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Antibacterial Activity of Silver Nanoparticles Synthesized from Plant Latex

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Abstract

Nanoparticles produced by plants are preferred in the medical field for its safe and unpolluted product; it is also accepted as an ecofriendly, non-expensive, and non-toxic nanomaterial. In this study, silver nitrate was successfully used to produce silver nanoparticles (AgNPs) by the use extractsof 4 different latex-producing plants which belong to 2 families (*Moraceae* and *Euphorbiaceae*). The synthesis was proved by Atomic Force Microscopy (AFM).The sizes of the AgNP grains were estimated by Granularity Cumulating Distribution (GCD). The results revealed the production of AgNPs in different sizes of 103 and 82 nm using the *Moraceae* family and 77 and74nm using the *Euphorbiaceae* family.Antibacterial activity was also detected against both Gram positive and Gram negative pathogenic bacteria using the well diffusion assay. In conclusion, this source of nanoparticles can be a very useful industrial project in a goal to find new safe and economic alternatives to antibiotics.

Keywords: Plant latex, Silver nanoparticles, Green method, Antibacterial activity.

الفعالية ضد البكتيرية لجسيمات الفضة النانوية المصنعة من لاتكس النبات

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الخلاصة

إن الجسيمات النانوية المنتجة من النباتات تكون مفضلة في الحقل الطبي لكونها منتجات أمينة وغير ملوثة وكذلك فهي صديقة للبيئة ,ورخيصة وغير سامة. في هذه الدراسة استخدمت نترات الفضة لانتاججسيمات الفضة النانوية AgNPs بنجاح من خلال استخدام لاتكس اربعة انواع مختلفة من النباتات تعود لعائلتين نباتيتين(*Moraceae* and *Euphorbiaceae*).تم تصنيعجسيمات الفضة النانوية باستخدام مستخلص اللاتكس للنباتات وتم اثبات تكونها بواسطة مجهر القوة الذري وقياس حجم الجسيمات بواسطةGCD.أظهرت النتائج تكون جسيمات الفضة النانويةبأحجام مختلفة (103 و 82 نانوميتر) لعائلة ال*Moraceae* و (77 و74 نانوميتر) لعائلة ال*Euphorbiaceae* , كذلك تم التحري عن فعالية جسيمات الفضة النانوية ضد البكتريا الممرضة الموجبة والسالبة لصبغة غرام باستخدام طريقة الانتشار في الحفر. نستنتج أن المصدر النباتي لهذه الجسيمات النانوية ممكن ان تكون ذات فائدة كبيرة من الناحية الصناعية كهدف لايجاد بدائل امينة واقتصادية للمضادات الحيوية.

Introduction

The word ‘‘nano’’, meaning ‘‘dwarf ’’, was derived from a Greek word that refers to objects of one -billionth in size. The nanomaterials have been gathering a great attention due to their wide

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applications, including ‘‘Nano medicine’’ as well as their properties in different fields that lead to unique characteristics depending upon their overall size, shape, composition and distribution. Nan oparticles have shown significant change sdue to a wide range of applications in bio-medical, antimicrobials, sensors, electronics, catalysts, agricultural, optical fibers, bio-labeling and others [1].In spite of many applications of nanoparticles, there are certain concerns of toxicities which should be taken in mind [2], mainly including environmental applications of nanotechnology such as environmentally benign products(e.g. green chemistry or pollution prevention) and remediation of substances contaminated with hazardous materials[3].

Chemical and physical techniques have been used to synthesize nanoparticles, including heat evaporation [4], photochemical reduction [5] and many others [6, 7]. These techniques have many disadvantages and efforts have been made to find sources with less damage. There is a need to explore and develop environmental friendly techniques of nanoparticle synthesis that do not include toxic materials. Biological processes for nanoparticle synthesis using bacteria, fungi, algae enzymes, and plants or plant extracts have been studied as ecofriendly alternatives tochemical and physical methods[8, 9].The use of plants for nanoparticle synth esiscan be much better than other biological processes. Different types of nanoparticles have been used with different mechanisms for fighting microbial resistance, where the synthesized silver nanoparticles showed activity against many clinical strains of bacteria [10].

The first gold and silver nanoparticles were produced by living plants [11], then many plants and plant products were used to produce nanomaterial such as green tea [12], *Aloe vera* plant extract [13], starch [14],and lemon grass leaves extract [15].

Thus, this study was interested in investigating the possibility of using the plant latex of two latex plant families to facilitate the formation of silver nanoparticles (AgNPs) and then their use as anantibacterial material.

