. pneumoniae	0.0047	0.0086	0.0142	0.0465	0.0837	0.1689	0.1686	0.1692

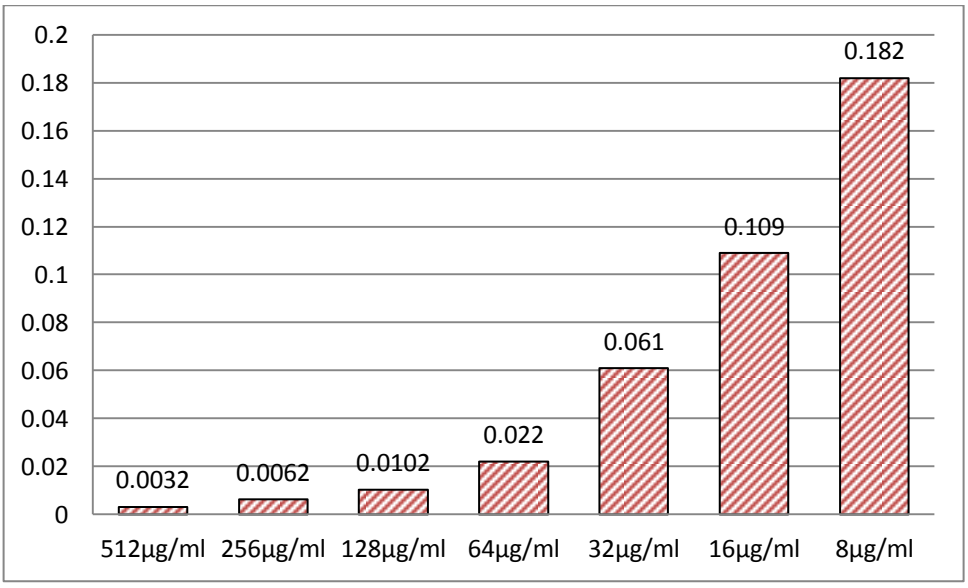


Figure 3-40: Distribution of mean concentration of latex AgNPs *Ficus elastica*

It is clear from the Figure (3-41) which represents the distribution of the mean concentration according to bacteria treated with latex AgNPs of *Ficus elastica* (*K.pneumoniae, E.coli, E.faecalis,* and *S.aureus*) that the strongest effect of silver nanoparticles prepared by *Ficus elastica* latex is against *E. coli*, followed by *S. aureus* with a rate of 0.0453 and 0.0494 respectively, then

E.faecalis at a rate of 0.0604 and finally *K. pneumoniae* with a rate of 0.0707. But when conducting the statistical study, there were no significant differences, as the P-value was equal to 0.929 as shown in the Table (3-29)

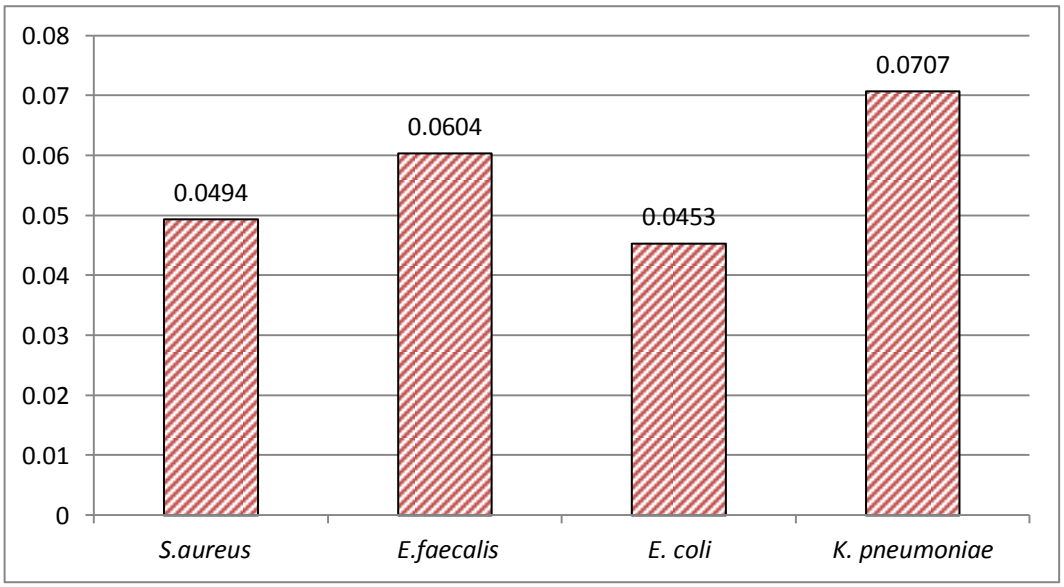


Figure 3-41: distribution of the mean concentration according to bacteria treated with latex AgNPs of *Ficus elastica*

