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BIOSYNTHESIS OF SILVER NANOPARTICLES USING EXTRACT OF ZIZIPHUS LEAVES

Nazar Abdulameer Hamzah

Department of Biology, College of Science, Al-Qasim Green University, Iraq.

Abstract

Ziziphus extract was exploited for the produce of the silver nanoparticles by $AgNO_3$ solution. Characterizations of the Ag nanomolecules were accomplished by UV-vis spectrophotometer, Scanning Electron Microscope and Energy Dispersive-x-ray Spectroscopy after change of nanoparticles solution from yellowish to red brown to dark brown. The synthesis and stability of the reduced nanomolecules were examined using UV-vis spectrophotometer, the maximum absorbance recorded at 440 nm for 1, 24 and 48 hours. Scanning Electron Microscope was used for calculate mean of the size (40-90 nm) and the shape of the nanomolecules. Silver element was showed by Dispersive-x-ray Spectroscopy which showed strong signal of the Ag compared with other elements. Application of the Ag nanomolecules as an antimicrobial against some pathogenic bacterial species was noticed toward G^+ and with a less degree toward G^- bacteria.

Key words: Ziziphus, Silver nanoparticles, SEM, EDS, Antibacterial activity.

Introduction

Nanotechnology is modern area of science rapidly progressed for synthesis and utilize the nanomolecules from a many metal ions such as iron, silver, copper titanium and gold (Natarajan *et al*., 2010). Nanoparticles are show new improved features of the metals include size, arrangment and shape compared with original state (Ahmad *et al.,* 2003). In addition, structure, crystallization, wide surface area than small volume ratio and homogeneity modification also offers new properties (Ramezani *et al*., 2008). Manufacturing of these particles were ranged from 1 to 100 nm required one of the physical, chemical or biological methods (Cao and Hu, 2009). Nanoparticles generated according tow strategy, Top-down and Bottom-up. In the first strategy nanoparticles obtained from large scale structure of different techniques such as mechanical milling, chemical etching and laser ablation. Where as in second such as in bio reduction, nanoparticle structures are made atom by atom or molecule by molecule by self-assembly or self-organization (Kasthuri and Veera pandiran, 2009; Dubey *et al*., 2010). The chemical and physical procedures were adopted for the manufacture of nanomolecules can produce of the toxic, dangerous and pollutants materials that caused biological damages (Sastry *et al*., 2003). Although of many biosynthetic ecofriendly methods to production of Ag nanomolecules mediated by microbes, it's not very appropriate for industrial scale because of need to high degree of sterilization conditions and maintenance (Kalishwaralal *et al*., 2010). Synthesis Ag nanomolecules by using of the plants extracts have several advantages involve fast results, eco-friendly, avoid pathogenic organisms or substances, low cost technique and providing a single step protocol for the green synthesis operation (Huang *et al*., 2007). Primary and secondary metabolites in the plants such as amino acids, proteins, enzymes sugars, vitamins, alkaloids, glucosides, phenolics, coumarins saponins, terpenoids, volatile oils and tannins have ability to reducing and stables of the Ag ions producing silver nanoparticles. (Kulkarni and Muddapur, 2014). Ag nanomolecules were synthesized from extract of different parts and species of plant reviewed by Roy and Das, 2015 and their activity against microbes were detected. According to importance of plants in green nanotechnology, our work focused on prepare Ag nanomolecules using a leaves extract of Ziziphus although may be some researches were previously showed same interest.

***Author for correspondence:** E-mail: nazar.hamzah@science.uoqasim.edu.iq

Materials and Methods

Samples collection

Leaves of Ziziphus were collected from college of Biotechnology/ University of Al-Qadisiyah during period from November to December 2018. Leaves were washed 3 times with tap water followed with rinse by dd H_2O. Samples were dried at 25°C then were grinded with electrical grinder to obtain fine powder. The powder was stored in clean dry containers at 4°C for next experiments.

Apparatus and chemicals

Sensitive balance, warn blender, electrical grinder, electrical oven, hot plate, UV-vis spectrophotometer, Scanning electron microscope (SEM), Energy Dispersive-x-ray, silver nitrate. barium chloride, sulfuric acid, sodium chloride, previously prepared Muller-Hinton Agar.