Materials and methods

Sample collection

Plants were selected from gardens in Baghdad city Figure-1 and transferred to the laboratory where they were identified by a botanist (Table-1).Crude latex was collected by cutting the edge of stems of the 4 plants and collecting the milky latex in Eppendr of tubes with avolume of approximately 2ml. The collected milky white latex was then stored at -20℃ for further experiments.

Table 1-tCharacteristicsof plants used in this study

Plant species (Common name)	Family	Description	Latex color
Ficus carica L (common fig)	*Moraceae*	This plant is from Asian and North America, the common name is figs and considered a good fruit to eat.	Milky white
Ficus elastic (rubber bush)	*Moraceae*	Native to tropical Asia, India, and has been known to West Indies. The plants is elliptic to oblong leaves, acuminate at apex, rounded at base, glabrous, smooth, leathery, and gray to brown when dry.	Milky white
Euphorbia milii (crown of thorns)	*Euphorbiaceae*	Native to Madagascar,this plant is straight, slender spines, and the sap is moderately poisonous.	Milky white
Euphorbia tireuall (pencil tree, milk bush)	*Euphorbiaceace*	Native to deserts of southern Africa and Madagascar, the plant shares the features of having a poisonous, milky, white, latex-like sap, and unique floral structures.	Milky white

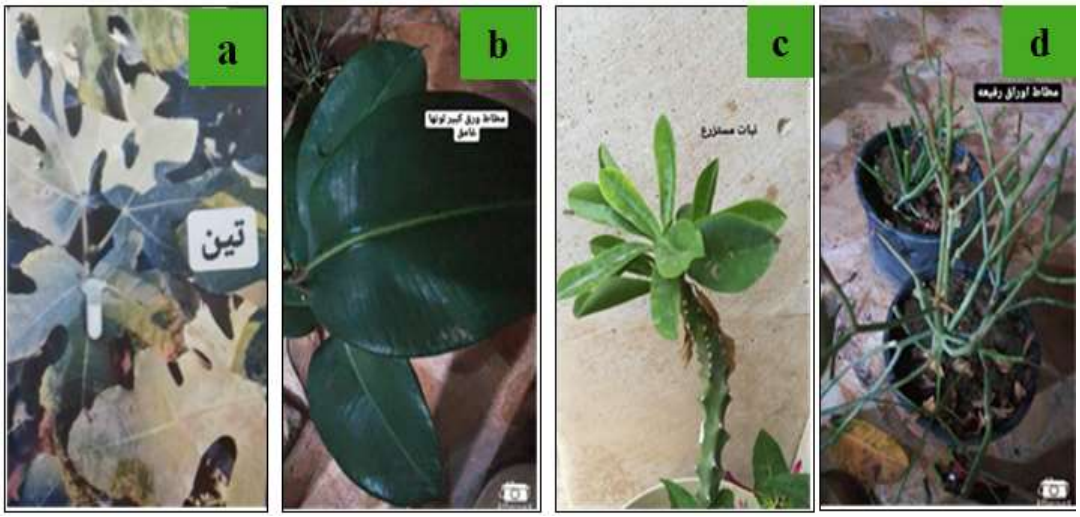


Figure 1-Plants in the irnormal habitat. a) *Ficus carica*, b) *Ficus elastica*,c) *Euphorbia milii*, d)*Euphorbia tirucall*i.

Synthesis of Silver nanoparticles

A concentration of 1Mof $AgNO_3$ was prepared using distilled water.2% (v/v) of crude latex was prepared using deionized distilled water at a final volume of 100 ml, then 25 ml of this solution was heated at 60℃ with stirring for 15min in water bath. Separately, 25ml of1M$AgNO_3$ aqueous solution was heated at 60℃ with stirring for 15min in water bath. Then, latex solution was mixed with $AgNO_3$ solution and heated at 80℃ for 30min.Finally, AgNPs were synthesized gradually [16]. Synthesized AgNPs were centrifuged, dried, and a final stock concentration of 500µg/ml was prepared.

Description of synthesized AgNPs

The synthesized AgNPs were analyzed by Atomic Force Microscope (AFM) pictures for characterization of morphology and size of AgNPs, which enable microscopic information plot topographies showing the structure of the surface and surface alleviation [17]. Scanning Probe Microscopy (SPM) was used to image samples over a wide area to observe dimensions at better resolution.

