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SYNTHESIS OF SILVER NANOPARTICLES FROM LEAF EXTRACT OF OLIVE AND FIG WITH SILVER NITRATE AND EFFECT ON ECTO-5'- NUCLEOTIDASE (5'-NT), ADA AND AMPDA ENZYMES IN SERA OF ARTHROSCLEROSIS PATIENTS

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ABSTRACT

There is an increasing commercial demand for nanoparticles due to their wide applicability in various areas such as electronics, catalysis, chemistry, energy, and medicine. Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used are quite often toxic and flammable. In this work, we describe a cost effective and environment friendly technique for green synthesis of silver nanoparticles from 1 mM $AgNO_3$ solution through the extract of leaf extract of olive and fig as reducing as well as capping agent. Nanoparticles were characterized using UV-Vis absorption spectroscopy, atomic force microscopy (AFM) and scanning electron microscope (SEM). Also the effect of these nanoparticles on adenosine aminohydrolase (ADA), AMP-aminohydrolase (AMPDA) and Ecto-5'-nucleotidase (5'-NT) activities in sera of 80 patients with atherosclerosis and 70 control person. The AFM and SEM analysis showed the average particle size of 102.76 nm with olive, 88.23 nm with fig, while the sizes of silver nanoparticles from mix fig and olive as a ratio 1:1, 1:4, 1:6 and 3:5 were (103.00, 77.49, 103.58 and 97.48) nm, respectively. The current study suggest that the nanoparticles are a good inhibitory effects on 5'-NT, ADA and AMPDA enzymes in sera of control and patients with arthrosclerosis.

Key words: Fig, Olive extract, Silver nitrate, Silver nanoparticles.

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INTRODUCTION

In the recent years, we note a wide attention of researchers in the field metal nanoparticles. Due to their properties electronic, mechanical, optical, magnetic and chemical properties that are significantly different from those of bulk materials. These properties may be attributed to their small sizes and large specific surface area. Metallic nanoparticles are used in many applications in deferent fields as catalysis, electronics, and photonics[1].

Silver nano crystals are one of the most attractive inorganic materials. Its has a large applications in photography, catalysis, biosensor, bimolecular detection, diagnostics, and particularly antimicrobial activities also its has environmentally nature. A number of methods were used in the past for synthesis silver nanoparticles like reduction in solutions, radiation assisted chemical and photo reduction in reverse micelles, thermal decomposition of silver compounds and recently green-synthesis route[2]. The common Fig (*Ficuscarica*, family Moraceae) is a blessed tree, Allah the Al-mighty has sworn by it in Quran to draw our attention to their great benefits to humans. It has been employed as an important food crop for thousand years, it contains copious milky sap which called latex. The latex and extract from leaves of Fig are much employed in folk medicine and have several pharmacological properties in traditional medicine and the benefits have generally been ascribed to its antibacterial effect[3].

In this study, we used the natural olive oil as a stabilizer and which is derived without any chemical reaction. As we know that the olive oil is improbable to cause allergic reactions, so that its save to use used in preparations for lipophilic drug ingredients. The olive oil is very active in controlling of heart disease, cholesterol level, stroke. Furthermore, the usage of iron oxide nanoparticles with olive oil coating during the treatment may have the advantage to the blood cells. Olive oil is a triacylglycerol of long chain fatty acids with free fatty acids (FFA), polyphenols, peroxides, polycyclic aromatic hydrocarbons (PAHs), vitamin E and vitamin K[4]. In this study, we investigated the synthesis of stable silver nanoparticles using aqueous Olive foliate extract and Fig foliate.

EXPERIMENTAL

Methods

Preparation of leaf extract

Olive and Fig leaves were sun dried after washed with water to remove the residual moisture. About 10 g of leaves and 200 mL of distilled water were boiled for 10 min until

the color of solution changes light yellow with olive, direct yellow with Fig and yellow of mixture. The extract was cooled at room temperature and filtered through filter paper. The supernatant was stored at room temperature for additional nanoparticles synthesis process.

Silver nanoparticles synthesis

Five mL of leaf extract was added to 50 mL of 1×10^{-3} M silver nitrate and incubated at room temperature. Formation of gray color with olive extract mixture, while light gray with fig extract after 60 min was indicates silver nanoparticles synthesis.

