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Synthesis of Silver Nanoparticles from *Malva parviflora* Extract and Effect on Ecto-5'- Nucleotidase(5'-NT), ADA and AMPDA Enzymes in Sera of Patients with Arthrosclerosis

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Abstract:
The present research included synthesis of silver nanoparticle from($1*10^{-3}$,$1*10^{-4}$ and$1*10^{-5}$) M aqueous $AgNO_3$ solution through the extract of M.parviflora reducing agent. In the process of synthesizing silver nanoparticles we detected a rapid reduction of silver ions leading to the formation of stable crystalline silver nanoparticles in the solution. The characteristics of silver nanoparticles were studied by using UV-Visible absorption spectroscopy, and atomic force microscope (AFM) analysis. The AFM measurements showed that the average size of silver nanoparticles synthesized using ($1*10^{-3}$,$1*10^{-4}$ and$1*10^{-5}$) M aqueous $AgNO_3$ solution through the extract of *M.parviflora* were 102 to 114nm. UV-Vis spectra of the aqueous medium containing silver nanoparticles showed a surface peak at 220nm and 445nm for ($1*10^{-3}$,$1*10^{-4}$ and$1*10^{-5}$) M aqueous $AgNO_3$ solution through the extract of *M.parviflora*. The study of nanoparticles due to the possible application for the development of new technologies such as exhibited inhibitory effects on Ecto-5'-Nucleotidase (5'-NT), ADA and AMPDA enzymes in Sera of control and Patients with Arthrosclerosis. Further studies on other biological activities are required to exploit their full potential.

Keywords: *M.parviflora*, extract, Silver nitrate, Silver nanoparticles

Introduction:

Nanotechnology concerns with the development of empirical processes for the synthesis of nanoparticles of different sizes and shapes. That equipping an efficient monitoring abundant of the physical and chemical properties and their potential implementation in photoelectronic recording media sensing devices catalysis and medicine. To date, metallic nanoparticles are especially prepared from nobel metals (ie, Ag, Pt, Au and Pd). Among the nobel metals, (Ag) is the metal of choice in the field of biological system, living organisms and medicine [1].

Silver nanoparticle was used in wide range of applications such as, food industries, farming, weaves industries, water treatment as antimicrobial, cosmetics and salve[2]. Silvery nanoparticles exhibit new or improved properties depending on forming, distribution and size. Different ways approaches using plant extract have been used for the synthesis of metal nanoparticles [3]. Many technicality of synthesize silver nanoparticles like chemical reduction of silver ions in aquatic solutions with or without stability agents, thermic decomposition in organic solvents, alchemical shorthand while photoreduction in invert micelles, and radiation chemical reduction[4]. *M.parviflora* L. belongs to the family Malvaceae that includes trees, shrubs and herbs. In Lesotho, dried powder or an infusion made from leaves and roots of *M.parviflora* use to clean wounds and sores. A hot scumble made from leaves is also used to treat wounds and tumefaction and is incorporated into a lotion to treat bruised and broken limbs. The leaves of *M.parviflora* use by the Xhosa people of South Africa for drawing swollen, inflamed purulent wounds. If ingested it is toixc such *M.parviflora* is remind to cases mortality in foraging livestock, such as sheep, horses and cattle. Sheep are the most often affected and develop clinical signs including arched back and labored breath. This toxicity that may be due to the presence of malvalic acid, an

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unsaturated fatty acid formerly referred to as halphen acid [5].

In this study, we have synthesized silver nanoparticles using *M.parviflora* extract. Than the study of nanoparticles on exhibited inhibitory effects on Ecto-5'- Nucleotidase (5'-NT), ADA and AMPDA enzymes in Sera of control and Patients with Arthrosclerosis.

Materials and Methods:

Preparation of the Extract

Figure 1. Picture of *M.parviflora* (malvasylvestris)

Freshly leaves of *M.parviflora*, Fig. (1) were amass from various *M.parviflora* farms in Jadre of Baghdad university. *M.parviflora* were washed repeatedly with water to remove the dust particles and then sun dried to remove the residual moisture. *M.parviflora* extract used for the reduction of silver ions (Ag^{+}) to silver nanoparticles (Ag^{0}) was prepared by placing 10g of washed dried fine cut leaves in 250mL glass beaker along with 200mL of sterile distilled water. The mix were than boiled for (10)min. Till the color of the aquatic solution changes from green to green yellow .The solution was cooled to room temperature and filtered, Then the extract solution filter in centrifuging at 3500rpm for5 minutes to remove the heavy biomaterials .Extractor solution stored at room temperature in order to be used for further study .