Methods

• Silver nanoparticles biosynthesis: The extract of the Ziziphus was prepared by boiled 20 g of the powder in beaker contain 50 ml of the distilled water using hot plate for 5 minutes. The boiled mixture was cooled at the room temperature then filtered with 4-layer of the cheesecloth followed with additional filtration using Whatman No. 1 filter paper. Liquid solution of 1 mM of the $AgNO_3$ was prepared by dissolve 8.4 mg in 50 ml of the distilled water. Five ml of the extract was added to 50 ml of the liquid solution of $AgNO_3$ then wrapped with aluminum foil to avoid photo-oxidation. Color changing was monitoring at 1, 24 and 48 hours. The solution turned from yellowish to red brown to dark brown.

• Silver nanoparticles characterization: The spectra of absorption of the samples were estimated via UV-vis spectrophotometer. The wave lengths were 300-500 nm, the synthesis of the Ag nanomolecules was recorded during 1, 24 and 48 hours by following of the maximum absorbance. The blank was distilled water. According to Caroling *et al*., 2013 identification of the shape and size of the silver nanoparticles were examined through Scanning Electron Microscope (SEM) in electron microscope unit, University of Kufa, Faculty of Science. The small drop from Ag nanomolecules liquid was spotted on the slide. After drying of the slide was subjected to the SEM then the operating system was adjusted. The voltage of the electron acceleration traveled through the electron column was 15 kV using different magnification, low vacuum, a spot size was 4 and the working distances between the lens and the drop was 9.4 mm. The Ag element in solution of nanomolecules was determined by Energy Dispersive-x-ray Spectroscopy (EDS) combined with SEM in the electron microscope unit, Faculty of Science/ University of Kufa. Accelerating voltage, spot size and working distances were 10 kV, 5 and 1 mm respectively for preparation reaction of the source of X-ray excitation and a sample (Sarvamangala *et al*., 2013).

• Drying of Ag nanomolecules solution: All of the Ag nanomolecules liquids were prepared in several times pooled in beaker to obtain final volume of 200 ml. The pooled solution was subjected to oven heat 60°C for 12 hours with intervals 2 hour. The dried yield of the Ag nanomolecules was stored for detection of antibacterial activity.

• Antibacterial activity of Ag nanomolecules:

Stock and diluted solutions of Ag nanomolecules: Ten mg of the dry and solid material **(**Silver Nanoparticles**)** was dissolved in 10 ml of dd H_2O for obtaining 1mg/ml concentrated stock solution. Six concentrations (0.1-1 mg/ml) with interval 0.2 were prepared separately.

McFarland standard tube: Prepared according to (Baron and Finegold, 1990), 1.175 g of the barium chloride was dissolved in 100 ml of the dd H_2O then 9.5 ml of the sulfuric acid (0.1 ml) solution was added to 0.5 ml of the barium chloride solution to obtain 1.5×10^6 cell/ml standardized suspension of each tested bacteria. The mixture was stored in transparent glass tube in dark place at 4°C for next experiments.

Normal saline solution: NaCl (8.5 g) was dissolved in 100 ml dd H_2O then volume reached to 1000 ml. The solution was autoclaved the pH was adjusted at 7 and stored in 4°C until use (APHA, 1985).

Serial dilutions of the bacteria: Normal saline was used for serial dilutions of the bacteria for estimate approximately number (1.5×10^6 cell/ ml) compared with the McFarland standard tube.

Antimicrobial activity: Antimicrobial activity towards varied types of positive and negative gram stained clinical bacteria utilizing agar wells diffusion method. Three replicates of four sterile Muller-Hinton agars in Petri dishes were swabbed individually with 1.5×10^6 cell/ml of the *Staphylococcus aureus*, *Streptococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* then dried at room temperature. Six wells (5 mm) were formed by cork borer punching then each well loaded with 100 µl of the 0.1, 0.2, 0.4, 0.6, 0.8, 1 mg/ml respectively while seventh well used as control that contain only dd H_2O. The Petri dishes were incubated in 37°C for 24 hrs. Inhibition zones of seperate concentration were calculated in its own well as mm unit of the diameters.