Characterization of green synthesized silver nanoparticles

Silver nanoparticles (Ag^0) was spectrometerically recognized by UV-Vis spectrophotometer (Perkin Elmer, Singapore) (400-700 nm). Morphological characters such as size and shape of green synthesized silver nanoparticles was analyzed by Atomic Force Microscope.

Effect of AgNP on bacterial growth

We studied the growth of *E. coli, Pseudomonas, Bacillus and staphylococcus* bacteria in the presence of silver nanoparticles and were grown in liquid LB medium. The LB liquid medium were diluted in to optical density (OD600) 1×10^{-3} M. Different concentrations of AgNP solution was added into the cell culture and incubated at 37°C, 250 rpm.

Determination of AgNPs on the human sera adenosine aminohydrolase (ADA), AMP-aminohydrolase (AMPDA) activities and Ecto-5'-nucleotidase activity (5'-NT)

The effect of AgNPs on the levels of ADA, AMPDA and 5'-NT activities in sera of 80 patients with atherosclerosis and 70 healthy persons to be used as control ranging between (45-75) years. These patients were hospitalized at Research Institute for Educational Laboratories in the city of Medicine of the Ministry of Health in Baghdad. Five millilitre of blood was collected and it was allowed to clot for 10-15 min. Serum was removed after centrifuged. The ADA activity was determined according to Giustimethod[5]. The activity was measured using spectrophotometer, ADA unit defined as is the amount of enzyme, which forms one micromole of ammonia in (1) min. Determination of AMPDA activity was carried out according to Gromashevskaia method[6]. Ecto-5'-nucleotidase activity was measured in serum according to Wood and Williams's method[7].

RESULTS AND DISCUSSION

UV-VIS Spectroscopy analysis

The synthesized silver nanoparticle using Olive, Fig and the mixture of olive and extracts (Fig. 1) showed the UV–visible spectra of silver nanoparticle formation using constant $AgNO_3$ concentration (1×10^{-3}M) with olive extract concentration sat room temperature after 60 min. The UV-Vis spectrum of silver nanoparticles solution from olive and fig extracts have maximum absorbance peak at 441.40 nm and 230 nm, respectively, while the mix olive with fig extract has maximum absorbance peak at 236 nm.