Synthesis of silver nanoparticles

In a typical reaction procedure, 5 mL of *M.parviflora* extract were add to 50mL of ($1*10^{-3}$,$1*10^{-4}$ and$1*10^{-5}$)M aqueous $AgNO_3$ solution at room temperature , the resulting solution become grey –black in color with ($1*10^{-3}$M) aqueous $AgNO_3$ solution, light yellow with ($1*10^{-4}$ M) aqueous $AgNO_3$ solution and direct yellow with ($1*10^{-5}$ M) aqueous $AgNO_3$ solution after 60 minutes ,indicating the formation of Ag NPs.

The Effect of AgNP on bacterial growth

To determine the growth curve in the presence of silver nanoparticles, *E. coli, Pseudomonas, Bacillus and staphylococcus* bacteria were grown in liquid LB medium. Fresh LB liquid medium were diluted in to optical density (OD600) $1*10^{-3}$,$1*10^{-4}$ and$1*10^{-5}$M. AgNP solution was added into the cell culture medium at different concentrations, and the culture was incubated at 37°C and 250 rpm. Growth rates and bacterial concentrations were determined by measuring OD at 600 nm at different time points (Fig.7).

Determination of Ag NPs on the sera adenosine aminohydrolase (ADA), AMP-aminohydrolase activities and Ecto-5'-nucleotidase activity.

The effect of Ag NPs on the levels of adenosine aminohydrolase (ADA), AMP-amino 11hydrolase and Ecto-5'-nucleotidase activities. In sera of patients with atherosclerosis and 50 healthy persons. This study was conducted on a group of 60 patients with atherosclerosis and 50 healthy persons to be used as control ranging between (40-75) years. These patients were hospitalized at Research Institute for educational laboratories in the city of Medicine of the Ministry of Health. Five millilitre of blood was collected and it was allowed to clot for 10-15 min. Sera was removed after centrifuged .The ADA activity was determined according to Giusti method[6]. 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[7]. Ecto-5'-nucleotidase activity was measured in serum according to Wood and Williams's method[8].

Results and Discussion

Analysis of Silver nanoparticles:

SEM analysis:

To determine the morphology of the synthesized silver nanoparticles the sample was analyzed with Scanning electron microscope (SEM). The scattered silver nanoparticles were dried in an oven for 1 hours to obtain a powdered form. Then, 2.5 mg of the sample was redispersed in ethanol and the sample was prepared in thin films on carbon coated copper grid. Scanning electron microscopy provided further insight into the morphology and size details of the silver nano particles. Comparison of empirical results showed that the diameter of prepared nanoparticles in the solution was about 50 nm. Fig. 2 shows the scanning electron micrograph of the plant extract as apositive control obtained from the proposed bioreduction method at various magnifications[9].

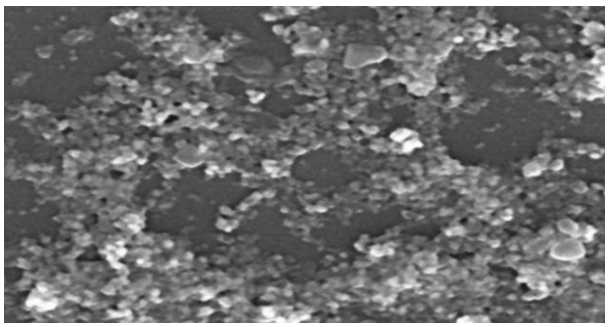


Figure 2. SEM image of the silver nanoparticle synthesized from *M.parviflora* leaf extracts.