Table (3-29) exhibited a significant and extremely significant difference between the control group and all concentrations, with the exception of 8 µg/ml, where the difference is non-significant at a P-value of 0.05. As a consequence, differences in concentrations inside *Ficus elastica* are highly significant, but differences in bacteria within *Ficus elastica* are non-significant at a P-value of 0.05.

Table 3-29: Distribution of different concentrations in *Ficus elastica*.

Conc. / **species**	**Control**	**512 µg/ml**	**256 µg/ml**	**128 µg/ml**	**64 µg/ml**	**32 µg/ml**	**16 µg/ml**	**8 µg/ml**	**Mean**	**SD**	**P-value (ANOVA)**
S. aureus	0.1853	0.0019	0.0049	0.0083	0.0165	0.0487	0.0923	0.1736	0.0494	0.06360	0.929 (N.S)
E. faecalis	0.2303	0.0028	0.0046	0.0089	0.0169	0.0689	0.0954	0.2253	0.0604	0.08110	
E.coli	0.1712	0.0034	0.0069	0.0097	0.0102	0.0433	0.0794	0.1643	0.0453	0.05924	
K. pneumoniae	0.1692	0.0047	0.0086	0.0142	0.0465	0.0837	0.1689	0.1686	0.0707	0.07232	
Mean	0.189	0.0032	0.0062	0.0102	0.022	0.061	0.109	0.182			
SD	0.0284	0.0011	0.0018	0.0026	0.0162	0.0186	0.0405	0.0284			
P-value (t-test)	-	0.001 (H.S)	0.001 (H.S)	0.001 (H.S)	0.001 (H.S)	0.001 (H.S)	0.018 (Sig.)	0.774 (N.S)			
P-value (ANOVA)	-				0.001 (H.S)						

Sig = significance, N.S = non significance, H.S= highly significance P value ≤ 0.05

As for the nanoparticles prepared using latex of *Euphorbia tirucalli*, they were as follows in Table (3-30) and Figure (3-42) which show the fluctuation in average AgNPs *Euphorbia tirucalli* concentrations, with the first concentration having the greatest inhibitory influence at 512 g/ ml and the lowest inhibitory concentration at 32 µg/ml.

The P-value for the five concentrations (512, 256, 128, 64, 32) µg/ml is 0.001 as a high significant value, as shown in Table (3-30). The results of the sixth and seventh concentrations, which had values of 16 and 8 µg/ml, respectively, were very similar to the control value. There was no significant difference in P-value (0.055, 0.034) between the value of 16 and 8 µg/ml concentrations and the value of the control in statistical analysis using (the t-test).

Table 3-30: Value of absorbance in AgNPs *Euphorbia tirucalli* latex

Conc. / **species**	**512 µg/ml**	**256 µg/ml**	**128 µg/ml**	**64 µg/ml**	**32 µg/ml**	**16 µg/ml**	**8 µg/ml**	**control**
S. aureus	0.0017	0.0054	0.0089	0.0129	0.0376	0.1327	0.1897	0.1939
E. faecalis	0.0016	0.0056	0.0088	0.0172	0.0628	0.1234	0.1663	0.1829
E. coli	0.0036	0.0065	0.0112	0.0395	0.0821	0.2153	0.2243	0.2331
K. pneumoniae	0.0043	0.0082	0.0111	0.0421	0.1434	0.2272	0.2343	0.2429