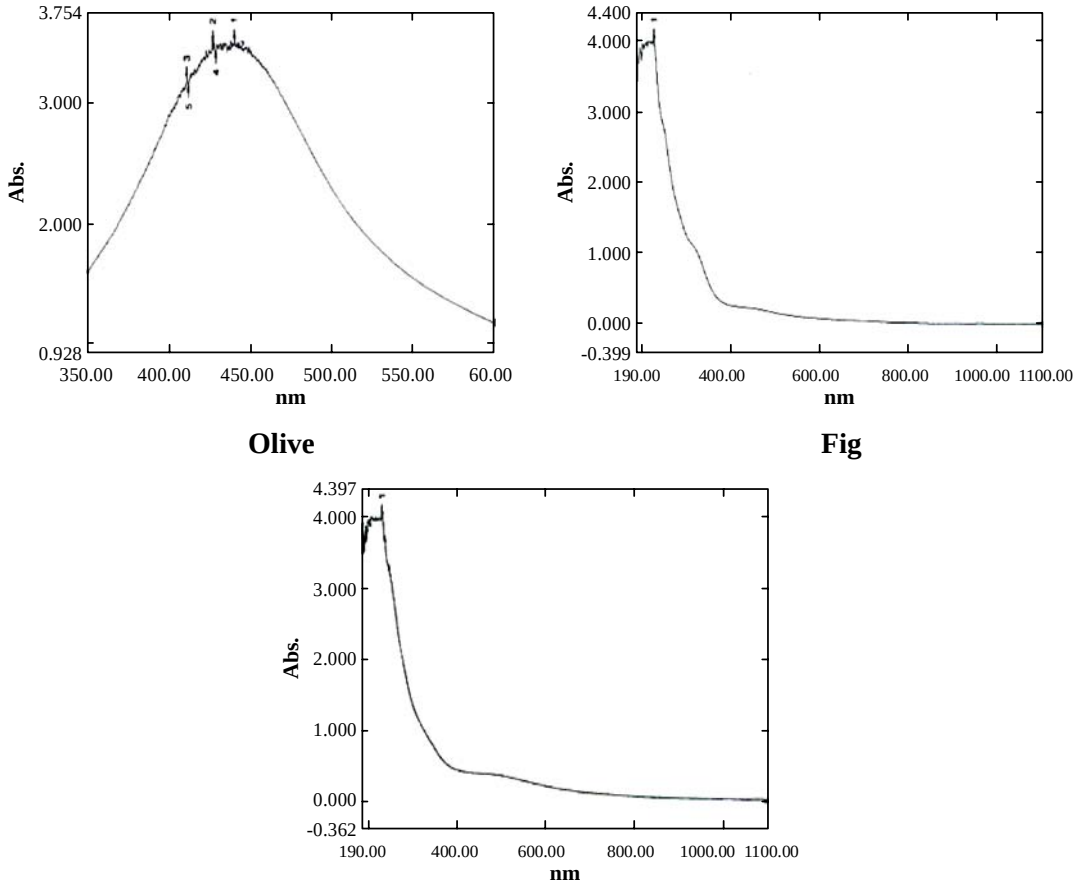