Detection of antibacterial activity of plant latex

The antibacterial activity of synthesized AgNPs was detected using the well diffusion assay. Dilutions of AgNPs(250, 125 and 62.5 µg /ml) were prepared from the stock concentration(500µg/ml). Indicator bacteria were activated in nutrient broth for 18hr. at 37℃, and the concentration of the culture was subjected to$1.5*10^8$cell/ml that confronts McFarl and turbidity tube of 0.5. Sterilized nutrient agar plates were cultured with pathogenic bacteria using cotton swab. After 5-10 minutes, wells were cut out using the end of a sterilized pasture pipette.AgNPs solution (100µL) from each concentration was addedin wellsand incubatedat 37℃ for 24hr. Inhibition zones were measured in mm [18].

Statistical analysis

The data were analyzed using SPSS IBM version 20 IBM. Results of study groups and assays were expressed as Mean± Standard Error, while One-sample T-test was used to calculate the significance where P-values ≤ 0.05were considered statistically significant.

Results and Discussion

Characteristic of the synthesized AgNps

The latex extract of the plants showed change in color and consistency, where the color changedfrom whit e(emulsion) to deep brown with the precipitation of the particles during the process of synthesizing AgNPs.

The technique of AFM deals with images that permit quantitative measurements of the material surface as average roughness (Ra), root mean square roughness (Rq) and analysis of different angels containing 3D simulation [19]. The 2D and 3D AFM images of AgNPs synthesized by plant latex are illustrated in Figure-2. For *Ficus carica* and *Ficus elstica*, average grain size of AgNPs was 103.98 and 82.82 nm, respectively (Figure-3), whereas the AFM images of AgNPs synthesized by plant extract of *Euphorbia milli* and *Euphorbia tirulli*are shown in Figure-4. The average grain size of

AgNPs was 74.63 and 77.22 nm respectively (Figure-5)which was estimated by the granularity cumulating distribution chart. The largest size of AgNPs was produced by *Ficus caria* compared to the other plant latex extracts used in the study, where this size was also higher than those produced by algae extracts (25-55 nm) and other bacteria as *Pseudomonas aeruginosa*(35-46 nm) [20,21].

