American Journal of BioScience
2022; 10(4): 153-159
http://www.sciencepublishinggroup.com/j/ajbio
doi: 10.11648/j.ajbio.20221004.15
ISSN: 2330-0159 (Print); ISSN: 2330-0167 (Online)

Anti-*Helicobacter pylori* Activity of Biosynthesized Silver Nanoparticles

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To cite this article:
Randa Mohammed Dhahi, Bushra Shehab Hamad. Anti-*Helicobacter pylori* Activity of Biosynthesized Silver Nanoparticles. *American Journal of BioScience.* Vol. 10, No. 4, 2022, pp. 153-159. doi: 10.11648/j.ajbio.20221004.15

Received: June 3, 2022; **Accepted**: August 19, 2022; **Published**: August 31, 2022

Abstract: *Background:* Recently, there was a great demand for a new, safe, natural, ecofriendly and low cost production of nano-sized silver based antibiotics which become increasingly important in order to resolve the difficulties related with antimicrobial functionalities. *Objective:* The present study investigates the antibacterial activity of the biosynthesized silver nanoparticles from the hot aqueous extract of the red cabbage *Brassica oleracea var capitata f. rubra* against the *Helicobacter pylori* after isolating and characterizing it by microbiological and biochemical tests. *Method:* The silver nanoparticles have been characterized by using the ultra Violet Visible (UV-vis), X-Ray Diffraction (XRD), the Atomic Force Microscope (AFM) and by scanning Electron Microscope (SEM) coupled with Energy Dispersive X-Ray (EDX). The biosynthesized silver nanoparticles from red cabbage were tested for their antimicrobial activity by 96-wells micro titter plate method against pathogenic *H. pylori* that clinically isolated from biopsies of patient with stomach ulcers. *Result:* It was found that the silver nanoparticles have spherical shape with a mean size of 42.34 nm. The study also reveals that the silver nanoparticles have an efficient inhibitory on the bacteria. It was easy to synthesize the silver nanoparticles by this eco-friendly biological method. *Conclusion:* The biosynthesized silver nanoparticles were efficient to be used as natural antibiotic to treat the stomach ulcer.

Keywords: Silver Nanoparticles, *Helicobacter pylori*, Antibacterial Activity of Silver Nanoparticles, Red Cabbage

1. Introduction

Nanobiotechnology is a promising field concerned with the using of nanomaterials, which leads to the enhancement of new human curative against pathogenic microorganisms' modalities [1]. The nanoparticles are a certain group of materials having different applications. It is found that the nanoparticles have applications in determining and characterizing the antimicrobial and treatments [2]. The silver was known as an active antimicrobial and they have other applications in the labs [3].

The *Helicobacter pylori* are negative bacteria of gram stain which occupies the infectious epithelial layer of the person [4]. About 50% of the population in the world has this bacteria and the resistance of this bacteria against the antibiotics is due to Genetic variation and its ability to develop the biofilm [5].

The red cabbage is regarded as one of the winter leafy crops in Iraq and it belongs to the *Brassicaceae* family which originally grows in the east of the Mediterranean Sea. This plant grows in partially cold weathers. The terminal buds were the most important part of this plant [6]. The study was employed for AgNPs synthesized biologically for antibacterial activity that may have an important application in the field of nanomedicine.

2. Experimental

2.1. Preparation of Aqueous Extract

The aqueous extract was prepared by dissolving 25gm of red cabbage leaves in 150 ml of distilled water and heated in Soxhlet at a temperature of 45 for 30 minutes and then the extract was filtered by sterile filter paper and placed in the refrigerator at 4 until use. A high-performance liquid chromatography (HPLC) was used to detect the active substances existing in the aqueous extract of the red cabbage.

2.2. Biosynthesis of Silver Nanoparticles

Ten milliliters of aqueous extract of the red cabbage were mixed with 100 ml of silver nitrate ($AgNO_3$) solution in three concentrations (1mM, 2mM, 5mM) with continuous stirring using shaker with observing the colour change of the extract [7]. For silver nanoparticles, the solution turned from purple to bright brown.

2.3. Characterization of Silver Nanoparticles

2.3.1. Ultra Violet Visible (UV-Visible) Spectroscopy

The biosynthesized silver nanoparticles were analysed for surface plasmon resonance by use of a UV- visible spectrophotometer at the wavelength of (190-1100) nm. Deionized water was used as a blank and the background absorbtion was subtracted from UV-Vis spectrum of biosynthesized sliver nanoparticles.