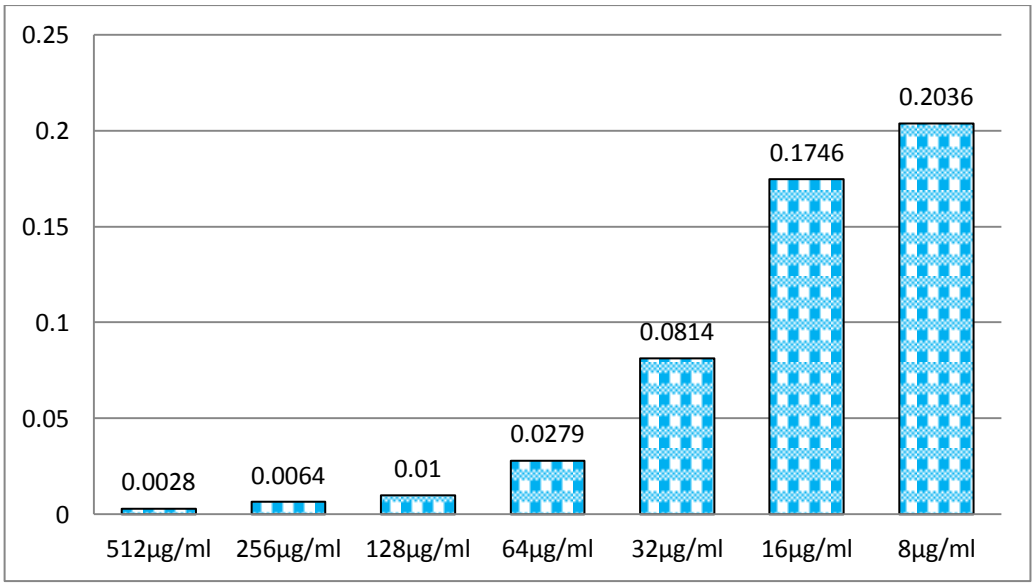


Figure 3-42: Distribution of mean concentration of latex AgNPs *Euphorbia tirucalli*

Figure (3-43) shows the mean concentration distribution for bacteria treated with latex AgNPs from *Euphorbia tirucalli*. With a rate of 0.0551 and 0.0555, respectively, silver nanoparticles made by *Euphorbia tirucalli* latex had the strongest impact against *E.faecalis* and *S.aureus*. *E.coli* and *K.pneumoniae* are next with rates of 0.0832 and 0.0958, respectively. However, there were no significant differences in the statistical investigation, as evidenced by the P-value of 0.746 in Table (3-31)

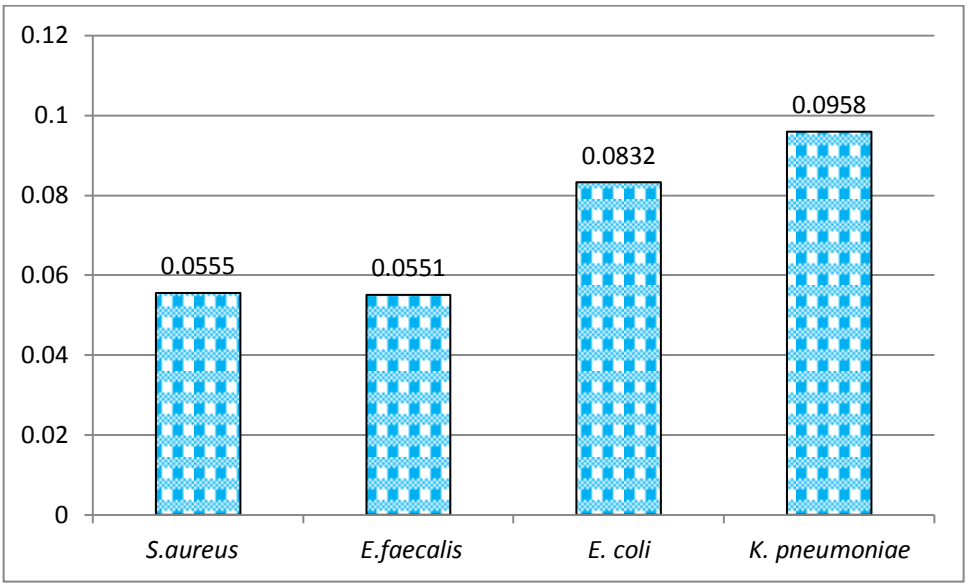


Figure 3-43: Distribution of mean concentration of latex AgNPs *Euphorbia tirucalli*

Except for concentrations of 16 and 8 µg/ml, which indicate a non-significant difference at a P-value of 0.05, Table (3-31) shows substantial and extremely significant differences between the control group and the utilized concentrations. At a P-value of 0.05, changes in concentrations within *Euphorbia tirucalli* are highly significant, whereas differences among bacteria within *Euphorbia tirucalli* are not.