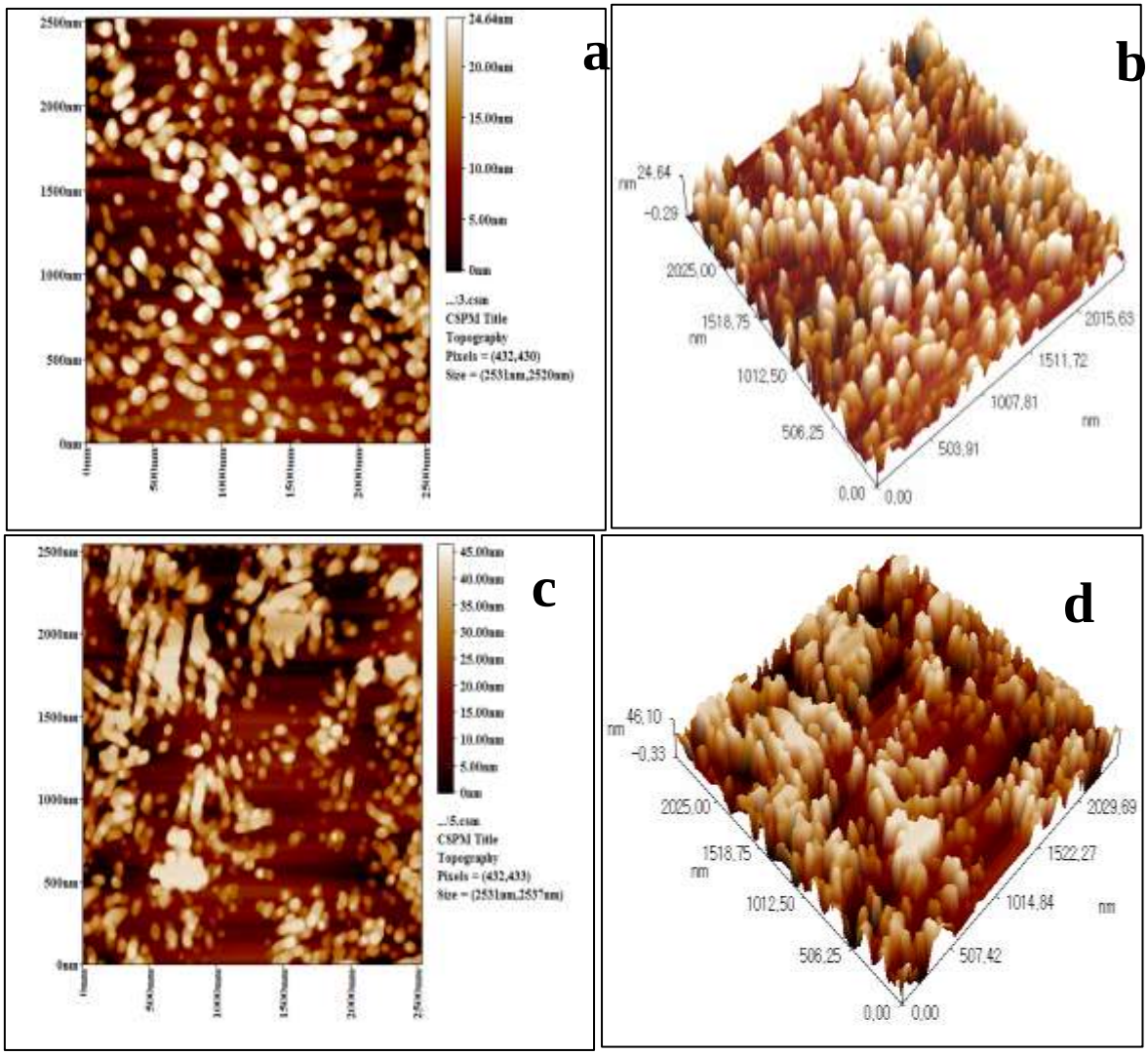
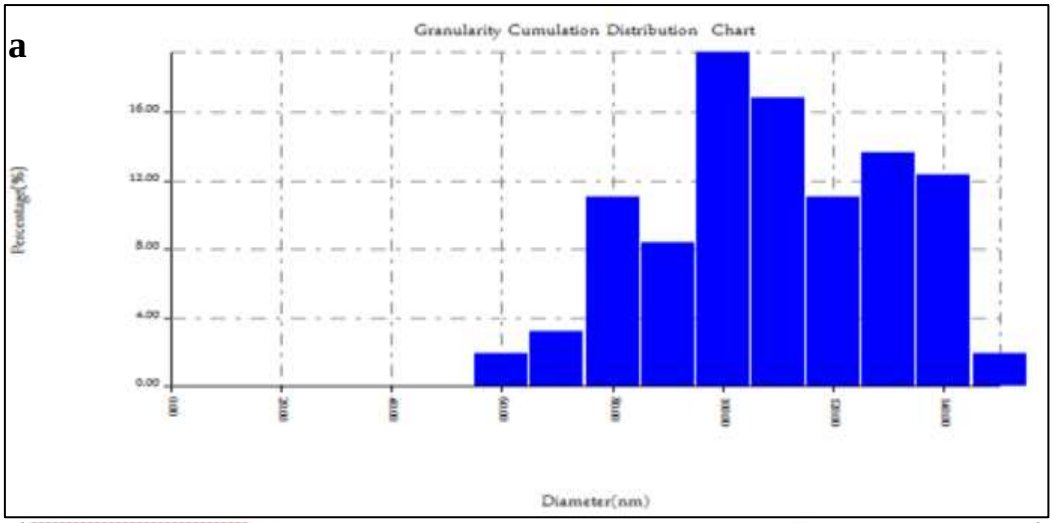
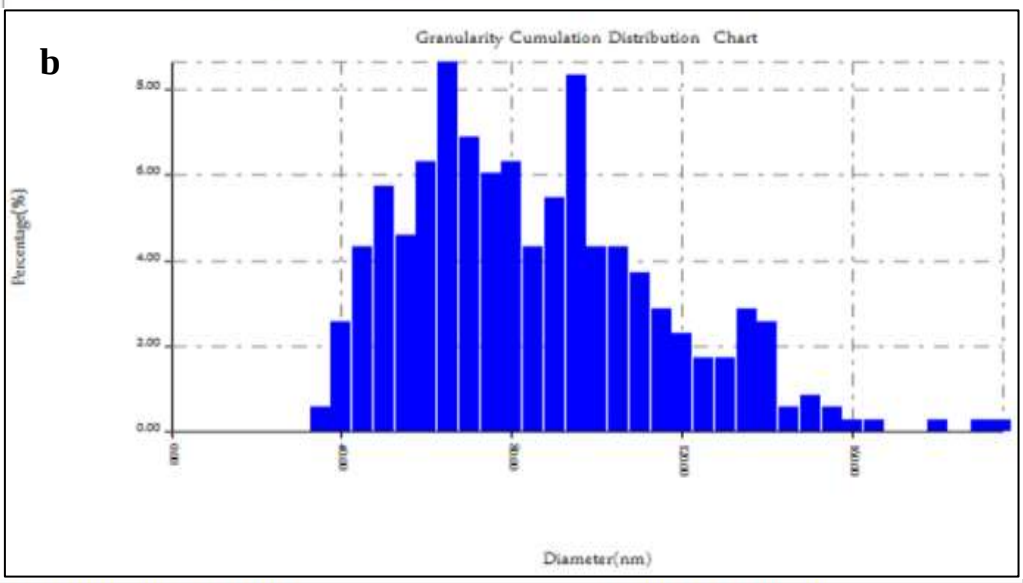