2.3.2. Scanning Electron Microscope Coupled with Energy Dispersive X-Ray (EDX)

The scanning electron microscope coupled with EDX was used for observing the morphology and size of nanoparticles. The elemental analysis of AgNPs was performed by using EDAX spectrometer. Samples for microscopy were prepared by dropping 10µl of biosynthesized AgNPs sample on a copper grid. Excess solution was removed by using a piece of soft filter paper. The copper grid was then dried for six hours in an oven at 80°C. [8].

2.3.3. X-ray Diffraction Analysis (X-Ray Diffraction)

The X-ray diffraction (XRD) pattern of powdered sample of AgNPs was recorded on XRD system for the purpose of determining the crystalline shape of the samples [9].

2.3.4. Atomic Force Microscope

The atomic force microscope was used for imagining the surface topography of silver nanoparticles and determining their size [10] AFM Image was taken with silicon cantilevers with 0.02–0.77 N/m force constant, tip height 10–15 nm, contact mod.

2.4. Isolation and Diagnosis of Helicobacter pylori

2.4.1. Bacterial Samples

Tissue biopsy samples were collected for isolating bacteria from patients with stomach ulcers and those referred to the endoscopy division of the Gastroenterology and Liver Diseases Hospital for the period from 3/11/2018 to 3/3/2019. Two biopsies were taken from each patient with stomach ulcers by means of special forceps Endoscope. One of the samples examined by the rapid urease test for ensuring the presence of bacteria in biopsy [11], and the others was transferred to the laboratory for performing bacterial culture in less than two hours [12].

2.4.2. Bacterial Culture

The tissue biopsies was allocated to isolate the bacteria (two biopsies for each patient) on a sterile glass slide, as each of them was cut into small pieces and mixed well and then transferred by the loop, It was cultured on Columbia blood agar base, where the dishes were placed in an anaerobic Jar sterilizer container, where the conditions for this bacterium are met [13]. The gas generating kit was used for the purpose of providing the required gas conditions, namely O_2, CO_2, N_2, where the anaerobic container is placed in the incubator at a temperature of 37°C for a period of (4-7) days [14].

2.4.3. Diagnosis of Bacteria

The bacteria were apparently diagnosed by colonial form and the use of a Gram stain. Diagnosis was made through biochemical tests using the enzymes: catalase, oxidase and urease.

2.5. Anti-H. pylori Activity

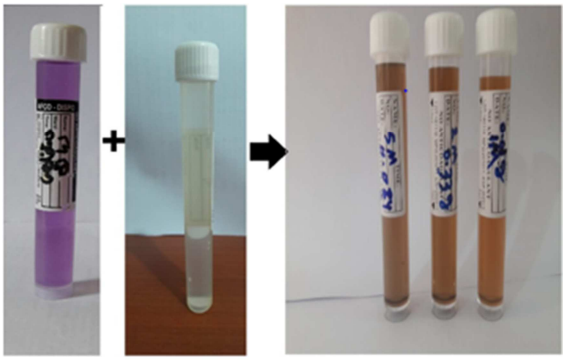
The biosynthesized silver nanoparticles from red cabbage were tested for their antimicrobial activity by 96-wells micro titter plate method against pathogenic *H. pylori*. The silver nanoparticles formed by using the aqueous extract of the red cabbage have been tested for inhibition of the biofilm of bacteria using 96-well micro plates and screening for inhibition by using the UV spectrum, where four bacterial isolation in the Brian heart infusion broth medium has been activated and diluted. The diluted bacterial culture medium was transferred to the micro titration dish and incubated for 24 hours at 37°C, poured the contents of the plates and washed with distilled water to remove the floating bacteria and left to dry at room temperature for 15 minutes, then silver nitrate, aqueous extract of red cabbage, esomeprazole and biosynthesized nanoparticles were added, then Brian heart infusion broth was added to the plates and incubated for 24 hours at a temperature of 37°C, then the crystal violet dye was added to the plates and left for 20 minutes, washed several times with distilled water and allowed to dry at room temperature for 15 minutes. Add ethyl alcohol to each well, and then read the optical density at wavelength 630 nm using the ELISA reader [15].

2.6. Statistical Analysis

Statistical analysis of the data was performed after tabulation with the SAS program according to the CRD design of the Dunkin test at a probability level of 0.05.