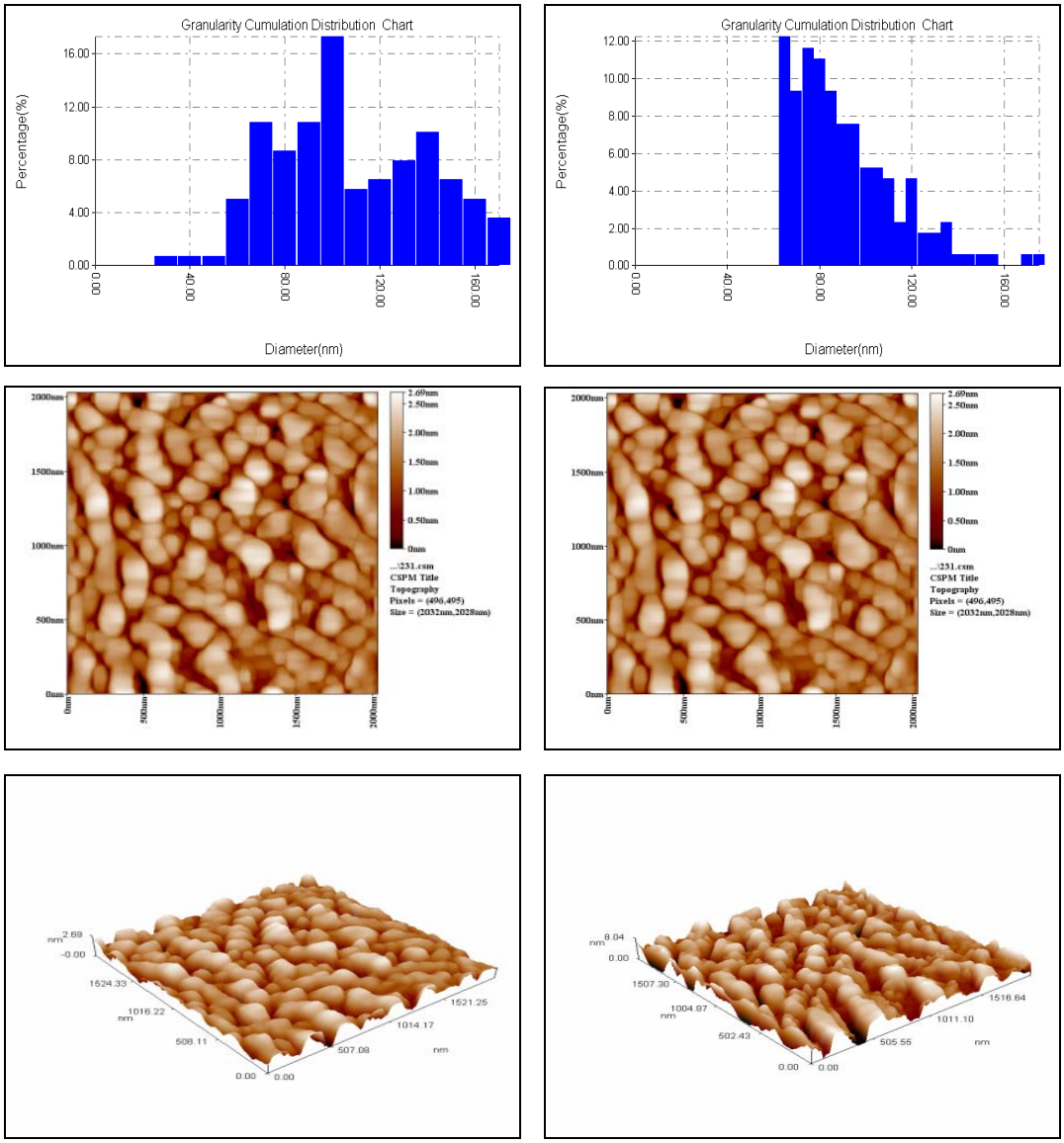