UV-Vis Spectroscopy:

The reduction of the Ag^{+} ions by the supernatant of the test plant extracts in the solutions and formation of silver nanoparticles was characterized by UV-visible spectroscopy monitored by sampling the aquatic component (2.0 mL) and gauge the UV-VIS spectrum of solutions. The UV-VIS spectroscopy of these specimens were measured on a UV-1800 Series spectrophotometer operated at a resolution of 1.0 nm. UV–vis spectroscopy is one of the most widely used techniques for structural characterization of silver nanoparticles. The reduction of silver ions into silver nanoparticles using *M.parviflora* extract was evidenced by the visual change of colour from yellow to reddish brown due to excitation of surface plasmon vibrations in silver nanoparticles. The UV-visible spectra show an absorption band at 445 nm with ($1*10^{-3}$M) aqueous $AgNO_3$ solution and extract nm which corresponds to the absorbance of silver nanoparticles (Fig. 3). (Fig. 4) and (Fig. 5) show decreased concentrations of silver nitrate show an absorption band at220nm with ($1*10^{4}$M) aqueous $AgNO_3$ solution and extract and 220nm with ($1*10^{-5}$M) aqueous $AgNO_3$ solution and extract resulted in a brown solution of nanosilver indicating the completion of reaction[10].

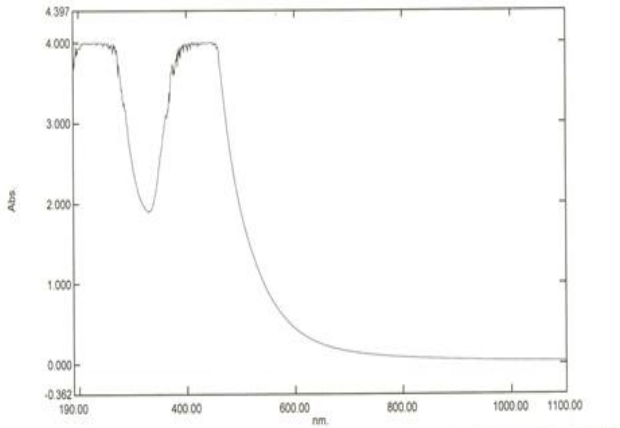


Figure 3. UV–vis spectrum showing absorption of 10^{-3} M aqueous solution of silver nitrate with *M.parviflora* extract

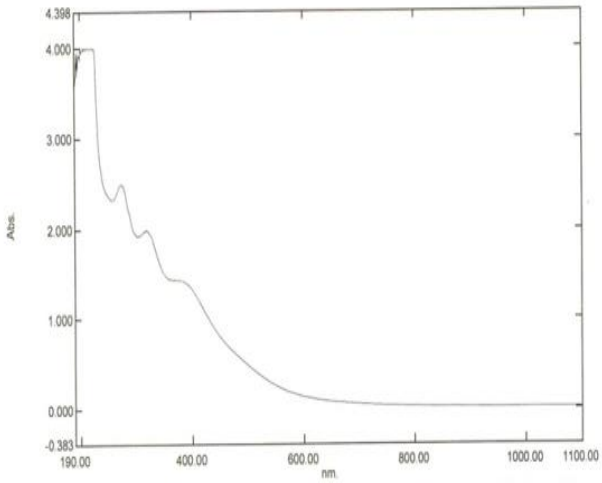


Figure 4. UV–vis spectrum showing absorption of (10^{-4} M) aqueous solution of silver nitrate with *M.parviflora* extract

