Egypt. J. Chem. **Vol. 65**, No. 3 pp. 627 - 635 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Kinetic and inhibition effect studies of ecofriendly synthesized silver nanoparticles on lactate dehydrogenase and ferritin activity of waxy crude oil

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Abstract:

The present work investigates the synthesis of silver nanoparticles by biological method using Myrtus communis leaves extract and silver nitrate as precursors. Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD), and Fourier transform infrared spectroscopy (FT-IR) were used in addition to UV-visible spectroscopy (UV) in order to characterize the AgNPs. The biosynthesized AgNPs exhibited inhibitory effects on ferritin and lactate dehydrogenase activity in sera of covid-19 patients compared with control subjects. Kinetic studies of ferritin and lactate dehydrogenase were performed. Further studies on other biological activities were required to exploit AgNPs full potential. Conclusion of this study is prepared using simple, cheap and environmentally green method to synthesis silver nanoparticles. This stage is more suited to large-scale manufacturing since it is speedy and removes the complex steps in other biobased methods (by using fungi and bacteria).

Keywords: silver nanoparticles, Myrtus communis leaves extract, biological synthesis, lactate enzyme, ferritin, biological activity.

1. Introduction

The high surface area that provides distinct features and possible applications compared to their bulk counterparts has sparked a lot of scientific interest in nanoparticle creation and characterization[1, 2]. Silver, gold, copper, and platinum nanoparticles have been created via physical, chemical, and biological methods. Due to their apparent simplicity, low cost of implementation and environmental friendliness, biological treatments is increasingly becoming a viable alternative to standard methods. Several studies have observed the development of silver nanoparticles with bacteria from biological sources[3], fungi [4]and plants [5]. Due to the extreme high rate of plant extract response and the lack of specific conditions necessary, Plant medium green chemistry has developed as one of modern nano-biotechnology research's active fields.[6, 7]. Production with more stable nanoparticles with maximum output is achieved when plants apply to controlled nanoparticles. Different parameters (Temperature, pH, reaction duration, Ag ion concentration and concentration of plant extract should be regarded when nanoparticles are produced by plant biosynthesis[8].

Silver nanoparticles have been employed in a wide range of applications, including electronics, biological equipment, and the production of several biological and pharmaceutical products[9]. This is most likely owing to the nanoparticles' stability, which is ideal for medicinal applications[10]. Antimicrobial agents such as silver are commonly employed, Silver ions can destroy a wide spectrum of microorganisms by modifying the structure and activities of the cell membrane. (10, 11). Bacteria is destroying at very low concentrations (less than 1–10 μM) of silver nanoparticles, hence they are utilized as a germicide in water purification systems[11]. Antibacterial activity against bacteria has been reported to be conflicting.[12]. Silver can be poisonous to mammalian animals at higher doses, freshwater and aquatic organisms[13]. Apparently, such micromolar silver concentrations have no detrimental impact on humans[14].

Lactate dehydrogenase, abbreviated LDH, is an enzyme that participates in the anaerobic cellular metabolism. It is categorized as an oxidoreductase and is recognized by the enzyme commission number EC 1.1.1.27. The objective of the enzyme is to catalyze the reversible conversion of lactate to pyruvate by converting

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EJCHEM use only: Receive Date: 18 June 2021, Revise Date: 21 August 2021, Accept Date: 05 September 2021
DOI: 10.21608/ejchem.2021.81194.4020

NAD+ to NADH and vice versa.[15] In a number of species including plants and animals, the enzyme is present. It is found in all tissues and functions as a crucial checkpoint in gluconeogenesis and DNA metabolism. According to a species-wide evaluation, LDH has a well-preserved structure with just a few changes in amino acid sequence across species.[16] Because of the structural similarities and small amino acid variations, functional compounds to modify the enzyme's catalytic potential and expression are a natural choice. The interaction of silver ions with sulphydryl groups of proteins or enzymes resulted in metabolic activation for silver nanoparticles (e.g. lactate dehydrogenase)[17].