Table 3-31: Distribution of different concentrations in *Euphorbia tirucalli*.

Conc. / **species**	**Control**	**512 µg/ml**	**256 µg/ml**	**128 µg/ml**	**64 µg/ml**	**32 µg/ml**	**16 µg/ml**	**8 µg/ml**	**Mean**	**SD**	**P-value (ANOVA)**
S. aureus	0.1939	0.0017	0.0054	0.0089	0.0129	0.0376	0.1327	0.1897	0.0555	0.07492	0.746 (N.S)
E. faecalis	0.1829	0.0016	0.0056	0.0088	0.0172	0.0628	0.1234	0.1663	0.0551	0.06580	
E.coli	0.2331	0.0036	0.0065	0.0112	0.0395	0.0821	0.2153	0.2243	0.0832	0.09718	
K. pneumoniae	0.2429	0.0043	0.0082	0.0111	0.0421	0.1434	0.2272	0.2343	0.0958	0.10393	
Mean	0.2132	0.0028	0.0064	0.0100	0.0279	0.0814	0.1746	0.2036			
SD	0.0292	0.0013	0.0012	0.0013	0.0150	0.0451	0.0541	0.0313			
P-value (t-test)	-	0.001 (H.S)	0.001 (H.S)	0.001 (H.S)	0.001 (H.S)	0.002 (H.S)	0.055 (N.S)	0.034 (N.S)			
P-value (ANOVA)	-	0.001 (H.S)									

Sig = significance, N.S = non significance, H.S= highly significance P value ≤ 0.05

The results show Silver nanoparticles of all plant species under study showed anti-bacterial activity against the bacteria used, and as it clear because of the presence of chemical compounds with anti-bacterial properties, which were previously mentioned in the table [(1-1)-(1-5) and [(3-1)-(3-7)], but the differenceses were in that there are substantial variations in the series of concentrations created from silver nanoparticles particular to each plant species, the highest concentration, which is 512g/ml, was the most inhibitory in all plant species, whereas the minimal inhibitory concentration varied depending on the plant species, as shown in Table (3-15). *Ficus carica*, *Ficus elastica*, and Calotropis procera all have MIC values of 16 µg/ml and (46.4, 45.4, and 47.8), respectively, whereas *Nerium oleander* and *Euphorbia tirucalli* have MIC values of 32 µg/ml and (50.5 and 51.6) respectively, where

The smaller the average size of the AgNPs, the antibacterial activity will be greater (Liu et al .,2010) Table (3-15).In general, where P-value ≤ 0.05 the statistical analysis results show non-significant differences among plant species under study in relation to concentration Table (3-32) as well as non-significant differences among plant species under study in relation to bacterial species Table (3-33).

Table 3-32: Differences among plant species under study according to concentration

		512 µg/ml	**256 µg/ml**	**128 µg/ml**	**64 µg/ml**	**32 µg/ml**	**16 µg/ml**	**8 µg/ml**
N	**Valid**	20	20	20	20	20	20	20
	Missing	0	0	0	0	0	0	0
Mean		0.0029	0.0059	0.013	0.0206	0.054	0.115	0.211
Std. Deviation		0.0013	0.0018	0.0162	0.0176	0.047	0.0603	0.039
Minimum		0.0012	0.0024	0.0069	0.0089	0.012	0.043	0.164
Maximum		0.0069	0.0099	0.0813	0.077	0.196	0.227	0.294
P-value		0.900 (N.S)	0.931 (N.S)	0.297 (N.S)	0.492 (N.S)	0.089 (N.S)	0.110 (N.S)	0.086 (N.S)

Sig = significance, N.S = non significance, H.S= highly significance P-value ≤ 0.05

Table 3-33: Differences among plant species under study according to bacterial species