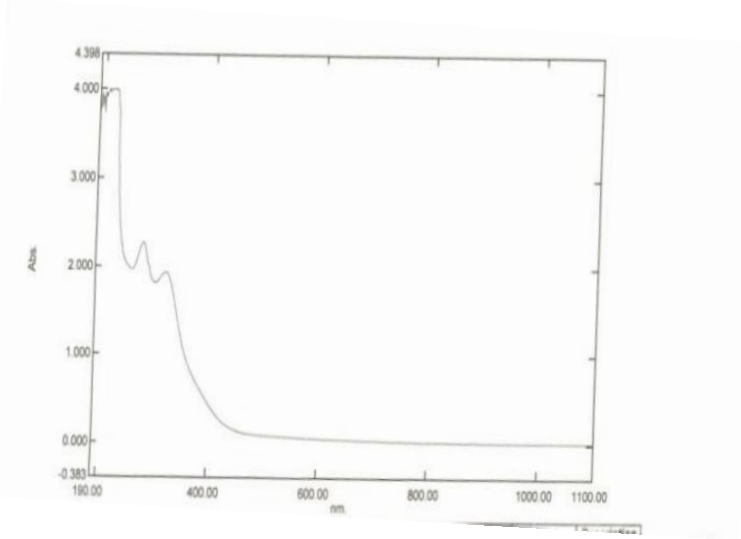
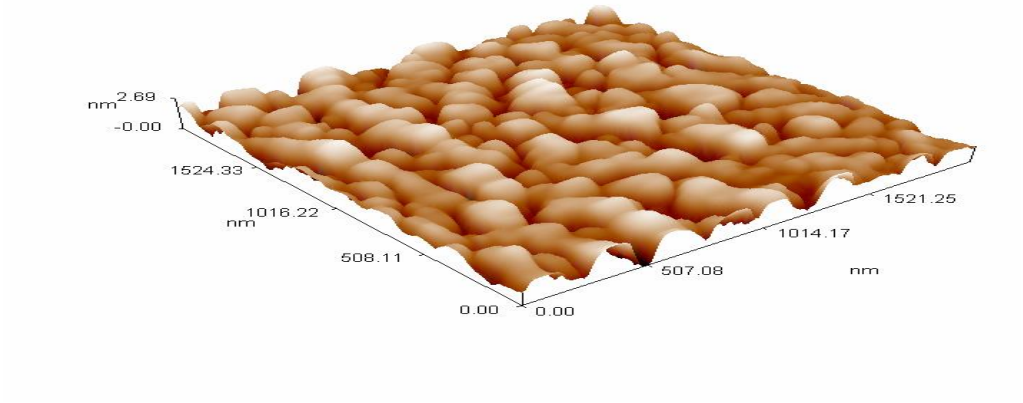


Figure 5. UV–vis spectrum showing absorption of (10^{-5} M) aqueous solution of silver nitrate with *M.parviflora* extract

Atomic force microscopy;