While Ferritin is a significant iron storage protein that contains ferroxidase activity and preserves a massive iron core in its cavity. A variety of ferritin present in prokaryotes are permitted by traditional 24-mer FTN molecules, BFR heme-containing, smaller 12-mer DPS and the recently identified EncFtn encapsule, which creates an absolutely enormous iron storage cabinet. Recent studies have shown ferritin function to be more dynamic than before and novel pathways for the recycling of ferritin iron have been found[18]. They play an important part in the control and control of cellular iron homeostasis as well as the manufacture of ferritine and they interact with the iron-dependent release mechanism. Some of these pathways are shared by individual and animal cells[19].

The aim of the present work were synthesis of silver nanoparticles using Myrtus communis leaves extract and study the biological applications of the synthesized AgNPs, through evaluation of their antibacterial activity against gram positive bacteria e.g.(staphococcus aurous) and gram negative bacteria e.g.(Escherichia coli). Also, study AgNPs effect on lactate dehydrogenase and ferritin activity in sera of patients with covid-19 disease and control subjects.

1. Experimental

2.1 Materials

Silver nitrate (stock solution) was obtained as supplied from sigma-Aldrich chemicals. The entire glassware rinsed on three occasions, with diluted nitric acid and deionized water then dried, in a heating oven. 2×10^{-2} M of $AgNO_3$ was made by dissolving 0.34 g from the stock solution in 100 ml of deionized water, Boiling 5.0 g of fresh Myrtus communis leaves powder in 100 ml of deionized water for 15 minutes at 70 °C with stirring and filtering the extract through filter paper resulted in a 5 percent (w/v) plant extract. The filtrate extract was kept in the dark at 2º C to be used as reducing agent for further work.

2.2 Synthesis of silver nanoparticles

One ml of plant extract (5%) of Myrtus communis leaves was added drop by drop to 2 ml of the stock solution 2 x 10^{-2} M Silver Nitrate . After completing the volumes to 20 ml with deionized water, then the sample was stirred with heating for one hour at 70º C. After that, its maximum absorbance was measured using U.V.-Visible spectrophotometer. Reducing of Ag^+ to Ag^o nanoparticles, evidenced by the color shift from yellow to brownish and eventually deep brown. The material was eventually dried in order to acquire the manufactured silver nanoparticles for examination.

2.3 Characterizations of silver nanoparticles by multi instruments:

The ultraviolet spectrum has been recorded at room temperature using a Shimadzo UV-1800 spectrophotometer. At room temperature, an infrared Fourier transform infrared (FTIR) spectra was obtained using a Shimadzo FTIR 84005 spectrometer. In order to Preparing an FT-IR analysis sample, The plant extract containing AgNPs was dried for one hour at 60 °C before being combined with an appropriate amount of KBr. A Shimadzu XRD-6000 diffract meter was used to acquire an X-ray diffraction (XRD) pattern to confirm the biosynthesis of AgNPs. The morphology and contact surface of silver nanoparticles were studied using AA300 Angstrom AFM Atomic Force microscopy. Aliquot of plant extract-filters containing silver nanoparticles were evaluated with the SEM S-4160 electron microscope (SEM).

2.4 Anti-bacterial activity

The technique for well-diffusion Staphylococus aureus and E. coli was used to determinate antibacterial activity of silver nanoparticles. The culture was infected with a plate technique. In the case of cultures, brain heart infusion (BHI) broth was incubated with 37˚C for 24 hours. Pathogenic bacteria incubation of Mueller-Hinton agar plates. Every plate has been equipped with sterile paper disk, with a diameter of 5mm and plant extract as control and various levels of produced silver nanoparticles. Afterwards, the dishes were incubated at 37 °C for 24 hours. Measured and tabulated the inhibitory zones.

2.5 Effect of silver nanoparticles on Lactate dehydrogenase (LDH) and ferritin:

Effect of AgNPs on the activity of Lactate dehydrogenase (LDH) and ferritin was examined in the present work. The study was conducted during the period (Abril 2021 to May 2021) on 50 patients (16-50 years) admitted to alyarmok teaching hospital, where they diagnosed with covid-19 infection. Fifty healthy individual (15-50 years) were participated as control group. Venous blood was collected and allowed to cold at room temperature for 30 minutes, serum was pipette and stored at 4℃.