3. Results and Discussion

The aqueous silver ions were reduced to silver nanoparticles when added to natural red cabbage extract, it was observed that the color of the solution turned from purple to bright brown (Figure 1) resulting from the excitation of the surface of the plasmon in the nanoparticle. The active substances present in the aqueous extract of the plant, such as flavonoids, anti-oxidant and anti-microbial phenols, were detected through the use of a high-performance liquid chromatography device. As a result of the reduction of the silver ion that shows the formation of nanoparticle and this is due to the phenomenon of plasmon surface resonance. This phenomenon is a physical process that can occur when light accumulated on a metal slice collides under conditions of total reflection. Another study has shown the same results, the silver nanoparticles have unique optical and electrical properties and are being combined into products that range from biological and chemical sensors [16].

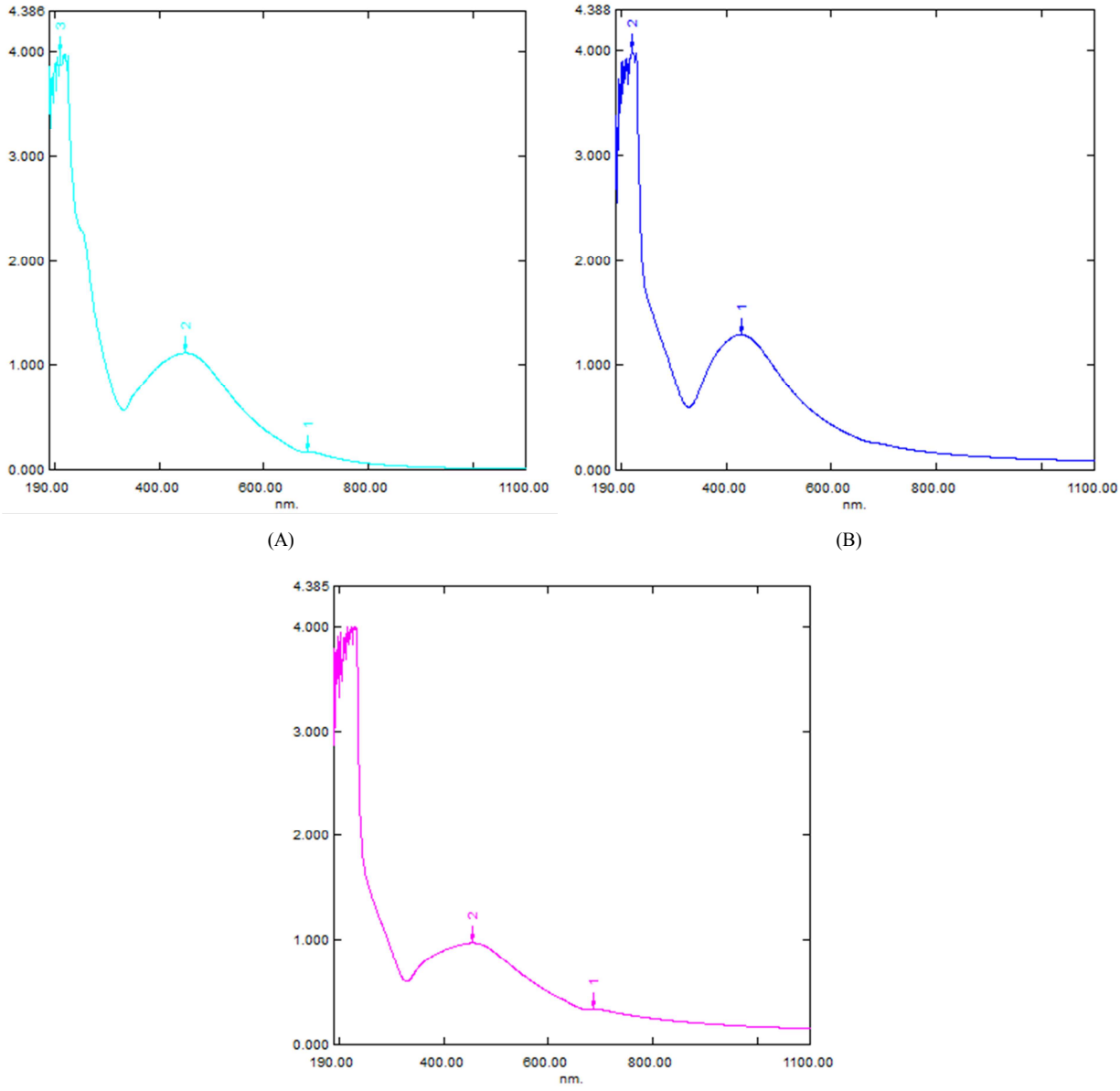


(A) (B) (C)

