Phytochemical Composition and Some Biological Application of Nanoparticles from ficus Elastica Roxb. (Moraceae) Latex

Rula Dhahir Al-Jayid[1], Huda Jasim M. Altameme[2]
[1, 2]Biology, College of Science for Women, University of Babylon, Babylon, Iraq. 1rulaE-mali: Email: aljayid.gsci82@student.uobabylon.edu.iq

Abstract
During the period from November 2021 to May 2022 collecting the Ficus elastica latex and analysis by Gas Chromatography-Mass Spectrometry. The results revealed the presence of 12 common chemical compounds in methanolic extract including amine, amide and esters which have biologically activities such as antibacterial and antioxidant properties.The current work was provided an easy method for producing silver nanoparticles from the latex of Ficus elastica. F.elastica latex silver nanoparticles (FE-AgNPs) were identified by many methods such as the formation of surface plasmon resonance (SPR) band was monitored by UV-Vis spectroscopy, Fourier transforms infrared spectroscopy (FT-IR) spectra, X-Ray Diffraction (XRD) Pattern and Scanning Electron Microscopy (SEM). then it used in some application which applied in biological activities, including as an antibiofilm, antibacterial, hemolysis, antioxidant and DNA defragmentation, the results appeared a vary according to the concentrations and types of bacteria that were used. Therefore, highly stable and biocompatible Ag-NPs may be produced with great efficiency using Ficus elastica Latex, making them appropriate for biomedical applications.
Keywords: Ficus elastica, Latex, GC-MS analysis, silver nanoparticles, biological application

1. Introduction

Ficus elastica Roxb. belongs to the Moraceae family and is indigenous to northeast India and southern Indonesia. It is also known as the rubber fig, rubber bush, rubber tree, rubber plant, or Indian rubber bush. Rubber is made using the branch bark latex that is collected. It grows in humid, warm, tropical climates, where it may reach heights of 7.5 to 10 meters and extend its branches widely [1].

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 [2]. 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 [3].

[4] 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 where [5] mentioned the presence of Coumarins: α-amyrin; bergapten which acts as a natural anti-inflammatory and antitumor agent, Ficin E (a serine centred protease) as an antibiofilm [6, 7]. Phenols with plant disease resistance properties, polyphenols manufacture various adhesives, phenolic resins, and antioxidants, steroids and alkaloids as analgestic, emetic, vermifuge, diaphoretic, and anti-tumorous [8]. Finally, proteases as antifungal and antimicrobial activity [9].

F.elastica was selected for this study because of its role in traditional medicine which is used to cure various diseases ,The limitation of scientific literature to validate the traditional knowledge has limited its application in modern medicine; in this regard, the aim of this study was to study *F.elastica* for its phytochemical composition, preparation of silver nanoparticles and biological applications such as an antibiofilm activity, antibacterial activity, haemolysis effect, antioxidant activity.

2. Materials and Methods

2.1 Collecting of latex samples

Latex samples were collected in November 2021 to May 2022 from different places in Babylon governorate, as shown Figure (1) by breaking the plant parts and storing them in clean, sterile containers in the refrigerator until use.

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Figure (1): Collecting of latex samples

2.2 phytochemical analysis

The methanolic extract obtained was subjected to GC-MS for the determination of bioactive volatile compounds according to the method mentioned by [10].

2.3 Preparation of silver nanoparticles

The AgNPs was synthesized by an environment-friendly method, according to [11].

2.4 Characterization of FE-AgNPs

In a Shimadzu UV-visible 1800, absorption spectral analyses were done. The surface shape and size of nanoparticles in composite films were determined using SEM. Different functional groups were detected using Fourier transforms infrared (FT-IR) spectra.

2.5 Antibiofilm activity

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 sample for two types of bacterial genera are gram-positive bacteria like *Staphylococcus aureus* and gram-negative bacteria like *E.coli* according to the method of [12], The 96-well microtiter plate technique was used to test the effectiveness of Ag-NPs in biofilm development.

2.6 Antibacterial activity

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 [13].