		S. aureus	*E. faecalis*	*E.coli*	*K. pneumoniae*	P-value
N	**Valid**	35	35	35	35	
	Missing	0	0	0	0	
Mean		0.0573	0.0543	0.0555	0.0745	
Std. Deviation		0.0728	0.0816	0.0733	0.0879	0.799
Minimum		0.0012	0.0016	0.0018	0.0012	(N.S)
Maximum		0.2432	0.2943	0.2243	0.2413	
P-value		0.990 (N.S)	0.999 (N.S)	0.745 (N.S)	0.691 (N.S)	

Sig = significance, N.S = non significance, H. S= highly significance, P value ≤ 0.05

3.3.2.6 DNA Fragmentation Effect

DNA is the most crucial genetic information contained in cells, and any damage to DNA can result in an organism's mutation or death, some cells naturally take steps to safeguard DNA molecules from damage (Abbas, *et al*., 2019). The impact of AgNPs Prepared from the latex of the five latex-producing plant species on the bacterial genome was investigated in this study the impact of Ag NPs that was prepared by five plant species producing latex on the genomic DNA of *E.coli* bacterium was observed by the DNA damage, and this increased as the concentration of AgNPs increased Except for the *Ficus elastica*, where the results showed that it did not cause DNA damage in the concentrations used, as shown in Figure (3-44).

The results demonstrate that DNA treated with AgNPs exhibits particular DNA smearing, which is a Hallmark feature of cell death, whereas untreated DNA did not exhibit fragmentation

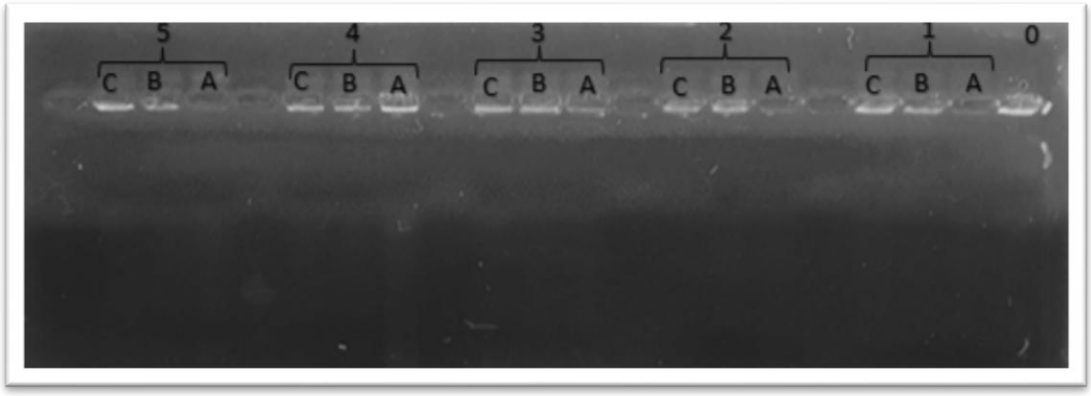


Figure 3-44: Fragmentation of DNA by biosynthesis Ag NPs, where:

0: genomic DNA of *E.coli* in 1% Agarose gel at 85 vol. for 2 hr.

1: DNA with Ag NPs latex of *Ficus carica*

2: DNA with Ag NPs latex of *Nerium oleander*,

3: DNA with Ag NPs latex of *Calotropis procera*

4: DNA with Ag NPs latex of *Ficus elastica*

5: DNA with Ag NPs latex of *Euphorbia tirucalli*

concentrations(A=512, B=256, C=128µg/ml).