Surface topology of the formulated silver nanoparticles was studied by atomic force microscopy (AFM) analysis. Fig. (6) show the topographic structures in 2D and 3D the type seem to be very smooth surfaces with grains nearly equal to the starting nano powder have the calculated sizes in the range of (102 ,112 and 114) nm for($1*10^{-3}$,$1*10^{-4}$ and$1*10^{-5}$)M aqueous $AgNO_3$ solution with *M.parviflora* extract appendixes [11].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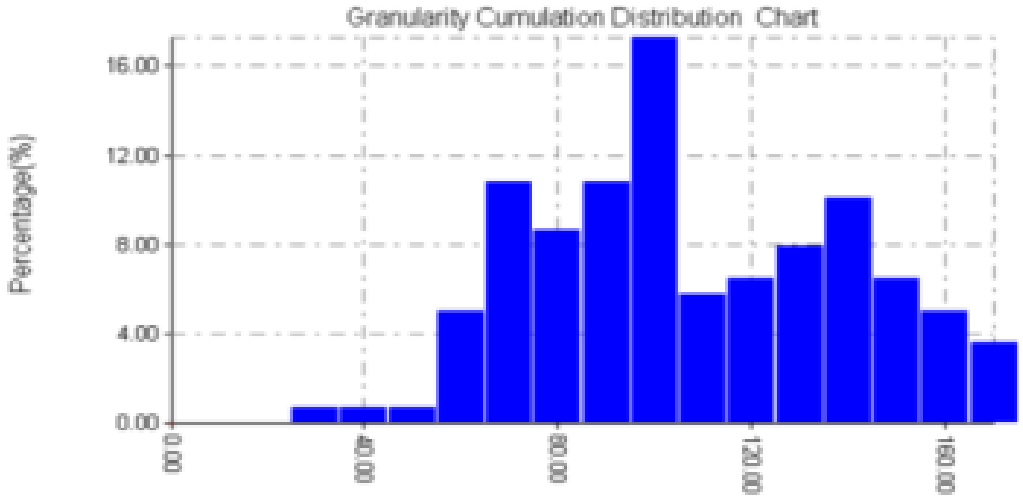
Granularity Cumulation Distribution Chart
Percentage(%)
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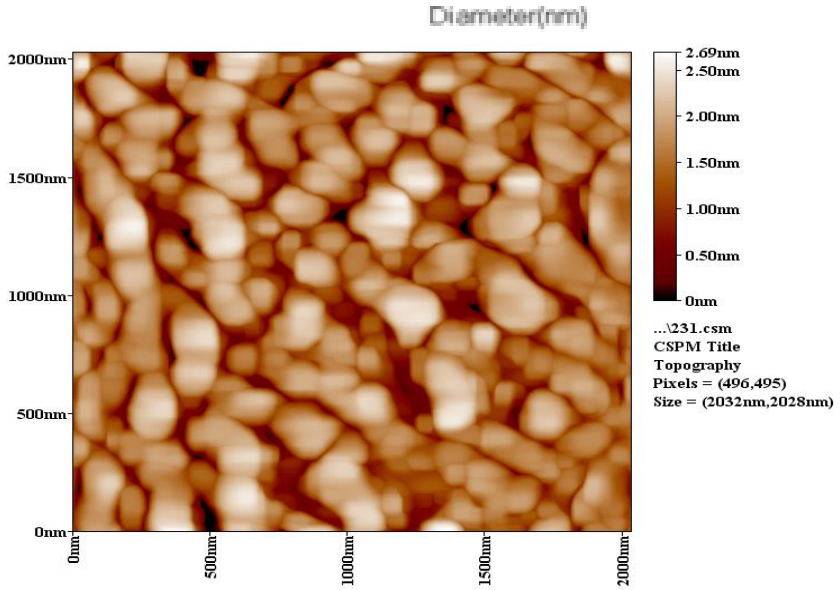
Diameter(nm)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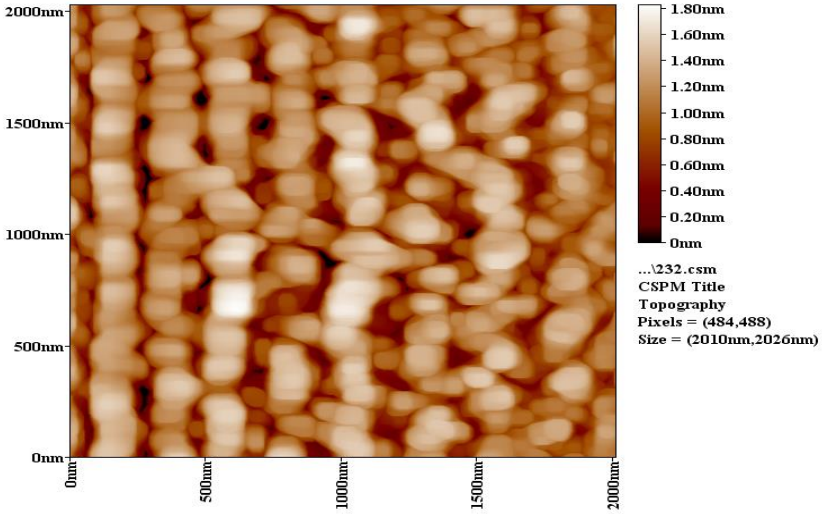
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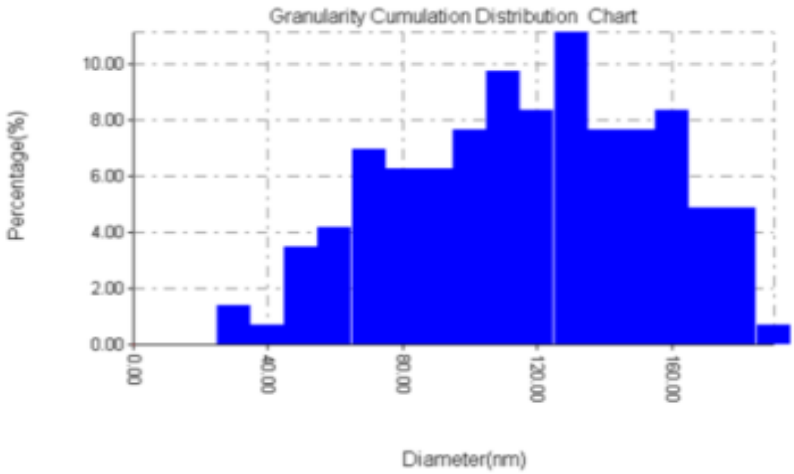
Granularity Cumulation Distribution Chart
Percentage(%)
10.00
8.00
6.00
4.00
2.00
0.00
0.00
40.00
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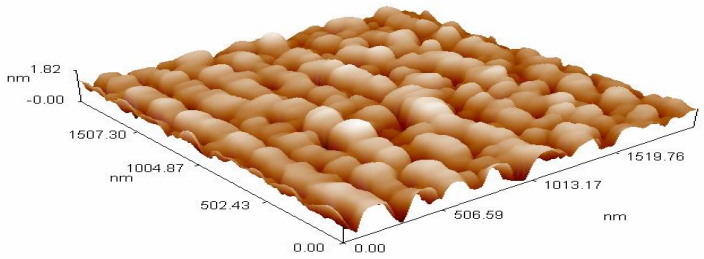
120.00
160.00
Diameter(nm)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1519.76
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B