Figure 2- AFM images of AgNPs. Two dimensional (a) and three dimensional (b) of *Ficus carica ;* Two dimensional (c) and three dimensional (d) of*Ficus elastic.*



Avg. Diameter:103.98 nm **<=10% Diameter:70.00 nm**
<=50% Diameter:100.00 nm **<=90% Diameter:130.00 nm**

Diameter (nm)<	Volum e(%)	Cumulati on(%)	Diameter (nm)<	Volum e(%)	Cumulati on(%)	Diameter (nm)<	Volum e(%)	Cumulati on(%)
60.00	1.95	1.95	100.00	19.48	44.16	140.00	12.34	98.05
70.00	3.25	5.19	110.00	16.88	61.04	150.00	1.95	100.00
80.00	11.04	16.23	120.00	11.04	72.08			
90.00	8.44	24.68	130.00	13.64	85.71			



Avg. Diameter:82.82 nm **<=10% Diameter:45.00 nm**
<=50% Diameter:75.00 nm **<=90% Diameter:125.00 nm**

Diameter (nm)<	Volum e(%)	Cumulati on(%)	Diameter (nm)<	Volum e(%)	Cumulati on(%)	Diameter (nm)<	Volum e(%)	Cumulati on(%)
35.00	0.57	0.57	85.00	4.31	56.32	135.00	2.87	93.97
40.00	2.59	3.16	90.00	5.46	61.78	140.00	2.59	96.55
45.00	4.31	7.47	95.00	8.33	70.11	145.00	0.57	97.13
50.00	5.75	13.22	100.00	4.31	74.43	150.00	0.86	97.99
55.00	4.60	17.82	105.00	4.31	78.74	155.00	0.57	98.56
60.00	6.32	24.14	110.00	3.74	82.47	160.00	0.29	98.85
65.00	8.62	32.76	115.00	2.87	85.34	165.00	0.29	99.14
70.00	6.90	39.66	120.00	2.30	87.64	180.00	0.29	99.43
75.00	6.03	45.69	125.00	1.72	89.37	190.00	0.29	99.71
80.00	6.32	52.01	130.00	1.72	91.09	195.00	0.29	100.00

Figure 3-The granularity cumulating distribution chart of AgNPs synthesized by a)*Ficus carica* and b)*Ficus elastic*.