For LDH activity determination, Lactate dehydrogenase (LDH) assay kit was purchased from (Spain react -Spain) to measure the level of LDH spectrophotically. The NADH produced in the lactate-to-pyruvate reaction (L —» P) when Lactate dehydrogenase catalyzes the reaction (L-lactate +NAD^+ <=== > pyruvate + NADH) has been measured at 340 nm by using spectrophotometer at 37 ℃ and pH 6.8 under specified conditions. 1 mL of biosynthesized AgNPs (5%) was added to sera of each groups (patient with covid-19 infection and healthy subjects), then LDH activity was determined once again.

Ferritin assay kit was purchased from (Monobind Inc. -USA) for quantitative determination of circulating ferritin concentration in human serum by micro plate enzyme immunoassay, colorimetric, at 37 ℃ and pH 6.8 under specified conditions. Also 1 mL of biosynthesized AgNPs (5%) was added to sera of each groups (patient with covid-19 infection and healthy subjects), then ferritin activity was determined once again.

2.6 The Kinetic behavior studies

Parameters of lactate dehydrogenase and ferritin in the absence and present of AgNPs were assessed. The reaction mixture was prepared and treated as mentioned in LDH and ferritin assay protocol, LDH and ferritin activity was determined at various constant reactions of both ferritin and LDH substrate for kinetic study. The data of these experiments were used to generate a linear relationship by plotting 1/v values against 1/[S] values for control and patients groups according to Limewater-Burk equation[20].

2. Results and discussion

This work has shown that extract from the Myrtus communis leaves was rapidly created to reduce silver nitrate to silver nanoparticles. UV-visual monitoring of the formation of silver nanoparticles has been performed within 60 minutes with a stirring and heating at 70° C. The reaction was completed. The colorless solution was tinted brown, indicating that silver nanoparticles are formed as seen in the fig (1).

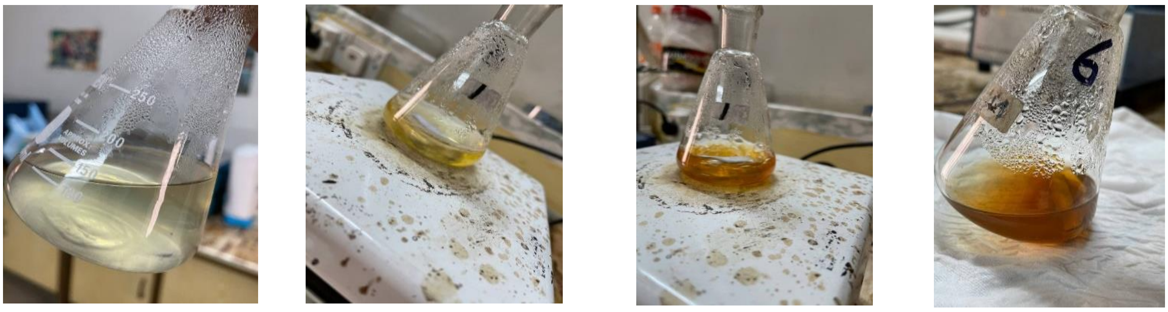


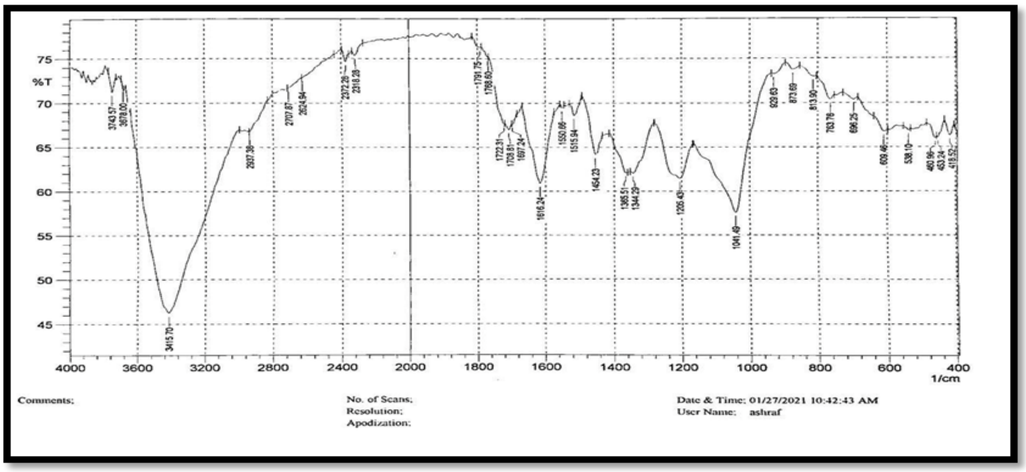
Figure (1) Color changes yellow to brown which indicates the formation of Silver nanoparticles using Myrtus communis leaves extract.