Fig. 1: UV–visible spectra of silver nanoparticle formation using constant $AgNO_3$ concentration (1×10^{-3}M) with Olive, Fig and Olive, Fig mixture extract concentration sat room temperature after 60 min

Atomic force microscopy

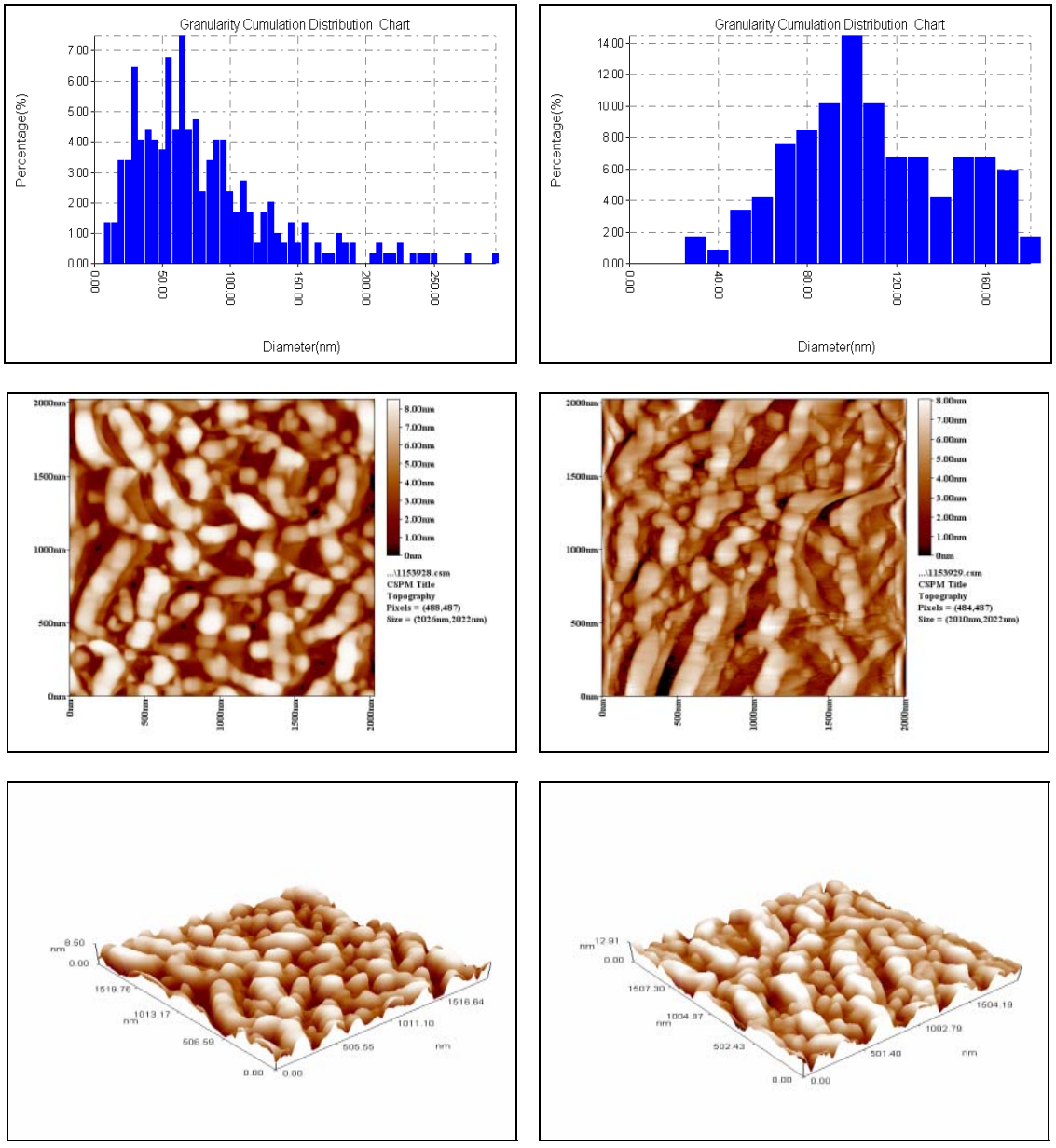
Atomic force microscopy analysis (AFM) was used to study the surface topology of the formulated silver nanoparticles (Fig. 2, A and B) for extract Olive and Fig.