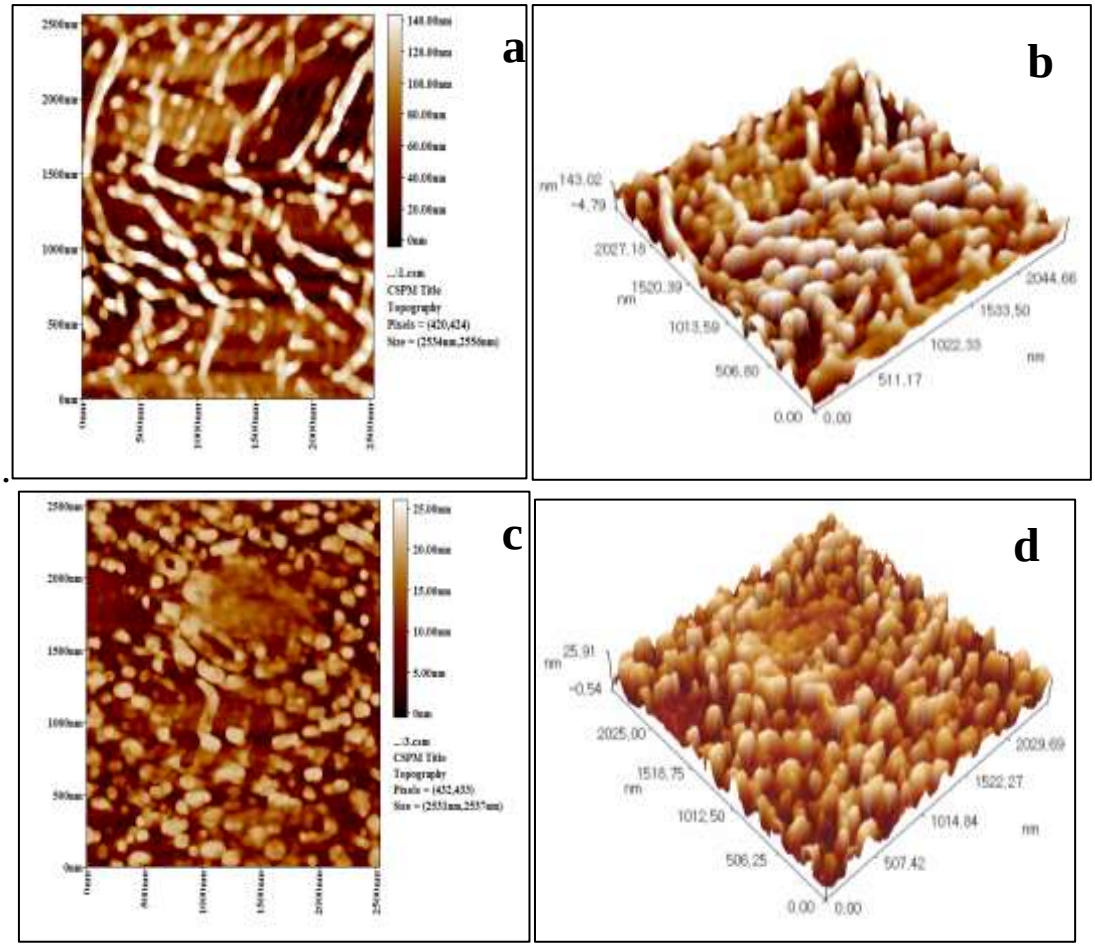
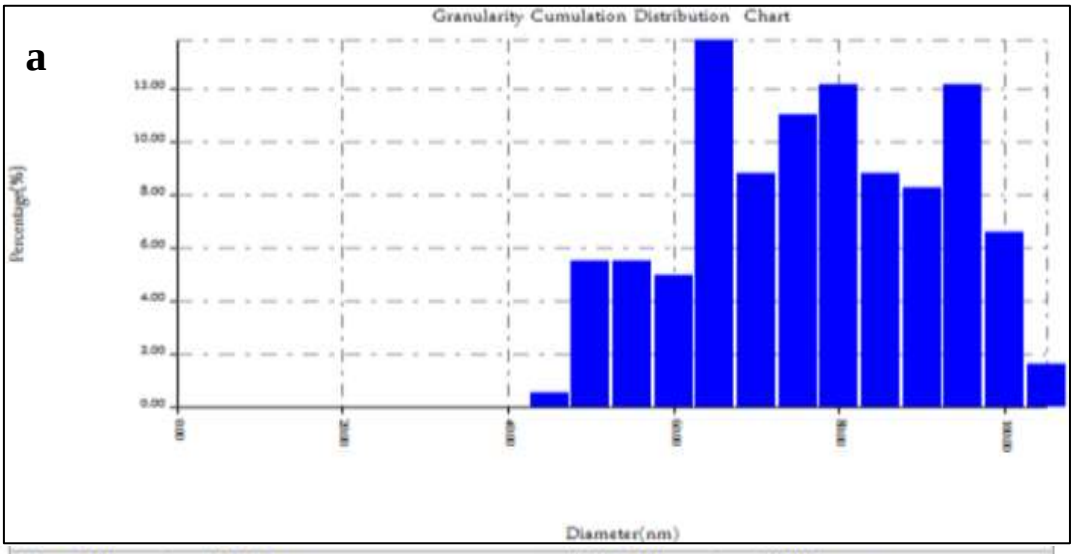
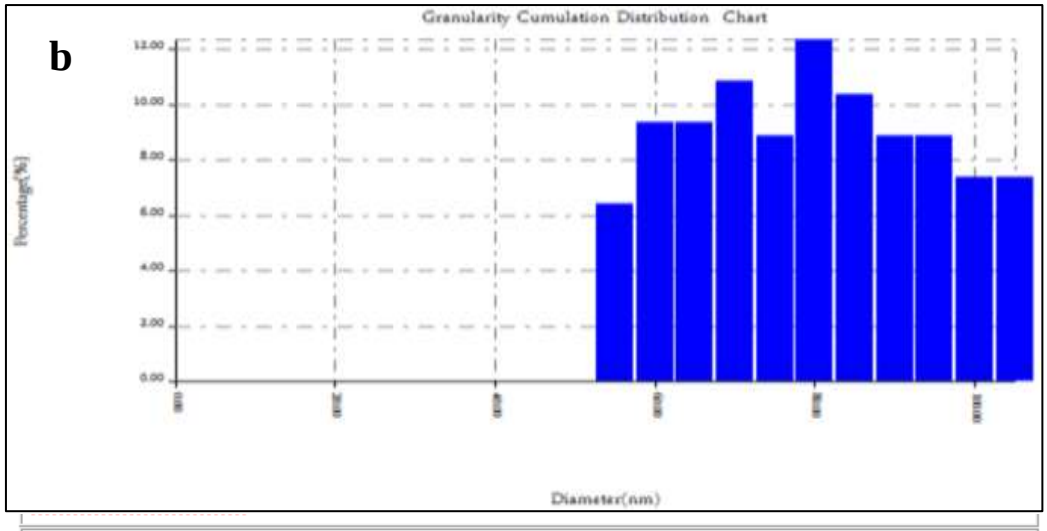