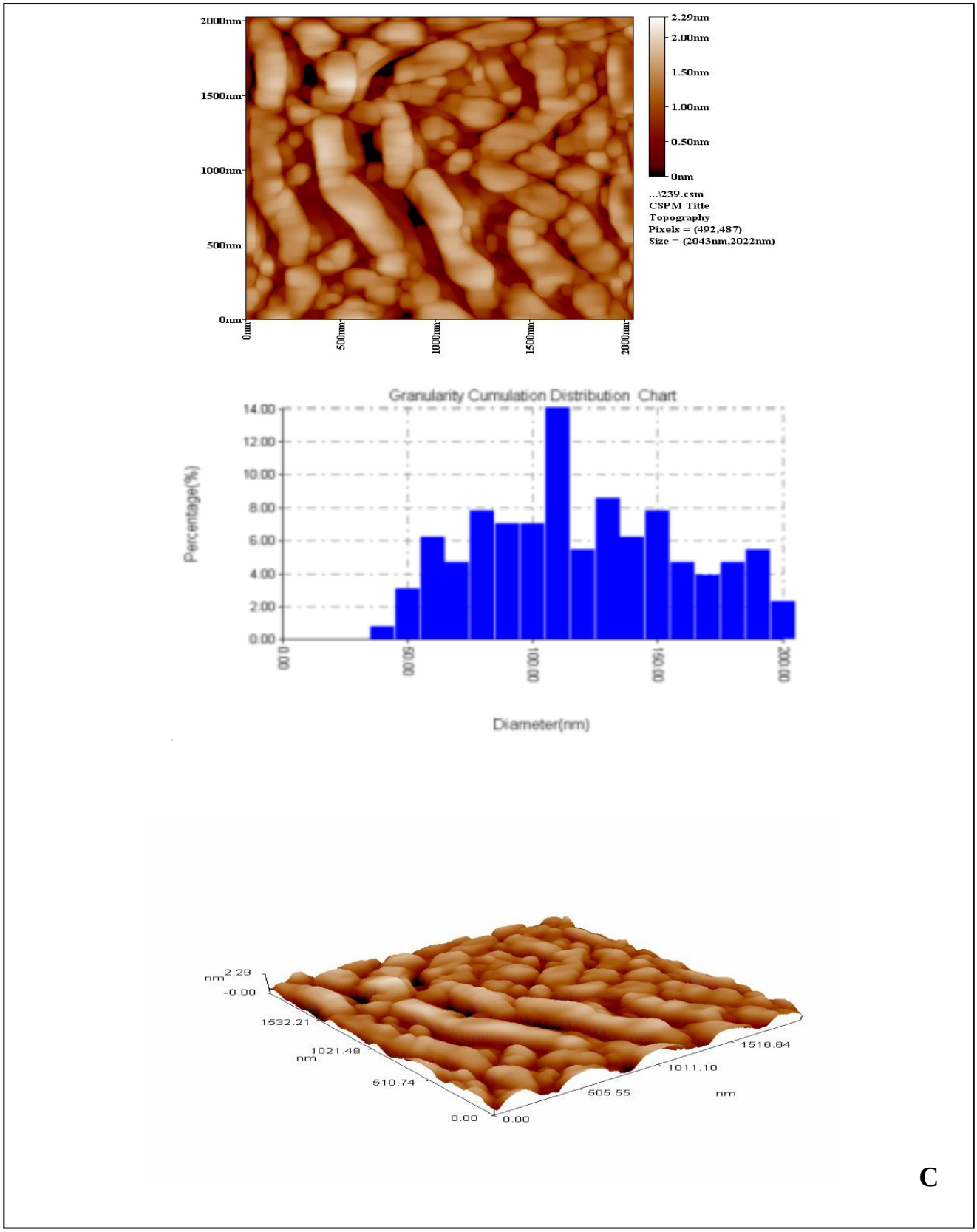
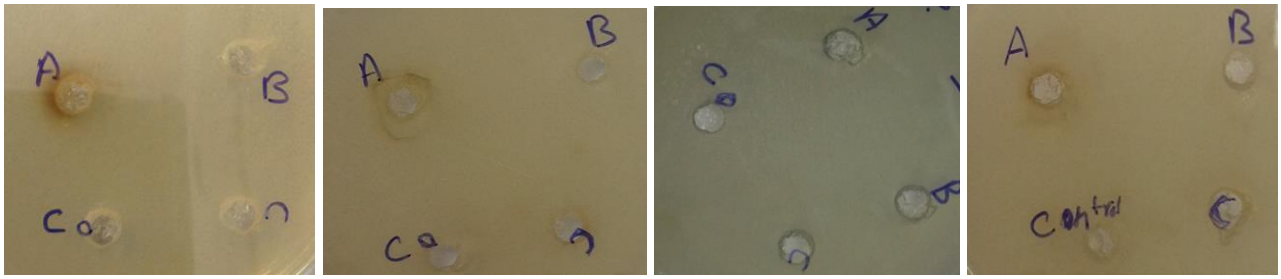