A [Olive] B [Fig]

Fig. 2: AFM image of silver nanoparticles from extract (A) Olive 2D image and 3D image, (B) Fig 2D image and 3D image

Fig. 3 showed the AFM image of synthesized silver nanoparticles from mixture of Fig and Olive; A- presentation 1:1 ratio, B-presentation 1:4 ratio, C-presentation 1:6 ratio and D- presentation 3:5 ratio.



A[1:1 ratio] B[1:4 ratio]

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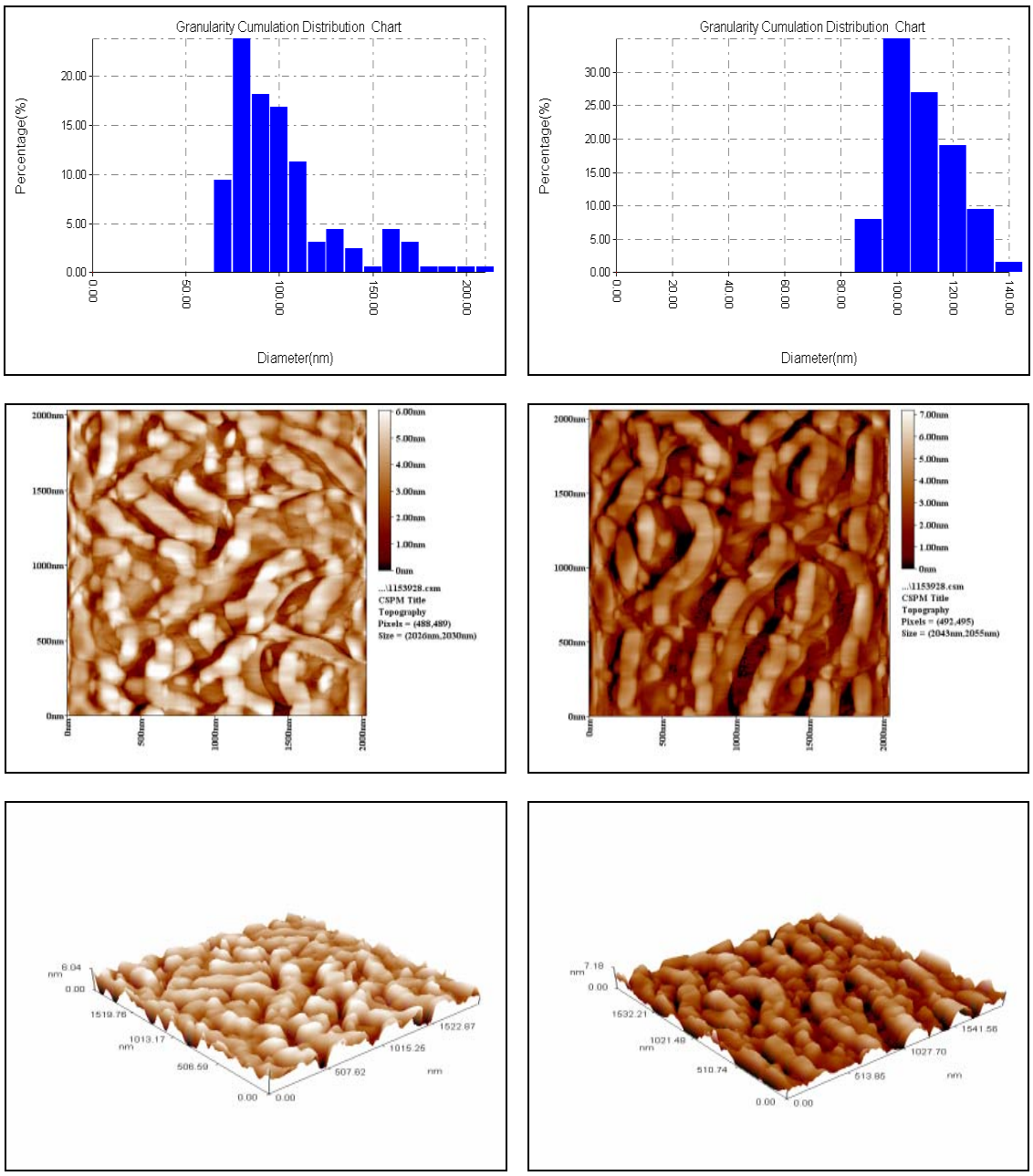