3.1 Nanoparticles characterization

3.1.1 Fourier Transfer Infrared spectroscope (FT-IR)

The (FT-IR) identify the different functional groups presented in *Myrtus communis leaves* extract which perform a position responsible for reduction $AgNO_3$ as capping and efficient stabilization of silver nanoparticles. (Fig 2) shows a typical Fourier Transfer Infrared spectroscope for *Myrtus communis leaves* extract and comparison with (Fig 4) which shows AgNPs synthesized using *Myrtus communis leaves* extract. Comparison of these two spectrum indicated that the FTIR spectra of silver nanoparticle show strong absorption band at 3433 Cm^{-1} Belonged to the stretching vibration of (O-H) the band at 1639

Cm^{-1} developed for (C=O) the stretching band of (C-C), (C-N) shown at 1384 cm^{-1}.



Figure(2)FT-IR spectra for *Myrtus communis leaves* extract.

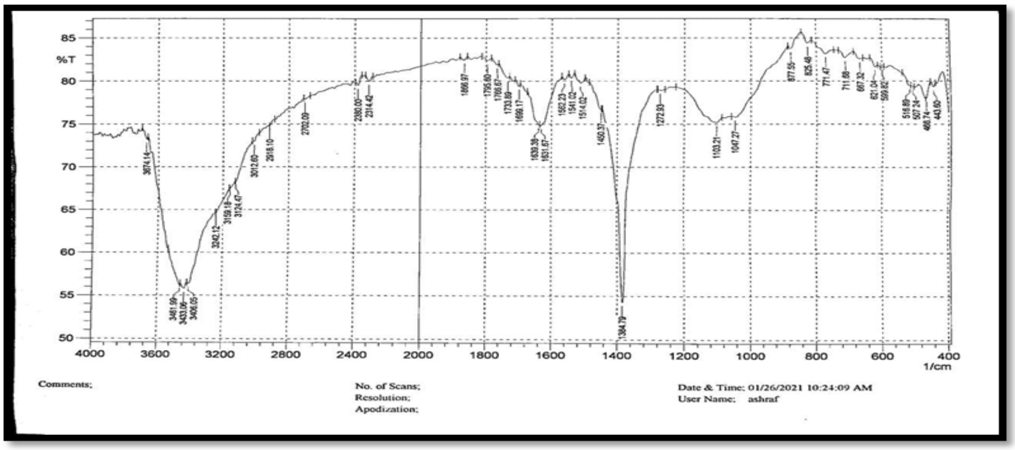


Figure (3): FT-IR spectra of silver nanoparticles prepared by extract of Myrtus communis leaves

3.1.2 The atomic force microscope (AFM)

The AFM study is used to display surface characteristics and determine topography. The (AFM) provides a three dimensional picture of the surface of a nanoparticles at a microscopic resolution. The averaged nano-scale particle diameter equal to the 59.2 nm (fig 4) illustrates the synthesized AgNPs' three-dimensional picture by Myrtus communis leaves extract.