Conclusions And Recommendations

Conclusion:

The most important conclusions of this study can be summarized in the following points:

1. Plant latex contains many chemical compounds of biological importance, some familiar in many plant species and some found in one species but not in other plant species.
2. Presence of the compound Benzaldehyde, 2-nitro-, diaminomet hylidenhydrazone, which has anti-bacterial properties, as it was found only in *Ficus carica*, but the compound Oleic Acid has antibacterial, antifungal and antioxidant properties was only found in *Calotropis procera*.
3. Octasiloxane, 1,1,3,5,5,7,7,9,911,11,13,13,15,15-hexadecamethyl which has anti-bacterial properties in both *Calotropis procera* and *Ficus elastica* also the compound Hexadecanoic acid, methyl ester with its anti-inflammatory properties and hypocholesterolemic, cancer preventive, hepatoprotective, their presence in recurrence *Nerium oleander* and *Ficus elastica*
4. Possibility of using secondary metabolites represented by the latex as a material for extracted DNA and using it in application techniques like RAPD marker.
5. The molecular study has proven the possibility of confirming the genetic fingerprint of the close relationships between the species *Ficus carica* and *Ficus elastica* that belong to the same family (Moraceae), as in the species *Calotropis procera* and *Nerium oleander* in Apocynaceae and the singular species *Euphorbia tirucalli* which belong to the family (Euphorbiaceae).
6. The current AgNP synthesis method, which uses latex extract as a reducing agent, completely eliminates the use of synthetic reducing agents. Latex is

very good at producing very stable and biocompatible AgNPs, which might be useful in biomedical applications.

Recommendations:

1. Plant latex is a rich source of medicines, insecticides, and immune system stimulants ,allergens, therefore it needs a future study as raw material in many bioengineering and biotechnological industries.

2. Isolation and purification of some effective compounds that were diagnosed by GC-MS method, which had pharmacological importance by using HPLC techniques.

3. More studies are needed to use more PCR-markers like SSR, ISSR and specific primers for specific genes to understand and expand the knowledge about the genes and pathways involved in laticifer plant cells

4. The use of purification methods when preparing silver nanoparticles, as impurities are one of the known negative aspects of the biological method for preparing nanoparticles.

5. Use other concentrations of silver nanoparticles in proportions higher than the concentrations used in this study when conducting the antibacterial test and the minimum inhibitory concentration and antioxidants.

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الخلاصة

تناولت الدراسة الحالية إجراء دراسة كيميائية وجزيئية لخمس عينات من مادة الحليب النباتي لخمسة أنواع نباتية منتجة للحليب هي (*Ficus carica* L. و*Ficus elastic* Roxb. ex Hornem و *Calotropis procera* (Aiton) W.T. Aiton. و *Nerium oleander* L. و *Euphorbia tirucalli* L.) باستعمال الكروماتوغرافيا الغازية لقياس الطيف الكتلي (GC–MS) وتقنية التضاعف المتماثل للتضخيم العشوائي Random Amplified Polymorphic DNA (RAPD) على التوالي ثم تكوين مادة نانوية من هذا الحليب واستخدامها في بعض تطبيقات الانشطة البيولوجية ، كمضاد للبكتيريا و تأثير المضاد الحيوي وانحلال الدم وتحلل الحمض النووي.

أجريت هذه الدراسة في مختبر النبات المتقدم في كلية العلوم للبنات / جامعة بابل بالتعاون مع مختبرات وزارة الصناعة والمعادن ومركز الأمين للبحوث التكنولوجية المتقدمة في محافظة النجف لإجراء بعض الفحوصات للجانبين الكيميائي والجزيئي وتقنية النانو للمدة من تشرين الثاني 2020 إلى اذار 2021.

أظهر التحليل الكيميائي للأنواع النباتية المنتجة لمادة للحليب المتضمنة في هذه الدراسة وجود عدد من المركبات المهمة بيولوجيًا، والتي كان بعضها مشتركًا في عدد من الأنواع النباتية قيد الدراسة، ومن هذه الدراسة تأكد الاختلاف بين المكون الكيميائي لمادة الحليب بين الأنواع النباتية قيد الدراسة والتي تظهر امكانيات مختلفة للأنشطة العلاجية.

ومن خلال نتائج الدراسات الجزيئية عن طريق التباين الجيني بين الأنواع المنتجة للحليب باستخدام خمسة بادئات عشوائيةهي (OPB18، OPC2، BH10، BH11، BH14)، بعد نجاح طريقة استخلاص الحمض النووي من المستقلبات الثانوية ومنها الحليب النباتي، فقد أثبتت إمكانية تأكيد البصمة الوراثية للعلاقات الوثيقة بين النوعين *Ficus carica* و *Ficus elastica* اللذان ينتميان إلى نفس العائلة Moraceae، وكذلك في النوعين *Calotropis procera* و *Nerium oleander* في العائلة Apocynaceae والنوع المفرد *Euphorbia tirucalli* الذي ينتمي إلى العائلة Euphorbiaceae.