Figure 6. AFM image of synthesized silver nanoparticles of (a) 10^{-3} M,(b) 10^{-4} M and (c) 10^{-5} M aqueous solution of silver nitrate with malvaparviflora extract.

The Effect of AgNP on bacterial growth

Chemical antimicrobial agents are progressively becoming resistant to a extensive spectrum of antibiotics. Another way to overcome the drug resistance of numerous bacteria is therefore instantly needed. Though, there are some limitations in using Ag salts as antimicrobial agents, these may be due to the interfering effects of salts. Using silver

in nano form can be removed these type of limitation, this may due to the increase of the surface area in nano case, the relate area between Ag(0) and that of the microorganism increases[12]. To use AgNP against microbes in various fields, it is paramount to formulate AgNP in a green milieu. Used allow concentricity of AgNPs(A) $1*10^{-3}$M, (B) $1*10^{-4}$M ,and (C) $1*10^{-5}$M, Fig.7.The effect was investigated by growing *E. coli*, *Pseudomonas*, *Bacillus and staphylococcus* on agar plates and،supplemented with AgNP. The bacterial growth was non inhibited in the presence of AgNP on the nutrient agarplate. This may be due to the inhibition solely depended upon the low AgNP concentration.



1 **2** **3** **4**

Figure 7. Antibacterial activity of AgNPs. Antibacterial activity of AgNP having different concentrations:, and (A) $1*10^{-3}$M, (B) $1*10^{-4}$M ,and (C) $1*10^{-5}$M, with (1) *E. coli*, (2) *Pseudomonas*, (3) *Bacillus and* (4) *staphylococcus* inoculated on nutrient agar plate. The 'Co' spot of the agar plate is for the blank test, having no AgNP.

The Effect of AgNPs on the sera adenosine aminohydrolase (ADA), AMP-aminohydrolase activities and Ecto-5'-nucleotidase activity.

In the previous study we referred to a highly significant elevated in the activates of Ecto-5'-nucleotidase(5'-NT), ADA and AMPDA enzymes U/L in patients with atherosclerosis compared to monitoring group. This is normally expressed in intravenous endothelial cells and in a broad range of impervious cells. Therefore, adenosine formative by extracellular nucleotide catabolism on endothelial cells and immune cells appears to be an important endogenous modulator of arteriogenesis and key transcription factor involved in inflammatory responses[13].The inhibitory influence of the (Ag) NP on the activity of ADA , AMPDA and Ecto-5'-nucleotidase(5'-NT) were (38.68, 30.62and 66.81%) respectively in patients group and (38.12, 30.95 and 66.92%) in control group when we used 50 µL of AgNPs ,(Table 1 and 2) .The percentage of inhibitory effect was showed highly when using $1*10^{-3}$ of AgNPs compared to $1*10^{-4}$ and $1*10^{-5}$ of AgNPs in both patients and control groups as shown in Table 1 and 2.

Table 1. The degree of inhibition of enzymes activities at [$1*10^{-3}$, $1*10^{-4}$, $1*10^{-5}$] M of AgNP, using 60 patients with atherosclerosis samples.