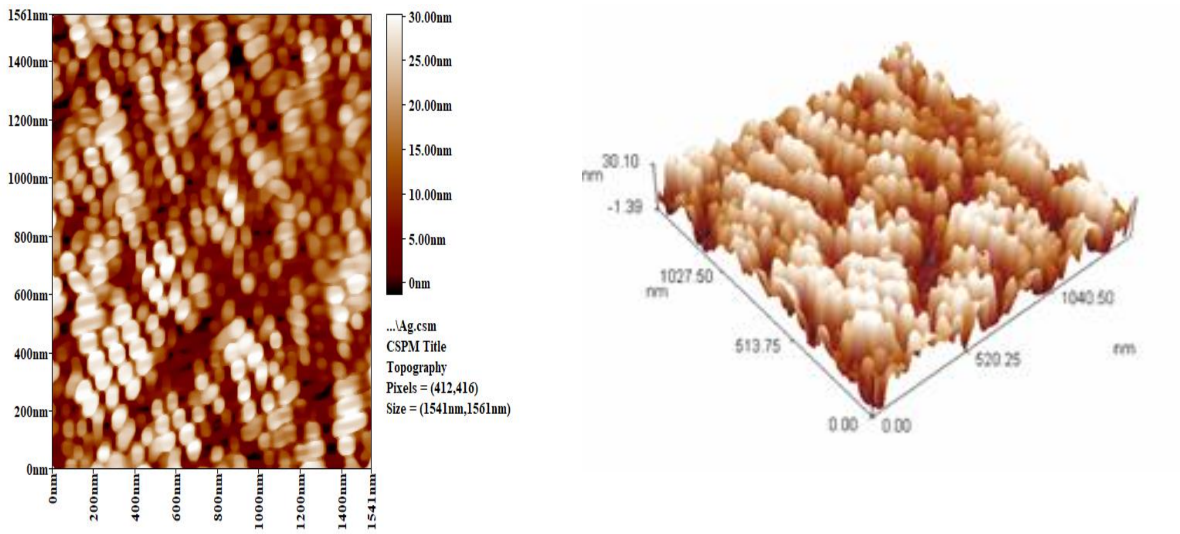


Figure (4): AFM image of synthesized silver nanoparticles using *Myrtus communis leaves* extract

3.1.3 Scanning electron microscopy (SEM)

The size, form, and distribution of produced silver nanoparticles by Myrtus communis leaves extract were studied using a scanning electron microscope (SEM). The particles are spherical, as seen in (Fig 5), with an average size of between (35 to 74 nm).

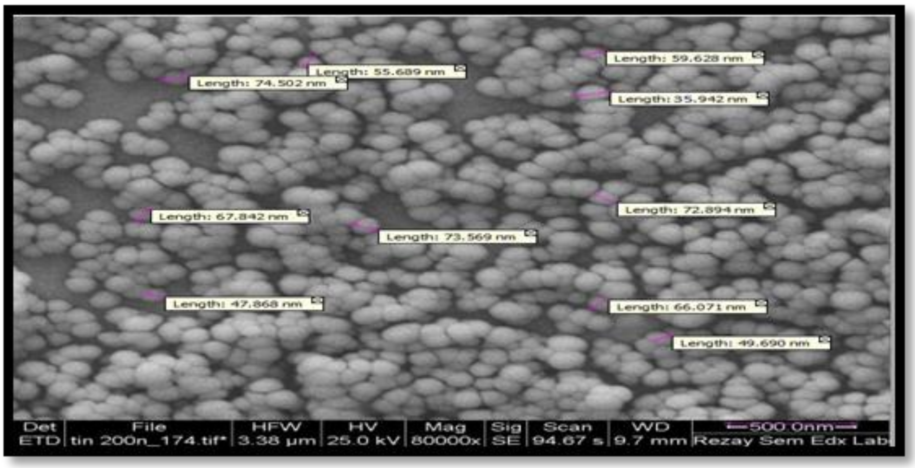


Figure (5) SEM image of silver nanoparticles prepared with Myrtus communis leaves extract

3.1.4 X-Ray diffraction (XRD)

The X-ray diffraction features of the silver nanoparticle formed by the bio reduction was determined using = $(0.9\ \lambda \times 180^{\circ})/\beta \cos \theta\pi$ and was estimated at 15.21 nm (Fig (6).