C[1:6 ratio] D[3:5 ratio]

Fig. 3: AFM image of synthesized silver nanoparticles from mixture of Fig and olive; A- presentation 1:1 ratio, B-presentation 1:4 ratio, C-presentation1:6 ratio and D-presentation 3:5 ratio

Scanning electron microscope (SEM)

For SEM analysis silver nanoparticles are completely dried. Fig. 4 showed (A) SEM analysis of Ag NPs synthesized by Olive extract, while (B) showed the SEM analysis of Ag NPs synthesized by Fig extract, (C) and (D) SEM analysis of Ag NPs synthesized by Fig and Olive mixture [1:1 and 1:6 ratio] extract, respectively.

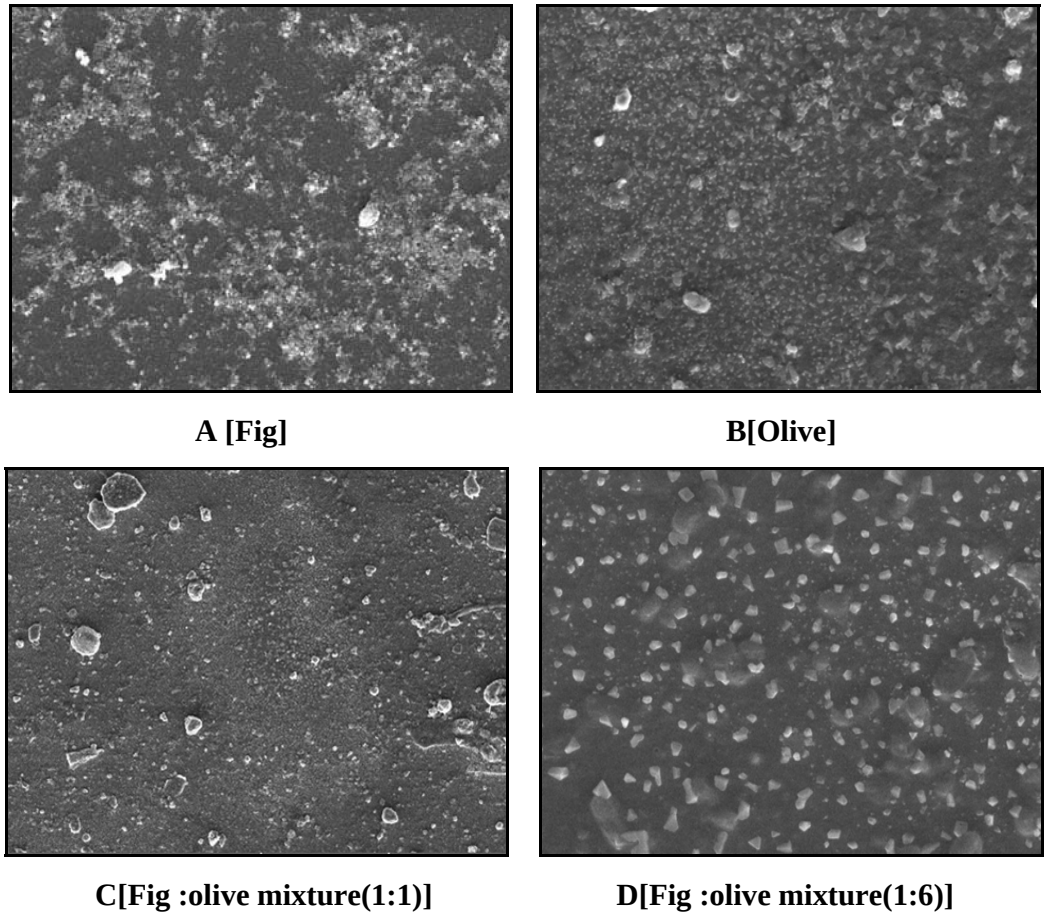

Fig. 4: (A) SEM analysis of Ag NPs synthesized by Olive extract and (B), SEM analysis of Ag NPs synthesized by Fig extract (C) and (D) SEM analysis of Ag NPs synthesized by Fig and Olive mixture [1:1 and 1:6 ratio] extract

Effect of AgNP on bacterial growth

The effect of silver nanoparticles from mixture of Fig and Olive; A- presentation 1:1 ratio, B-presentation 1:4 ratio, C-presentation 1:6 ratio and D-presentation 3:5 ratio were

investigated by growing *E. coli, Pseudomonas, Bacillus and staphylococcus* on agar plates and supplemented with AgNP. The result didn't referred any inhibition effect on bacterial growth with presence of AgNP on the nutrient agarplate.

Effect of AgNPs on the sera ADA, AMPDA and 5'-NT activities

In the previous study, we referred to a highly significant elevated in the activates of ADA, 5'-NT, and AMPDA enzymes in atherosclerosispatients[8]. The inhibitory influence of the silver nanoparticles from mixture of Fig and Olive; A- presentation 1:1 ratio, B-presentation 1:4 ratio, C-presentation 1:6 ratio and D- presentation 3:5 ratio on the activity of ADA, 5'-NT, and AMPDA were showed in (Table 1 and 2). The percentage of inhibitory effect were showed highly when using a mixture of Fig and Olive on 1:6 ratioin both patients and control groups (Table 1 and 2).

The UV-Vis spectrum of colloidal solution of silver nanoparticles from Olive and Fig extracts have maximum absorbance peak at 441.40 nm and 230 nm, respectively, while the mix Olive with Fig extract has maximum absorbance peak at 236 nm, which is proved the synthesis of silver nanoparticles in the colloidal solution. The shape and position of the (olive, fig and mix.) absorption depends on the particles shape, size and the dielectric constant of the surrounding medium. Generally, the (Olive, Fig and Olive, Fig mixture) extract bands are effect by morphology, size, shape, composition and dielectric environment of the prepared nanoparticles[9].

The micrographs shows that the formulated Ag NPs have spherical shape and the calculated sizes in the 102.76 nm with olive, 88.23 nm with Fig[10]. Figure 3 for mixture Olive and Fig have the calculated sizes in the (103.00, 77.49, 103.58 and 97.48).

To the best of our knowledge, no previous study showed to these result. Fig. 4 showed (A) SEM analysis of Ag NPs synthesized by olive extract and (B), SEM analysis of Ag NPs synthesized by Fig extract, (C) and (D) SEM analysis of Ag NPs synthesized by Fig and Olive mixture [1:1 and 1:6 ratio] extract. The dry specimen was mounted on specimen state using anathema which as epoxy resin or electrically conductive adhesive tape before examination in microscope. In scanning electron microscope, the analysis size of silver nanoparticles was between 80 nm-100 nm with different magnificationsnm[11].

Chemicals antimicrobial agents are progressively will be resistant to an extensive series of antibiotics. Another way to conquer the drug resistance of numerous bacteria hence instantly needed. Though, the use of Ag salts as antimicrobial agents subject to some of restrictions and limitations, these may be due to the interfering effects of salts.