Enzymes	Patients group [n=60]	$1*10^{-3}$ of AgNP			$1*10^{-4}$ of AgNP			$1*10^{-5}$ of AgNP		
		V[U/L]	% Inhib.	% Reco.	V[U/L]	% Inhib.	% Reco.	V[U/L]	% Inhib.	% Reco.
ADA [U / L] Mean ± SD	46.23±14.65	28.35 ±13.02	**38.68**	61.32	39.74±16.89	**14.03**	85.96	42.35±12.01	**8.40**	91.60
AMPDA[U /L] Mean ± SD	39.68±12.93	27.53 ±11.20	**30.62**	69.38	33.06±13.03	**16.68**	83.32	35.21±10.05	**11.27**	88.73
5'-NT [U/L] Mean ± SD	52.21±16.33	17.33 ±10.97	**66.81**	33.19	38.65±12.08	**25.97**	74.03	42.35±10.01	**18.89**	81.11

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

Enzymes	Control group [n=40]	$1*10^{-3}$ of AgNP			$1*10^{-4}$ of AgNP			$1*10^{-5}$ of AgNP		
		V[U/L]	% Inhib.	% Reco.	V[U/L]	% Inhib.	% Reco.	V[U/L]	% Inhib.	% Reco.
ADA [U / L] Mean ± SD	13.85 ±2.88	8.57 ±2.91	**38.12**	61.88	11.85 ±3.01	**14.44**	85.56	12.72±3.34	**8.16**	91.84
AMPDA [U /L] Mean ± SD	13.02 ±3.25	8.99 ±2.78	**30.95**	69.05	10.93 ±3.20	**16.05**	83.95	11.53±4.23	**11.44**	88.56
5'-NT [U/L] Mean ± SD	11.85 ±3.48	3.92 ±3.97	**66.92**	33.08	8.80 ±3.21	**25.74**	74.26	9.60 ±3.97	**18.99**	81.01

It can be seen in Table 1 and 2 the particle size was 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 Ecto-5'-nucleotidase(5'-NT) when it's used. This result needs to be further studied to determine the effect of particle size for activity of ADA, AMPDA and Ecto-5'-nucleotidase(5'-NT)in vivo ,in order to use the AgNPs 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 this work, we have presented easy method for the preparation of AgNPs with well-defined size and shape.Silver nanoparticles with an average size of 102, 112 and 114nm were synthesized using aqueous solution of silver nitrate with *M.parviflora* extract. The synthesized silver nanoparticle was characterized by UV-AFM measurements. This synthesis method is alternative chemical method, since it is cheap, pollutant free and eco-friendly. The results showed that of silver nitrate with *M.parviflora* extract plays an important role in the reduction and stabilization of silver to silver nanoparticles. The nanoparticles showed inhibitory effects on Ecto-5'- Nucleotidase (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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تحضيرالفضة النانوية من مستخلص نبات الخباز والتأثير على Ecto-5'- نوكليوتيداز(5'-NT)، ADA و AMPDA الانزيمات في مصول المرضى الذين يعانون من تصلب الشرايين

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[5] قسم التحليلات المرضية، كليات التقنيات الصحية والطبية، الجامعة التقنية الوسطى، بغداد، العراق.

الخلاصة:

يتضمن البحث دراسة تحضير الدقائق النانوية من محلول نترات الفضة بتراكيز(1* (10^{-3}و10^{-4} و10^{-5}) مولاري باستخدام مستخلص نبات الخباز كعامل مختزل من خلاله تحديد سرعة اختزال ايونات الفضة لتكون الدقائق البلورية الاكثر استقرارا للفضة بالمحلول.
تم دراسة الخصائص الطيفية باستعمال طيف (UV-visible)ومجهر القوة الذرية (AFM)للدقائق النانوية المحضرة حيث تراوحت حجم الدقائق النانوية (102-114) نانوميتر بينما ظهر طيف (UV-visible) قمم تراوحت بين 220_445 نانوميترعلى التوالي لتراكيز(1* (10^{-3}و10^{-4} و10^{-5})مولاري من محلول نترات الفضة من خلال مستخلص النبات، الدقائق النانوية تظهر تاثيرها كمثبط للانزيمات-Ecto-5 Nucleotidase (5-NT),ADA and AMPDAالمتواجدة في مصل الاشخاص الاصحاء والمرضى مع Arthrosclerosis .

الكلمات المفتاحية: مستخلص نبات الخباز، نترات الفضة، الفضة النانوية.