The XRD pattern for the silver nano particle observe four unique peaks, these peaks were at 40.15 pp, 44.95 pp, 64.45 pp and 77.95 pp, indexed respectively to the cubic face-centered silver planes at 111, 200, 220 and 311. The crystallite size of the sample was calculated from full width at half maximum (FWHM) of the peaks using Debye–Scherrer's approximation (Eq. 1)

$$d = k\lambda/\beta\cos\theta \qquad \ldots\ldots..(1)$$

Where d is the crystallite size, k is the wavelength of CuKa radiation (k = 1.542A°), β is FWHM for the diffraction peak under consideration (in radians), θ is the diffraction angle, and k is the broadening constant (k=0.9).

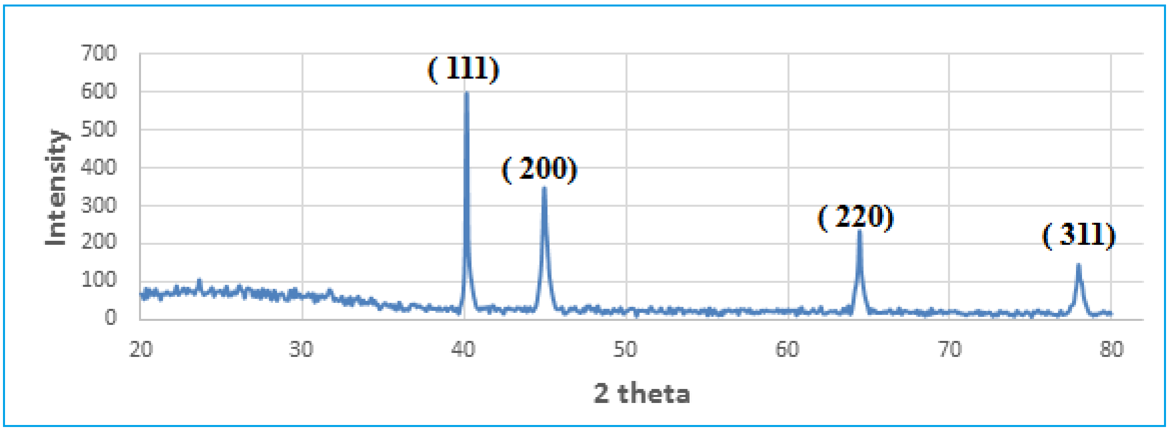


Figure (6) Silver nanoparticle XRD pattern synthesized using extract leaves of Myrtus communis

3.2 Antibacterial effect of synthesized silver nanoparticles

Synthesized silver nanoparticles of Myrtus communis leaves showed antimicrobial action against gram negative E. coli and Gram positive Staphyloccus aureus and assessed area of inhibition as shown in Fig 7 and tabulated in table 1.

The results shown that the varied concentrations of produced silver nanoparticles from the leaves of Myrtus communist show both Gram negative and Gram positive, efficient antibacterial action. Several studies have revealed that silver nanoparticles can kill bacterial spores by damaging membrane integrity [21, 22]. Other studies suggest that silver nanoparticles may interact with phosphorous and sulphide-containing compounds and can harm the DNA and the proteins resulting to cell death; it is obvious that silver nanoparticles might potentially be employed as an efficient bacterial agent against hazardous human infections and can be employed for vital applications in agriculture. AgNPs may readily infiltrate bacteria through the membrane protein sulfate groups which cause structural damage of the bacterium. AgNPs can also be transferred to the cytoplasmic fluid that can damage enzyme protein [23].