Figure 4-AFM images of AgNPs. Two dimensional (a) and three dimensional (b) of *Euphorbia milii ;* Two dimensional (c) and three dimensional (d) of *Euphorbia tireuall*



Avg. Diameter:74.63 nm **<=10% Diameter:50.00 nm**
<=50% Diameter:70.00 nm **<=90% Diameter:90.00 nm**

Diameter (nm)<	Volume(%)	Cumulation(%)	Diameter (nm)<	Volume(%)	Cumulation(%)	Diameter (nm)<	Volume(%)	Cumulation(%)
45.00	0.55	0.55	70.00	8.84	39.23	95.00	12.15	91.71
50.00	5.52	6.08	75.00	11.05	50.28	100.00	6.63	98.34
55.00	5.52	11.60	80.00	12.15	62.43	105.00	1.66	100.00
60.00	4.97	16.57	85.00	8.84	71.27			
65.00	13.81	30.39	90.00	8.29	79.56			



Avg. Diameter:77.22 nm **<=10% Diameter:55.00 nm**
<=50% Diameter:75.00 nm **<=90% Diameter:95.00 nm**

Diameter (nm)<	Volume(%)	Cumulation(%)	Diameter (nm)<	Volume(%)	Cumulation(%)	Diameter (nm)<	Volume(%)	Cumulation(%)
55.00	6.40	6.40	75.00	8.87	44.83	95.00	8.87	85.22
60.00	9.36	15.76	80.00	12.32	57.14	100.00	7.39	92.61
65.00	9.36	25.12	85.00	10.34	67.49	105.00	7.39	100.00
70.00	10.84	35.96	90.00	8.87	76.35			

Figure 5- The granularity cumulating distribution chart of AgNPs synthesized by a)*Euphorbia milii* and b) *Euphorbia tireuall.*

Many plant parts and extracts showed the ability to synthesize nanoparticles. The size of AgNPs synthesized by the leaves extract of *Alternantherta dentale* was 50-100nm [22],by the rhizomes of *Acorus calamus* synthesized AgNPs was 83nm [23], by the seeds of *Psoralea corylifolia*was 100-110nm [24], andby the fruits of *Vitis vinifera*was 30-40nm [25]. Natural latex extracted from *Aevea brasilensis* was used to synthesize colloidal AgNPs by easy green method with thermal treatment, with a resulted size of 10-20nm [26]. The high levels of flavonoids, carbohydrates, sapogenins and steroids act as reducing and capping agents that give stability to the synthesized nanoparticles [27].These studies show that plants could be a perfect source of nanoparticles which can be utilized in different kinds of applications.

Antibacterial activity of AgNps

Results of antibacterial activity of the AgNPs showed effects against all bacteria with variation in diameters of inhibition zones. The largest inhibition zone was detected against *E.coli*(20.5mm)while the smallestwas9.5mm was against *Staphylococcus aureus*at 500µg/ml of synthesized AgNPs (Figure- 6 and Table-2).

Figure 6-Antibacterial activity of AgNPS synthesized by latex extract of plants against :1)*Staphylococcus aureus*, 2)*Klebsiella* , 3)*Escherichia coli*, 4)*Pseudomonas aeruginosa*.Each well [1]: for*Euphorbia tirulli*, [2]: for*Euphorbia milli*, [3]:for*Ficus elstica*and [4]: for*Ficus carica*, conc. 500 µg/ml of synthesized AgNPs.