Figure 1. *Visual observation for the biosynthesized silver nanoparticles from the aqueous extract of red cabbage: (A) represents the aqueous red cabbage, (B) represents $AgNO_3$ solution and (C) represents three concentrations of nanoparticles with the color changed to brown within about 30 minutes.*

The UV-Vis absorbs waves of different sizes for nanoparticles that are due to the phenomenon of plasmon surface resonance [17], and this is one of the most frequently used reasons for diagnosing the composition of nanoparticles [18]. The figure 2 below shows the UV-Vis spectrum of nanoparticles with a range of 190-1100nm. This result showed that the highest peak of absorption of A was at the wavelength of 210nm, B was at the wavelength of 222nm and C was at 456nm. UV-VIS is known in the search for the shape and uniform size of nanoparticles.

Analysis of the SEM shows the nanoparticles size of (42.34) nm in addition to the spherical shape of the nanoparticles (Figure 3). The XRD was used in this study for the purpose of studying the structure and crystalline nature of nanoparticles. The results showed different diffraction values as in Figure 4.



(A) (B)

(C)

Figure 2. *Shows the nature of UV-Vis of the silver nanoparticles (A) = 1mM, (B) = 2mM, (C) = 5mM).*

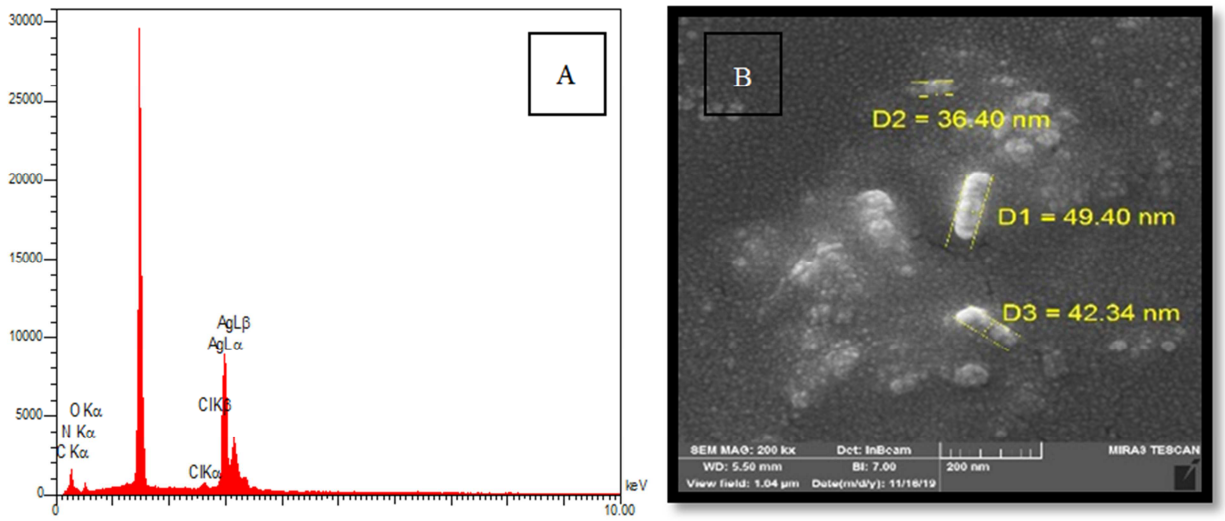


Figure 3. *(A shows a graph of EDX for silver nanoparticle synthesized from aqueous extract of the red cabbage.) (B) shows the shape of silver nanoparticles under (SEM).*