2.7 Haemolysis effect

According to the method of [14], Using one healthy donor's blood, haemolysis tests were performed.

2.8 Antioxidant activity

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 according to [15].

3. Results and Discussion

3.1 phytochemical analysis

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%. Figure (2), Table (1).

According to the results of the GC-MS study, *Ficus* latex included amine, amide and esters and revealed the presence of common chemical compounds which has a biologically important, as shown in Table (1) the latex of *Ficus elastica* containing the compound Oxime-, methoxy-phenyl which is used in the treatment of skin [16], Also, 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 [17], furthermore the Hexadecanoic acid, methyl ester compounds are common in the latex of *F.elastica* which used as an anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary [18], In addition to the presence of phenol with antioxidant properties [19].

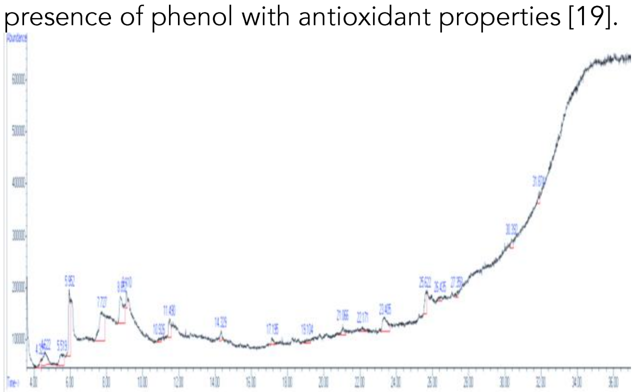


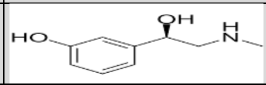
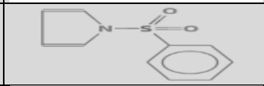
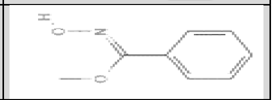
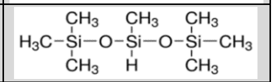
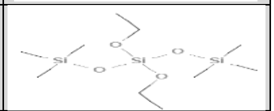
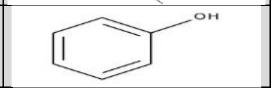
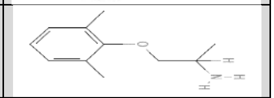
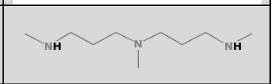
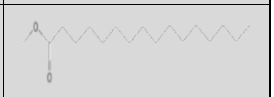
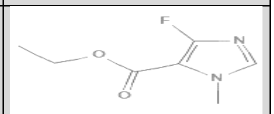
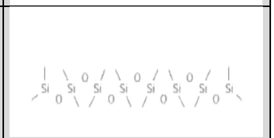
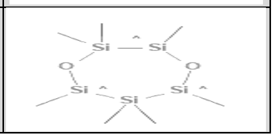
Figure (2): Chromatogram profile for GC-MS of Ficus elastica latex.

3.2 Characterization of silver nanoparticles

The Visual characterization

The bio-reduction of silver ions to silver nanoparticles using F.elastica latex extract was discovered as a consequence of the color change. The observable changes in color from brown to dark brown after adding 1mM of silver nitrate solution, owing to activation of surface plasmon vibrations, demonstrate the formation of AgNPs [20]. Spectral tests in the UV-Vis range verified it even more.