Table 1: The degree of inhibition of enzymes activities at mixture of Fig and Olive, using 60 patients with atherosclerosis samples

	ADA Activity[U / L] Patients group [n = 80]			**AMPDAActivity [U /L] Patients group [n = 80]**			**5'-NT Activity [U/L] Patients group [n = 80]**		
	48.85 ± 15.35 [U/L]			**40.03 ± 13.38 [U/L]**			**50.44 ± 13.20 [U/L]**		
	V[U/L]	**% Inhib.**	**% Reco.**	**V[U/L]**	**% Inhib.**	**% Reco.**	**V[U/L]**	**% Inhib.**	**% Reco.**
Fig to olive ratio [1:1] Mean ± SD	26.12 ± 13.35	46.53	53.47	20.21 ± 14.09	49.51	50.49	18.35 ± 5.78	63.62	36.38
Fig to olive ratio [1:4] Mean ± SD	29.93 ± 10.24	38.73	61.27	23.50 ± 12.55	41.29	58.71	27.21 ± 7.45	46.05	53.95
Fig to olive ratio [1:6] Mean ± SD	23.33 ± 10.97	52.22	47.58	19.35 ± 11.95	51.66	48.34	15.00 ± 6.35	70.26	29.74
Fig to olive ratio [3:5] Mean ± SD	28.33 ± 11.17	42.00	57.99	22.5 ± 12.32	43.79	56.21	23.50 ± 5.01	53.41	46.59

Table 2: The degree of inhibition of enzymes activities at [1 × 10^{-3}, 1 × 10^{-4}, 1 × 10^{-5}] M of AgNP, using 40 control samples

	ADA [U / L] Control group [n=70]			AMPDA [U /L] Control group [n=70]			5'-NT [U/L] Control group [n=70]		
	12.98 ± 2.23 [U/L]			12.25 ± 2.02 [U /L]			12.50 ± 3.44 [U/L]		
	V[U/L]	% Inhib.	% Reco.	V[U/L]	% Inhib.	% Reco.	V[U/L]	% Inhib.	% Reco.
Fig to olive ratio [1:1] Mean ± SD	6.90 ± 2.81	46.84	53.16	6.20 ± 2.15	49.39	50.61	4.60 ± 1.00	63.20	36.80
Fig to olive ratio [1:4] Mean ± SD	7.99 ± 2.33	38.44	61.56	7.25 ± 2.45	40.82	59.18	6.60 ± 1.23	47.20	52.80
Fig to olive ratio [1:6] Mean ± SD	6.40 ± 2.56	50.69	49.30	6.00 ± 2.15	51.02	48.98	3.75 ± 0.99	70.00	30.00
Fig to olive ratio [3:5] Mean ± SD	7.50 ± 3.84	42.22	57.78	7.00 ± 2.25	42.86	57.14	5.90 ± 0.97	52.80	47.20

Using silver in nano form can be removed these type of limitation, this may be cause increase of the surface area in Nano case, relate area between Ag(0) and increases of the bacteria[12]. These effects was investigated through growing *E. coli, Pseudomonas, Bacillus* and *staphylococcus* on agar plates and, accomplished with AgNP. The growth of bacterial non inhibited in the presence of AgNP on the nutrient agar plate. This may be due to the inhibition solely depended upon the low AgNP concentration**.**

The result in Table 1 and 2 the particle size were depend to increase the effect of percentage of inhibitory, especially on Ecto-5'-nucleotidase(5'-NT) activity. Other consequence showed that smaller particles would greatly inhibiting the activity of ADA, AMPDA and 5'-NT when it's used. These result needs to be further studied to determine the effect of particle size for activity of ADA, AMPDA and 5'-NT in vivo, in order to use the Ag NPs in biomedical applications such as the treatment and follow up by adjustment the levels of these enzymes of patients suffered from arthrosclerosis disorder.

CONCLUSION

In the present work, we have presented easy method for the preparation of AgNPs with well-defined size and shape. Silver nanoparticles with an average size of 103.00, 77.49, 103.58 and 97.48 nm were synthesized using aqueous solution of silver nitrate with mixture of Fig and Olive; 1:1 ratio, 1:4 ratio, 1:6 ratio and 3:5 ratio extract. The synthesized silver nanoparticles were characterized by UV-AFM measurements. The synthesis method in our study is cheap, eco-friendly, pollutant free. The nanoparticles showed inhibitory effects on activity of 5'-NT, ADA and AMPDA enzymes in sera of control and patients with arthrosclerosis. Additional studies on other biological activities on other enzymes are required to exploit their full potential.

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