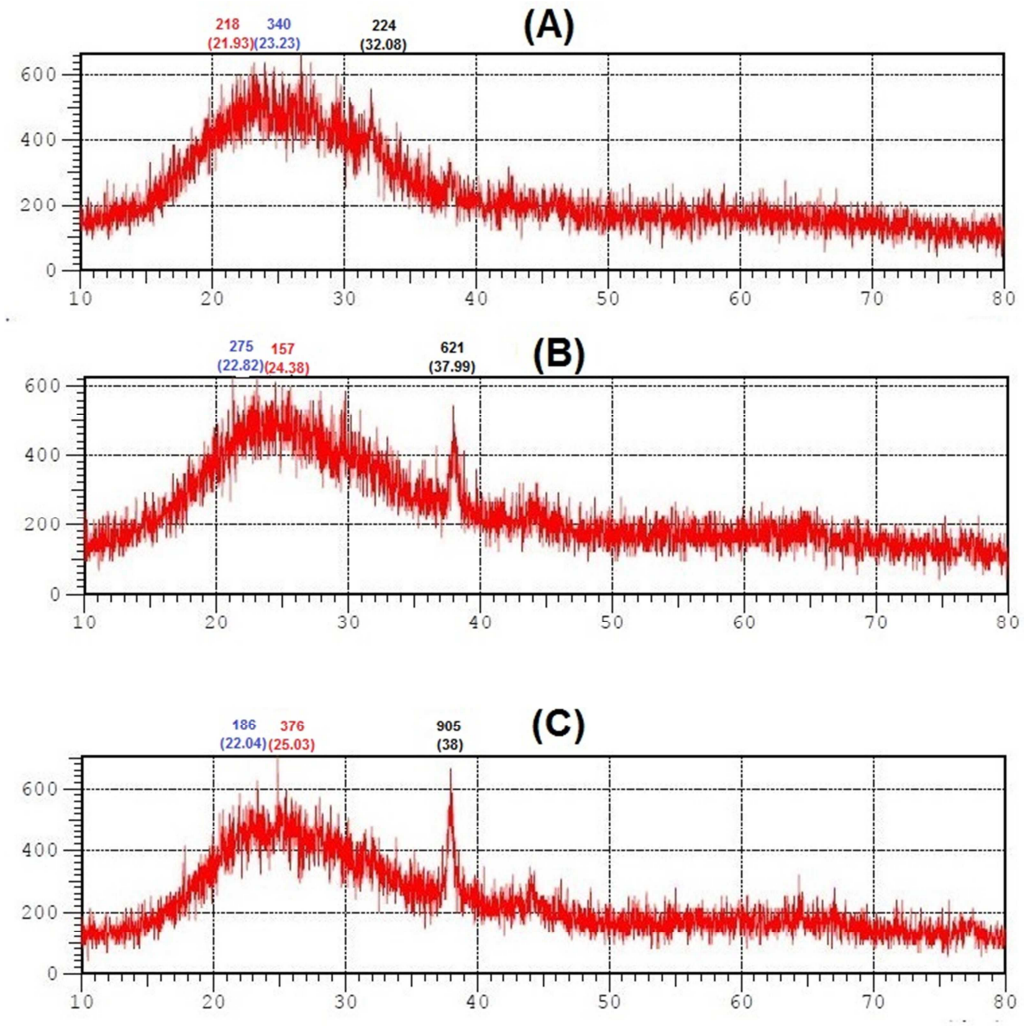


Figure 4. *Shows The XRD of three concentrations of silver nanoparticles (A=1mM, B=2mM, C=5mM) synthesized by biological method from the aqueous extract of red cabbage plant.*

The topographical image of spherical silver nanoparticles and mass of silver is reported in Figure 5, it was observed that the silver nanoparticles clustered and formed distinct nanostructures.