Table (1): GC–MS Chromatogram of the methanolic latex extract of Ficus elastica

No	Compound name	R.T / min	Area%	CAS Category of compound	Molecular Weight g/ml	Formula	Category	Structure
1	Phenylephrine	4.326	2.02	000059-42-7	167.21	$C_9H_{13}NO_2$C9H13NO2	Amine alcohol	
2	1-Benzenesulfonyl-1H-pyrrole	4.623	7.16	016851-82-4	207.249	$C_{10}H_9NO_2S$	heterocyclic	
3	Oxime-, methoxy-phenyl	5.515	5.93	1000222-86-6	151.16	$C_8H_9NO_2$	Amidoximes	
4	1,1,1,3,5,5,5-Heptamethyltrisiloxane	9.109	2.20	001873-88-7	222.50	$C_7H_{21}O_2Si_3$	silica	
5	Silicic acid, diethyl bis (trimethylsily) ester	10.927	1.29	003555-45-1	296.58	$C_{10}H_{28}O_4Si_3$	ester	
6	Phenol	11.488	5.16	000108-95-2	94.11	C_6H_6O	phenol	
7	Mexiletine	21.070	2.70	031828-71-4	179.26	$C_{11}H_{17}NO$	Amide	
8	N, N',N''-Trimethyldiitrimethylenet riamine	22.175	1.49	000123-70-6	173	$C_9H_{23}N_3$	Amine	
9	Hexadecanoic acid, methyl ester	23.407	8.56	000112-39-0	270.4507	$C_{17}H_{34}O_2$	ester	
10	4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester)	26.431	1.15	1000129-56-3	172.16	$C_7H_9FN_2O_2$	ester	
11	Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl	30.390	2.50	019095-24-0	577.2	$C_{16}H_{48}O_7Si_8$	organosilicon compound	
12	2,2,3,5,6,6,7-Heptamethyl [1,4,2,3,5,6,7] dioxapentasilepane	31.876	1.52	1000311-71-8	277.67	$C_7H_{21}O_2Si_5$	Hetero cyclic	

UV-Vi's analysis

The absorption spectra of silver nanoparticles formed in the reaction solution clearly show the interaction between silver ions and biomolecules found in the latex extract of *Ficus elastica*. A significant absorption band at 400nm was detected in the UV-visible spectra. The wide absorption band at 400nm corresponds to silver nanoparticles' Surface Plasmon Resonance (SPR). Silver nanoparticles vibrate when exposed to electromagnetic radiation, and this oscillation creates a distinct peak value [21]. According to [22], the observed widening of the peak shows that the nanoparticles were polydisperse, Figure (3).

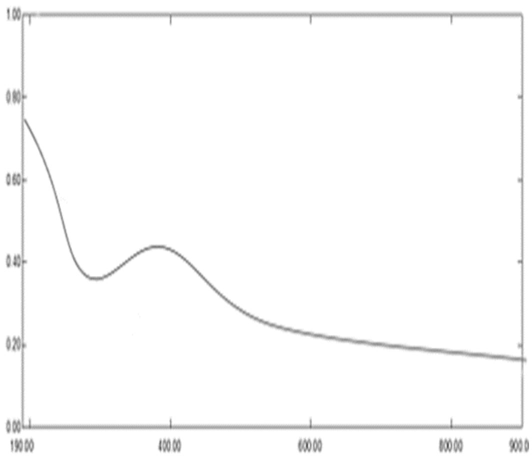


Figure (3): UV–visible absorption spectrum of AgNPs synthesized by F.elastica

Scanning electron microscopy (SEM)

It is clear in the Figure (4) which represents the FE-AgNPs latex, those clear homogeneous atoms with small nanoscale dimensions' range (47.8) nm, also the clear circular shape was noticed which shows that the latex particles completely surrounded the silver nitrate nanoparticles and gained this circular shape.