تضمنت هذه الدراسة أيضًا التحضير الناجح لجسيمات الفضة النانوية باستخدام مادة الحليب النباتي الخاص بأنواع النباتات الخمسة كعامل اختزال للفضة، وقد تم الاستدلال على ذلك من خلال تغير اللون الذي ظهر وباختبار التحليل الطيفي المرئي للأشعة فوق البنفسجية، نظرًا لأطياف

الامتصاص للجسيمات النانوية التي تم إنشاؤها يُظهر خليط التفاعل ذروة امتصاص عند 400 نانومتر لـ AgNPs، وتحليل SEM الذي يستخدم لتأكيد حجم وتشكل AgNPs. وأشارت نتائج أبحاث الفحص المجهري الإلكتروني إلى أن أنواع الحليب المختلفة من النباتات قيد الدراسة لها خصائص AgNPs الحيوية، إلى جانب حيود الأشعة السينية (XRD) وتحويل فورييه الطيفي للأشعة تحت الحمراء (FT-IR).

تم دراسة بعض التطبيقات البيولوجية لجسيمات الفضة النانوية مثل تأثير المضاد الحيوي حيث انخفض إنتاج الأغشية الحيوية بشكل ملحوظ بنسبة 40-99٪ بزيادة تركيز AgNPs مما قلل من كمية تطور الأغشية الحيوية في جميع الحالات.

كما كشفت دراسة التأثير المضاد للأكسدة أن قدرة الجسيمات النانوية على تقليل الجذور الحرة DPPH قد تحسنت مع زيادة تركيز AgNPs للحليب الحيوي، وتراوحت بين 65-78٪ وهي أقل وأعلى نسبة على التوالي. اما في تأثير انحلال الدم، فقدأظهر Latex AgNPs مع جميع التركيزات (64، 128، 256، 512 ميكروغرام / مل) معدل انحلال دم يتراوح من (0-4.9) لكامل الدم الذي تم اختباره والذي يعتبر طبيعيا، باستثناء النوع *Ficus elastica* اذ كانت معدلات انحلال الدم عالية عند التركيز الأول.

أظهرت دراسة التأثير المضاد للبكتيريا نشاط جزيئات الفضة النانوية ضد البكتيريا سالبة الجرام (*Escherichia coli* و*Klebsiella pneumonia*) والبكتيريا موجبة الجرام (*Staphylococcus aureus* و*Enterococcus faecalis*)، ووجود مناطق تثبيط واضحة وكشفت نشاطًا ضد جميع أنواع البكتيريا المستعملة في هذه الدراسة بأقطار مختلفة من منطقة التثبيط، نتيجة وجود مركبات كيميائية ذات خصائص مضادة للبكتيريا تم اكتشافها في مادة الحليب للأنواع النباتية الخمسة قيد الدراسة عند إجراء تحليل GC-MS، كما تم تحديد الحد الأدنى للتركيز المثبطMinimum) inhibitory concentration) (MIC) وكانت هناك نسبة عكسية بين حجم الجسيمات النانوية وقيمة الحد الأدنى للتركيز المثبط.

اما قياس تجزئة الحمض النووي، فقد أظهرت النتائج أن الحمض النووي المعالج بـ AgNPs اظهر تلطيخًا خاصًا للحمض النووي، وهو سمة مميزة لموت الخلايا، في حين أن الحمض النووي غير المعالج لا يظهر التجزئة.

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قسم علوم الحياة

دراسة مقارنة لمكونات الحليب النباتي في بعض الانواع النباتية وكيفية الاستفادة منها في بعض التطبيقات البايولوجية والتقنيات البايولوجية

رسالة مقدمة الى

مجلس كلية العلوم للبنات / جامعة بابل

كجزء من متطلبات نيل درجة الماجستير

في العلوم / علوم الحياة

من قبل

رلى ظاهر عبد المحسن كاظم

بكالوريوس علوم الحياة / جامعة بابل (2008)

بإشراف

أ.د. هدى جاسم محمد التميمي

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