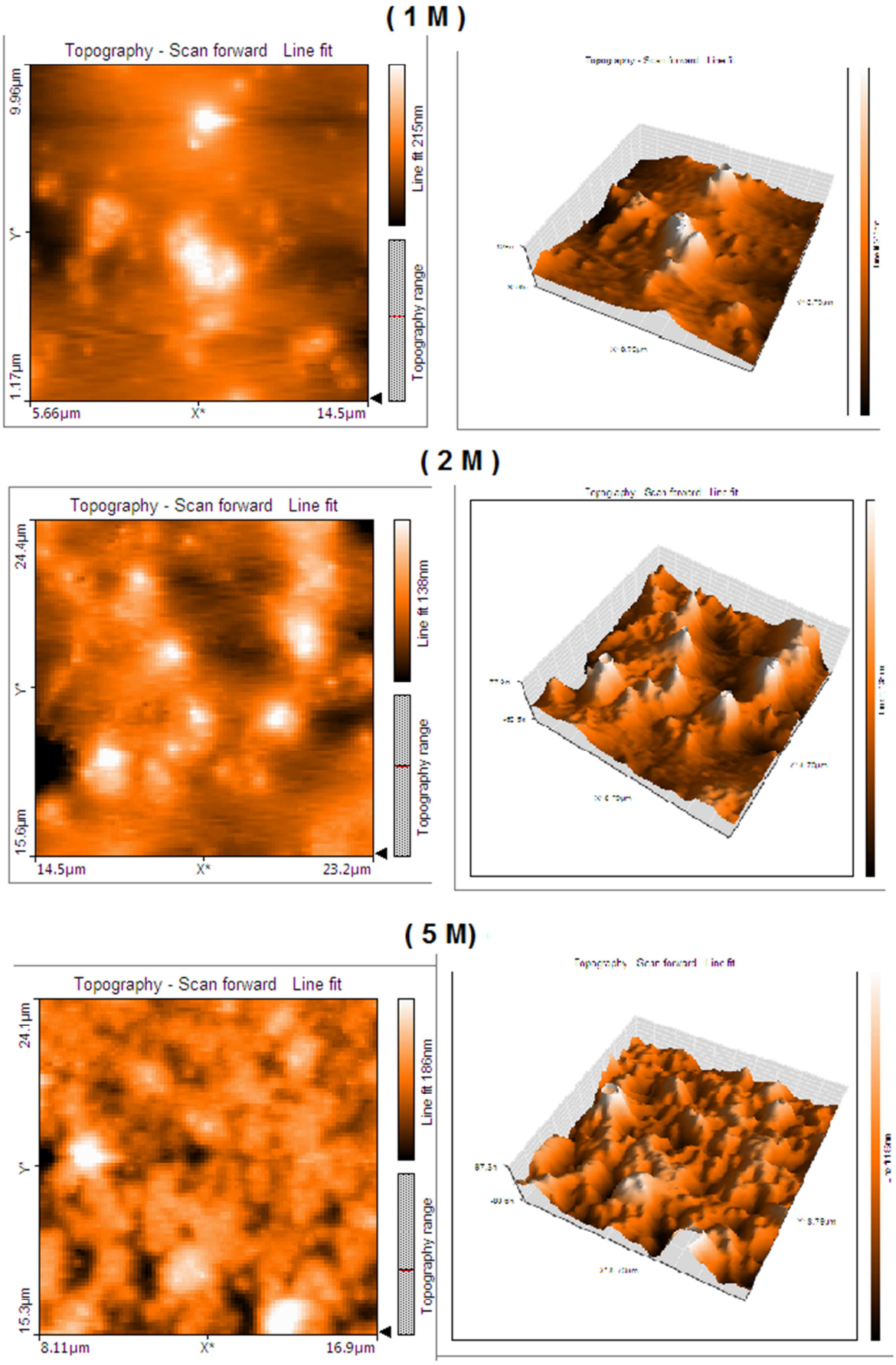


Figure 5. *Shows three-dimensional images of the topology of the surface by AFM of the silver nanoparticles in three concentrations (1mM, 2mM, 5mM).*

The bacteria were detected in tissue biopsy by rapid urease test, and isolates were diagnosed depending on tests followed for this purpose, which are Gram stain, where the bacteria appeared in the spiral and spherical shapes, and the diagnosis using urease, oxidase, and catalase tests showed all positive results. The effect of nanoparticles of silver nanoparticles has been tested against the biofilm of *H. pylori* bacteria using 96-well micro titration plates (table 1, Figure 6).

Table 1. *Shows the percentage of inhibition the biofilm for H. pylori in comparison with the control sample and different treatments (aqueous extract of the red cabbage, $AgNO_3$ in three concentrations (1mM, 2mM, 5mM), silver nanoparticles (1mM, 2mM, 5mM).*

strain	control	aqueous extract of the red cabbage	$AgNO_3$ (1mM)	$AgNO_3$ (2mM)	$AgNO_3$ (5mM)	AgNP 1mM	AgNP 2mM	AgNP 5mM
1	0	5.85	56.27	78.7	73.57	83.25	86.52	85.57
	A	I	H	E	F	D	B	C
2	0	9.82	54.82	76.5	72.25	84.22	84.27	87.82
		G	F	D	E	C	C	B
3	0	6.22	62.27	76.8	72.82	85.15	86.55	92.52
	A	I	H	E	F	D	C	B
4	0	1.52	59.82	78.2	79.7	85.25	85.75	86
	A	F	E	D	C	B	B	B

Note: the letters (A-I) represent the order of transactions according to Dunkin's method in statistical analysis.