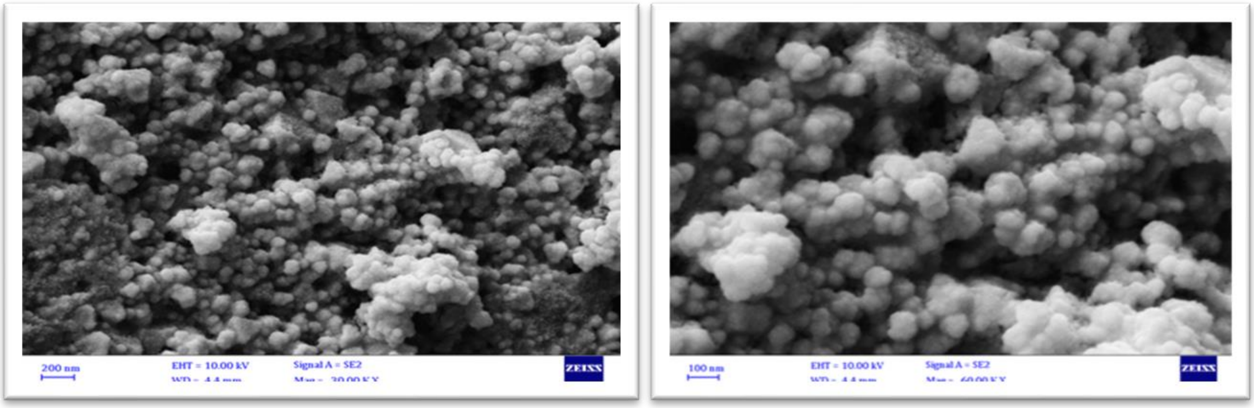


Figure (4): SEM of Silver nanoparticles prepared by Ficus elastica

FT-IR spectrum analysis

In latex FE-AgNPs was used to identify the probable biomolecules involved in reducing and capping the bio reduced silver nanoparticles. FT-IR was used to investigate silver nanoparticles' reducing, capping, and stabilizing capabilities. The absorption bands on the spectrum in Figure (5) and Table (2) indicated the existence of active functional groups in the latex of *Ficus elastica*.

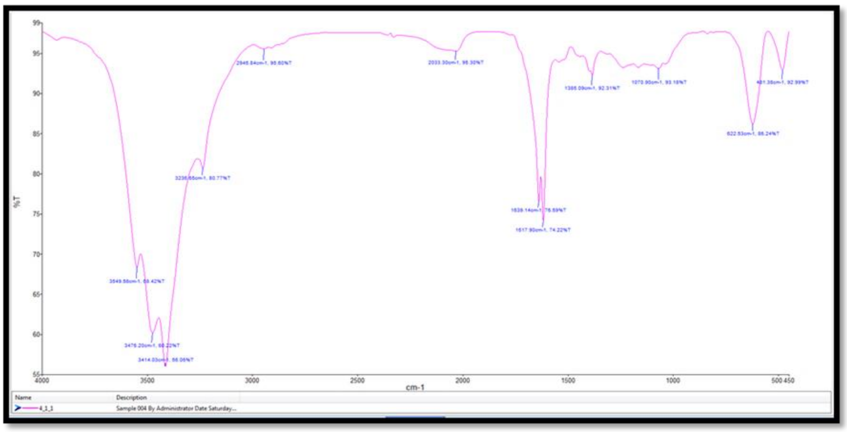


Figure (5): Fourier Transform Infrared Spectroscopy of Biosynthesized nanoparticles by F.elastica

The spectra of AgNPs made from *Ficus elastica* latex extract revealed absorption bands at (3549.56, 3476.2, 3414.03 cm^{-1}), which are typical of (O-H) [23]. Latex extract from *Ficus elastica*, 1mM $AgNO_3$ solution, and generated AgNPs, After AgNPs production, the spectra of AgNPs showed a change in transmission value and reduced in intensity to 2945.84 cm ^{1}, indicating that OH (alcohols) were responsible for the reduction of Ag^+. The fact that the peak has shrunk to 2033.3 cm^{-1} shows that hydrocarbons were involved in the conversion of Ag+ ions to AgNPs. The presence of alkane groups (C=C) is indicated by the peaks at 1639.14 cm^{-1}, and the existence of alkene (N-O) is indicated by the peak at 1617.9cm^{-1}.

Table (2): 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

3.3 Antibiofilm activity

The production of biofilms was significantly reduced by 46–98%. Increases in Ag-NPs concentration reduced the quantity of biofilm development in all situations. The Ag-NPs (at the concentration of 1024µg/ml) decrease the production of biofilms in both gram negative and gram-positive bacteria, up to 98% as shown in Figure (6).

The results indicate that silver nanoparticles prepared from the latex of Ficus elastica had a highly converged against both gram positive and negative bacteria in Ficus elastica and the reason was belonging to presences of chemical compounds with antibiofilm properties such as Ficin E (a serine centred protease) as an antibiofilm [6, 7].