Table 2-Inhibition zones(mm) of latex from the four plants used against pathogenic bacteria

Plant/ Pathogenic bacteria	Zone of inhibition(mm) Mean ± SE				
	AgNPs con.produced by latex extract (µg/ml)				
	500	250	125	62.5	
Ficus carica					
Staphylococcus aureus	10.5 ±0.06	10.0 ± 0.05	9.0± 0.04	9.0± 0.04	
Klebsiella	13.0±0.031	13.5± 0.035	12.0± 0.08	10.0± 0.05	
E.coli	17.5±0.06	17.0± 0.08	15.5± 0.04	13.0± 0.03	
Pseudomonas aeruginosa	15.0± 0.06	14.0± 0.09	14.0± 0.07	10.0± 0.08	
Ficus elstica					
Staphylococcus aureus	12.0± 0.06	11.5± 0.05	10.0± 0.06	9.0± 0.09	
Klebsiella	16.0± 0.08	14.0± 0.08	13.0± 0.08	11.0± 0.12	
E.coli	16.5± 0.12	16.0± 0.14	16.0± 0.09	14.0± 0.7	
Pseudomonas aeruginosa	16.0± 0.11	16.5± 0.09	15.5± 0.13	15.0± 0.07	
Euphorbia milli					
Staphylococcus aureus	11.0± 0.05	11.0± 0.07	10.0± 0.08	10.0± 0.06	
Klebsiella	17.0± 0.16	17± 0.08	15.0 ± 0.15	15.0 ± 0.05	
E.coli	19.0± 0.09	18.0± 0.08	18.0± 0.08	17.0± 0.13	
Pseudomonas aeruginosa	16.0± 0.15	16.5± 0.16	15.0± 0.09	11.0± 0.1	
Euphorbia tirulli					
Staphylococcus aureus	9.0± 0.06	8.5 ± 0.09	7.0± 0.04	7.0± 0.03	
Klebsiella	17.0± 0.16	16.5± 0.13	16.0± 0.05	15.0 ± 0.06	
E.coli	20.5± 0.08	20.0 ± 0.09	19.0± 0.06	18.0± 0.06	
Pseudomonas aeruginosa	18.5± 0.15	18.0± 0.09	17.0± 0.17	14.5± 0.05	

Mean±SE (represent triplicate experiments),con:concentration

These results agreed with related studies of synthesizing nanoparticles using plant extracts [28- 30]. Some specific plant parts or whole plant are used for the successful and sufficientsyn thesis of nanoparticle[31]. The variation in the diameters of inhibition zones may be due to the size or shapes of the synthesized nanoparticles that may affect the growth of bacteria and cause their inhibition,which was confirmed by analyzing enzymes and cell leakage [32]. These preparations can be used for various biotechnology and medical applications for controlling pathogenic bacteria with better dispersion and, consequently, bettere fficiency in aqueous environment. The bactericidal effect of metal nanoparticles was related to their small size and high surface to volume ratio, which let the minteract closely with microbial membranes and is not only due to the release of metal ions in solution[33].

The ionized form of silver with its positive charge provide the antimicrobial property of AgNPs. These ions form complexes with DNA, especially with nucleosides, and not with the phosphate group of the

nucleic acid [34]. As a bactericidal agent, some studies showed that electrostatic attraction is presented between positively charged NPs and negatively charged cells of bacteria. These NPs accumulate within the membranes of bacteria and penetrate inside the cells causing the damage [35]. Other studies showed that silver atoms bind to (-SH) group of bacterial enzymes, resulting in a S-Ag stable bonds which cause deactivation of the enzyme. Others proposed that Ag ions which enter the cell disrupt the pyrimidine and purine base pairs, finally disrupting the hydrogen bond between DNA parallel strands and causing denaturation. This interaction with macromolecules of the bacterial cell involves the electron release mechanism as well as free radical formation [36].

The inhibition of protein and cell wall synthesis induced by NPs has been explained by the accumulation of precursor envelope proteins or outer membrane destabilization, which finally leads to energy leakage [37].

According to the results of this study, Gram negative bacterial was more susceptible to the AgNPs than the Gram positive bacteria. This can be explained as the cell wall of Gram positive bacteria is thicker and built of peptidoglycan molecules, as well as its negative charge that causes the positive charge of Ag ions to be trapped outside the cell, as compared to Gram negative bacteria with a very thin cell membrane [38].

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