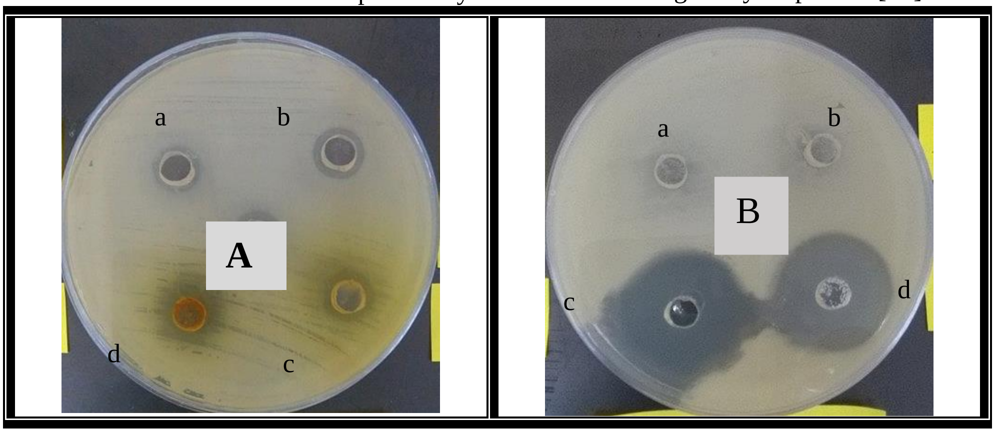


Figure:(7) antibacterial activity for synthesized AgNPs against *(A) E.coli* and (B) *Staphylococcus* using different concentration of *Myrtus communis leaves* extract; a) control b)1 mL c) 1.5 mL d) 2 mL, using *Myrtus communis leaves extract*

Table (1) Zone of inhibition (millimeter) of different concentration AgNPs synthesized using *Myrtus communis leaves* extract against pathogen.

Name of Organism	Inhibition zone (mm)			
	AgNPs(1ml)	AgNPs(1.5ml)	AgNPs (2 ml)	*Myrtus communis leaves* extract
E-coli	6	16	22	0.0
Staphylococcus	9	22	28	0.0

3.3 Effect of silver nanoparticles on Lactate dehydrogenase

The effect of silver nanoparticles (AgNPs) on the activity of enzyme LDH was shown in table (2) for patient and control groups. It is obvious that AgNPs have inhibition effects on LDH activity. The inhibition percent of LDH by AgNPs were (78.8%) and (66.3%) for patient and control groups respectively, as shown in table (2) this decreasing in LDH activity may due to the interaction between silver nanoparticles (AgNPs) and thiol group of the amino acids (e.g. cysteine), which found in enzyme structure [24, 25].

Table (2) Effect of silver nanoparticles on Lactate dehydrogenase activity of control and patients group

	Control groups	**Patient groups**
LDH activity (U/L)	330	994
LDH activity (U/L)with AgNPs	111	210
Percent of inhibition	(66.3%)	(78.8%)

3.4 Effect of silver nanoparticles on ferritin

The effect of silver nanoparticles (AgNPs) on the activity of ferritin was shown in table (3) for patient and control groups. It is obvious that AgNPs have inhibition effects on ferritin activity. The inhibition percent of ferritin by AgNPs were (63.5%) and (87.87%) for patient and control groups respectively, as shown in table (3) this decreasing in ferritin activity may due to the interaction between silver nanoparticles (AgNPs) and thiol group of the amino acids (e.g. cysteine), which found in enzyme structure [26, 27].

Table (3) Effect of silver nanoparticles on Lactate dehydrogenase activity of control and patients group

	Control groups	**Patient groups**
ferritin **activity (U/L)**	137	990
ferritin **activity (U/L)with AgNPs**	50	120
Percent of inhibition	63.5	87.87

3.5 Kinetic studies of LDH

Analyzing LDH binding data was performed using Linweaver-Burk equation [20]. The results showed in table (4) that inhibition type of AgNPs in control group was non-competitive. The enzyme activity reduces; where AgNPs binds equally well to the enzyme whether or not it has already bound to the substrate. V max, Km and Keq was calculated using Linweaver-Burk equation[28].

Table (4): Vmax, Km and Keq in presence and absence of silver nanoparticles in patient group.

Group	**Vmax (U/L)**	**Km (mM)**	**Keq (mM^{-1})**
Patient	1000	0.01	100
AgNPs-patient	250	0.015	66.7

4-Conclusion

In the present work the simplest and cheapest green chemistry methodology was used to produce silver nanoparticles that had improved stability. This one-step approach is particularly adapted for large-scale manufacturing, as it is very quick and removes complex steps used in other organic methods (by using fungi and bacteria). Interestingly, silver nanoparticles showed rather modest concentration of efficient bacterial activity. Based on the current observations, silver nanoparticles may be employed as a bacterial agent to control various pathogenic agents, however additional investigation is required to understand the specific process by which silver nanoparticles infiltrate the wall of the bacterial cell.

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