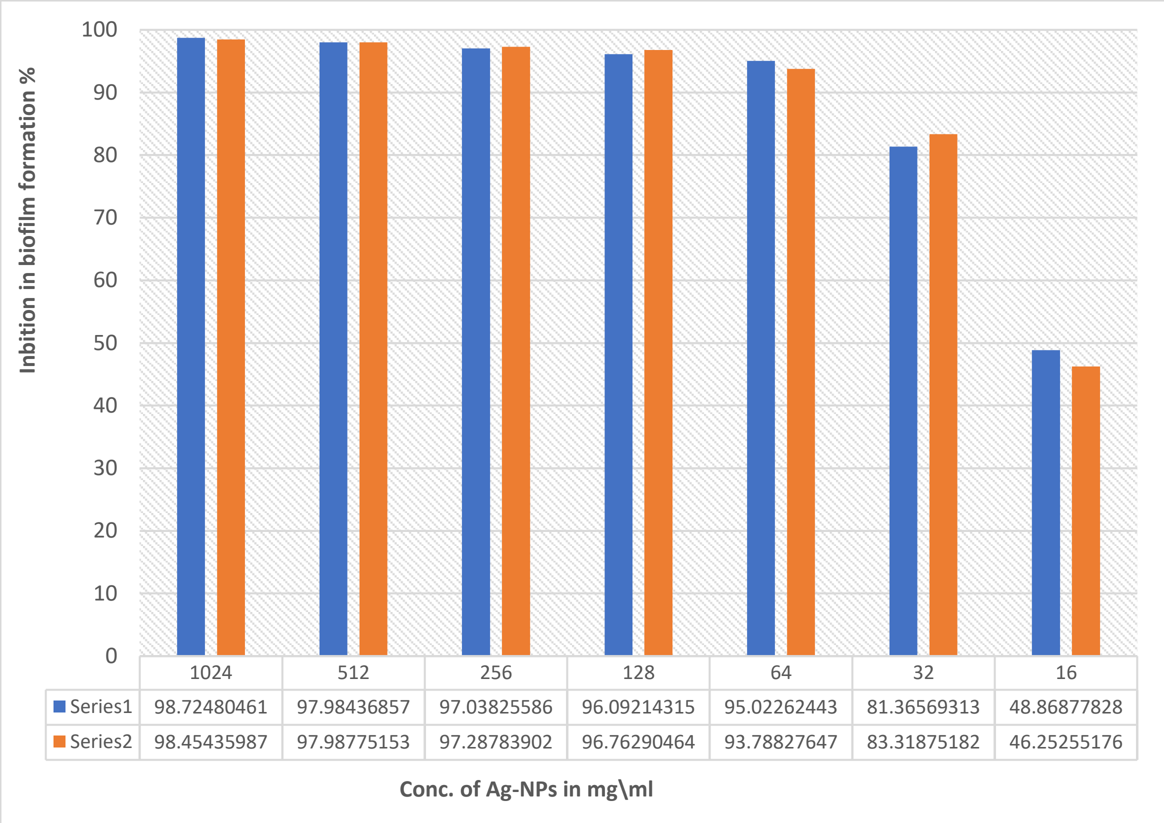


Figure (6): Determination of percent antibiofilm inhibition of Ficus elastica synthesized silver nanoparticles (Ag-NPs) on S.aureus, and E.coli, by microtiter plate method.

3.4 Antibacterial activity

The antibacterial activity of FE-AgNPs was tested at four concentrations (100, 50, 25, 10 µg/ml) against the four bacterial species used in this study (gram-negative bacteria Escherichia coli, Klebsiella pneumoniae) and (gram-positive bacteria Staphylococcus aureus, Enterococcus faecalis) and show varying inhibition zone diameters. The greatest inhibition zone was seen against E. coli and E.faecalis with an 18 mm zone of inhibition followed by a 15mm zone of inhibition against Staph.aureus, while the zone of inhibition against and K.pneumoniae were 14, all with concentrations of 100µg/ml. On the other hand, the greatest zone of inhibition with 50 µg/ml concentration appeared against E. coli and E.faecalis with an 14 mm zone of inhibition followed by a 12 mm zone of inhibition against Staph. aureus while the zone of inhibition against K.pneumoniae were 10 but the concentration of 25 µg/ml showed a zone of inhibition against E.coli only at 10mm, while the last concentration (10µg/ml) never show any zone of inhibition against all bacteria used in our study Table (3), Figure (7).