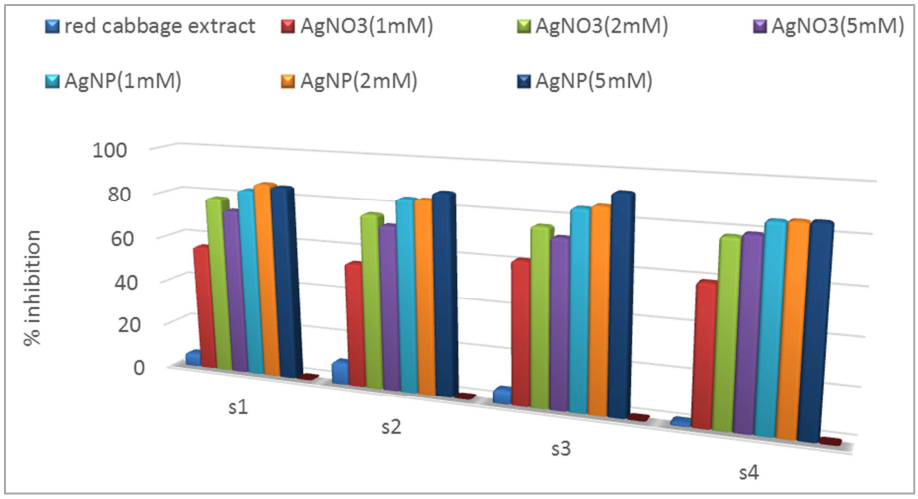


Figure 6. *Shows the percentage of inhibition the biofilm for H. pylori in comparison with the control sample and different treatments (aqueous extract of the red cabbage, $AgNO_3$ in three concentrations (1mM, 2mM, 5mM), silver nanoparticles (1mM, 2mM, 5mM).*

In this study, the effect of nanoparticle minutes against four strain of *H. pylori* bacteria was evaluated where results showed that spherical nanoparticles had The growth and replication of the four isolates were inhibited with different inhibitory percent for the three concentrations as shown in the table 1. As the highest rate of inhibition of nanoparticles in the first strain was (86.25%) in relation to the concentration of (2mM) of nanoparticles, while the lowest rate of inhibition of nanoparticles in the same strain was (83.25%) with respect to the concentration of (1mM). In the second strain, the highest inhibition rate for nanoparticles reached (87.82%) with respect to the concentration of (5mM) for nanoparticles and the lowest inhibition rate for nanoparticles reached (84.22%) for concentration (1mM) for nanoparticles. As for the third strain, the highest rate of inhibition of minutes reached (92.52%) for concentration (5mM) for minutes and the lowest inhibition rate was (85.15%) for concentration (1mM). In the fourth strain, the highest rate of inhibition of minutes reached (86%) in relation to the concentration of (5mM) and the lowest rate of inhibition reached (85.25%) of concentration (1mM) of minutes, compared to the control sample (99.57%), in addition to a comparison with other treatments including the aqueous red cabbage extract, the aqueous solution of the silver nitrate with three concentrations. As their study showed that the gold nanoparticles produced from *Moricandia nitens* plant have an inhibitory effect on these bacteria by a structural change in the membrane [19]. This can lead to an increase in the permeability of the bacterial cell and this leads to an uncontrolled transfer across the cytoplasmic membrane, and ends with the death of the cell [20]. The differences between bacterial strains in terms of inhibition ratios may be due to the genetic makeup of the bacterial strains. these results find the small sizes of AgNPs loading on make it a promising tool for biomedical applications.

4. Conclusion

The silver nanoparticles have been produced by *Brassica*

oleracea var *capitata f. rubra* hot aqueous extract, by cost-effective, effective and eco-friendly method. UV–visible spectroscopy, XRD, AFM and SEM coupled with EDX techniques have confirmed the reduction of silver nitrate to Ag nanoparticles. The biofilm inhibition by micro titrer plate test showed that the silver nanoparticle synthesized biologically has the effective anti *H. pylori* activity that could be suggested as an efficient natural antimicrobial for medical applications.

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