It is clear from the above that the effect of the first concentration, which is 100 µg/ml, was equal for two different bacteria, one of them positive and the other negative of gram stain. therefore, 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 [24, 25]. 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 [26] Patil et al. (2012).

Table (3): Diameters of inhibition zone for FE-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 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

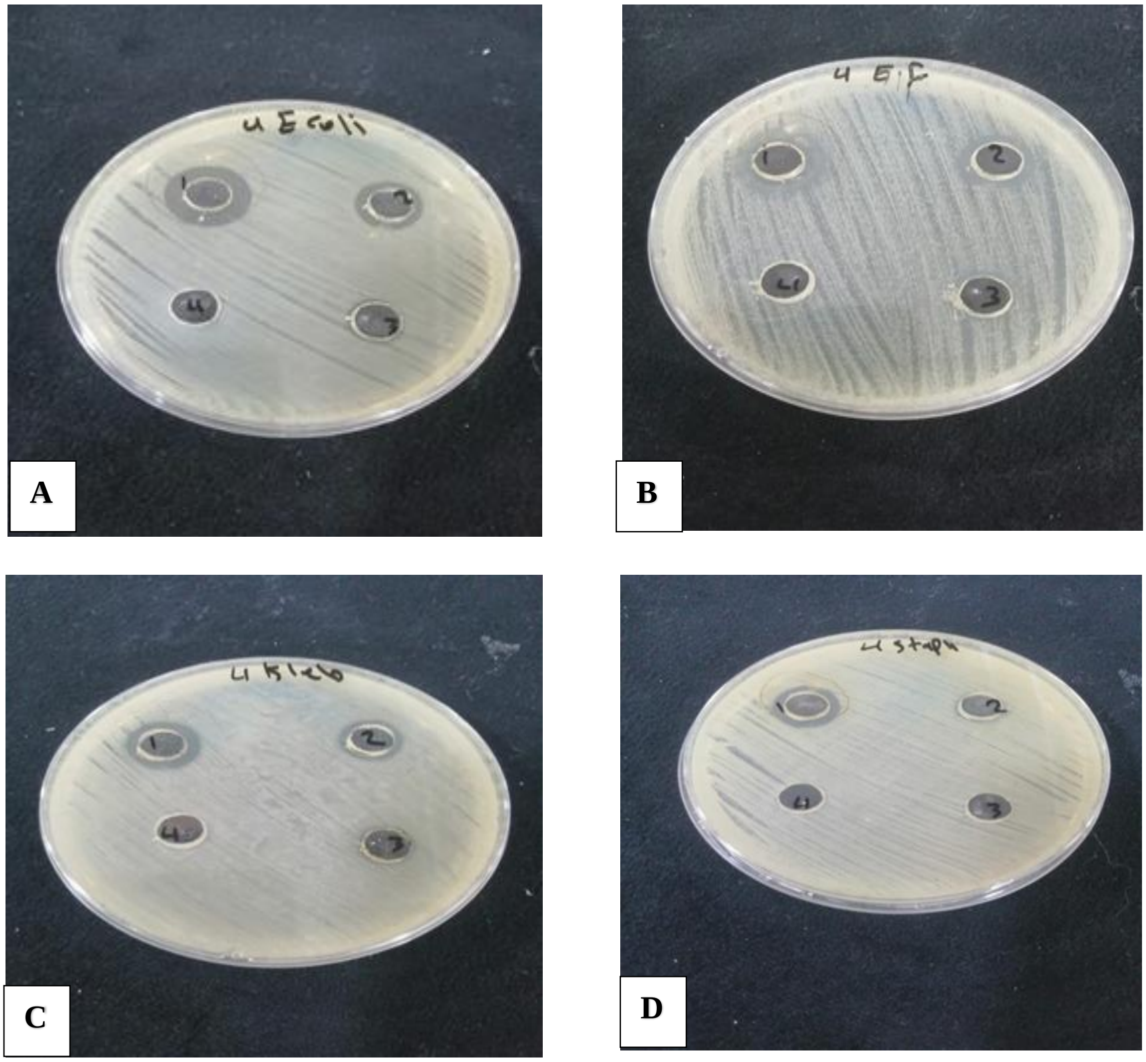


Figure (7): The Antibacterial activity of AgNPS produced from the latex of Ficus elastica (with four *concentrations) against A- Escherichia coli, B- Enterococcus faecalis, C- Klebsiella Pneumoniae, D- Staphylococcus aureus*

3.5 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) [27]. 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. The percent of haemolysis was estimated by reading the supernatant in a 96-well plate with an Eliza 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.

FE-AgNPs with all concentrations (64, 128, 256, 512 µg/ml) show a hemolysis rate ranging from (0-9) for the whole blood tested, which is considered null, except for the first concentration, which is the highest concentration which is the value of 512 µg/ml, that may be attributed to the different chemical compounds or chemical content of latex, as is clear from the results of the GC - MS analysis in this study Tables (1), Figure (8).

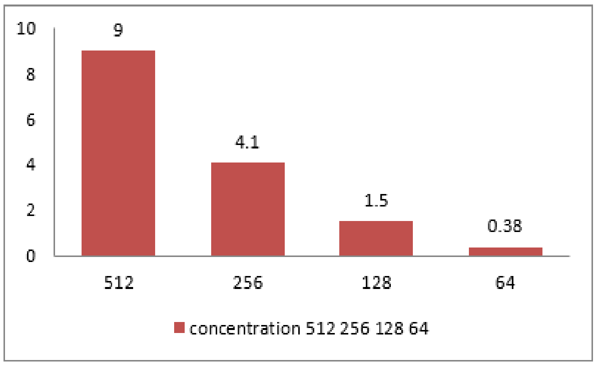


Figure (8): Percent of hemolysis of the latex AgNPs

3.6 antioxidant activity

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 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 and the highest percentage in 512µg/ml were 72 %, (Figure 9).

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, due to higher concentrations of phytochemical substances, *Ficus elastica* latex 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 addition to the results shown in the analysis of the latex extracts in this study using GC-MS technology, which showed the presence, phenol and some other compounds with antioxidant properties.

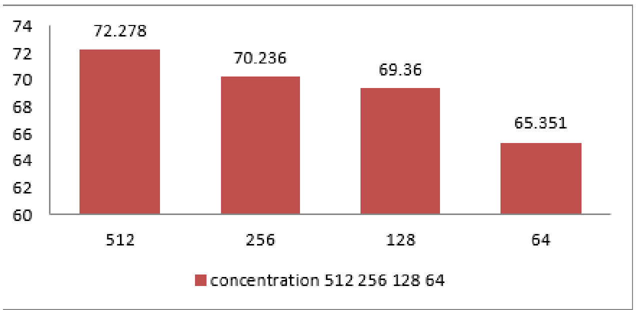


Figure (9): Percentage of nanoparticles to reduce DPPH.

4. Conclusion

Plant latex is a rich source of several secondary metabolites, including many bioactive substances with physiologically significant properties. Additionally, the current work outlines the synthesis of FE-AgNPs using a green approach method, which is well characterized by UV-visible, FT-IR, and XRD methods. Silver nanoparticles are employed in applications for antibacterial, antioxidant, antibiofilm, and hemolysis properties. Highly stable and biocompatible Ag-NPs may be produced with great efficiency using *F. elastica* Latex, making them appropriate for biomedical applications.

Financial Disclosure: There is no financial disclosure.
Conflict of Interest: None to declare.
Ethical Clearance: All experimental protocols were approved under the college of Science for Women and all experiments were carried out in accordance with approved guidelines.

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