Results and discussion

UV-vis spectrophotometer

The Ag^+ in aqueous solution reduced to Ag

Fig. 1: Color changing from yellowish to red brown to dark brown after 1, 24 and 48 hours from extract- $AgNO_3$ solution reaction.

nanomolecules (Ag^o) when added extract of Ziziphus. The color in the solution was changed with time progress of the reaction from yellowish to red brown to dark brown (Fig. 1) which refer of the Ag nanomolecules was synthesized. Chemical products of the Ziziphus like alkaloids, terpenoids, flavonoids, coumarins, phenols, polysaccharides and proteins have ability to reduce and stable of nanomolecules (Shankar *et al*., 2004). Ag nanomolecules show brown color in the liquid due to excitation of surface plasmon vibrations in Ag nanomolecules that lead to changing in optical features and alter of the color (Jain *et al*., 2009).

The absorbance measured via UV-vis spectrophotometer was increased gradually with increase of wave length and the period of the incubation (1, 24 and 48 hours) of Ag nanomolecules solution, the maximum value indicated in 440 nm (Fig. 2). The reported absorbance showed at 440 nm related to the surface plasmon resonance of Ag nanomolecules. The increase absorbance peak reflects increase number of the Ag nanomolecules formed by reduction. The absorbance of surface plasmon band in the Ag nanomolecules liquid is typical at range of the 446-448 nm (Banerjee *et al*., 2014). For characterization of size, shape and distribution of Ag nanomolecules scanning electron microscope was used.

Scanning electron microscope analysis

The magnitude result was appeared regular dispersed semispherical while some of the silver nanoparticles were present an irregular structure of shape having 40-90 nm in size (Fig. 3). Ability of plants extracts for nanoparticles synthesis was routinely examined by SEM and Transmission electron microscope (TEM) for surface shape and nanometric scale (Schaffer *et al*., 2009). The molecule size and dispersed of Ag nanomolecules were also detected by more advanced technique, histogram of dynamic light scattering (DLS) in varied solution of plants organs (Roy and Das, 2015). Other technique, X-Ray Diffraction was used for determination and identification of the crystal composition of the Ag nanomolecules (Sun *et al*., 2000).

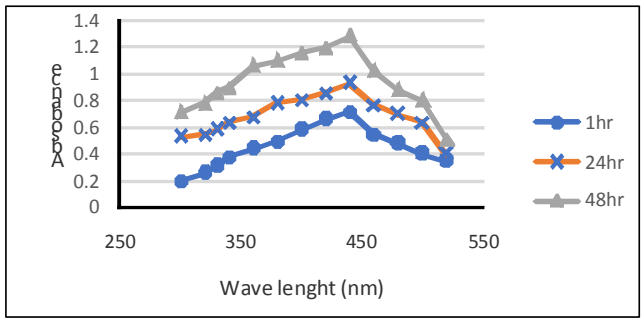


Fig. 2: UV-vis spectrophotometer of synthesis of the Ag nanomolecules.

Energy dispersive-x-ray spectroscopy analysis

The further characterization was presence of silver metal in prepared Ag nanomolecules solution was quantified by EDS technique via peak of silver elements absorbance. The EDS spectrum was recorded in the peak's profiles of the sample. Signals of the silver was strong whereas signals of oxygen and other elements

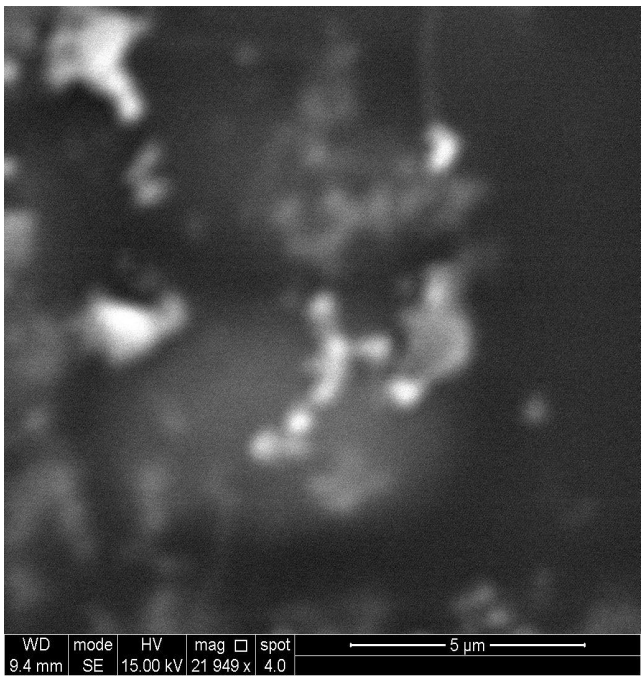
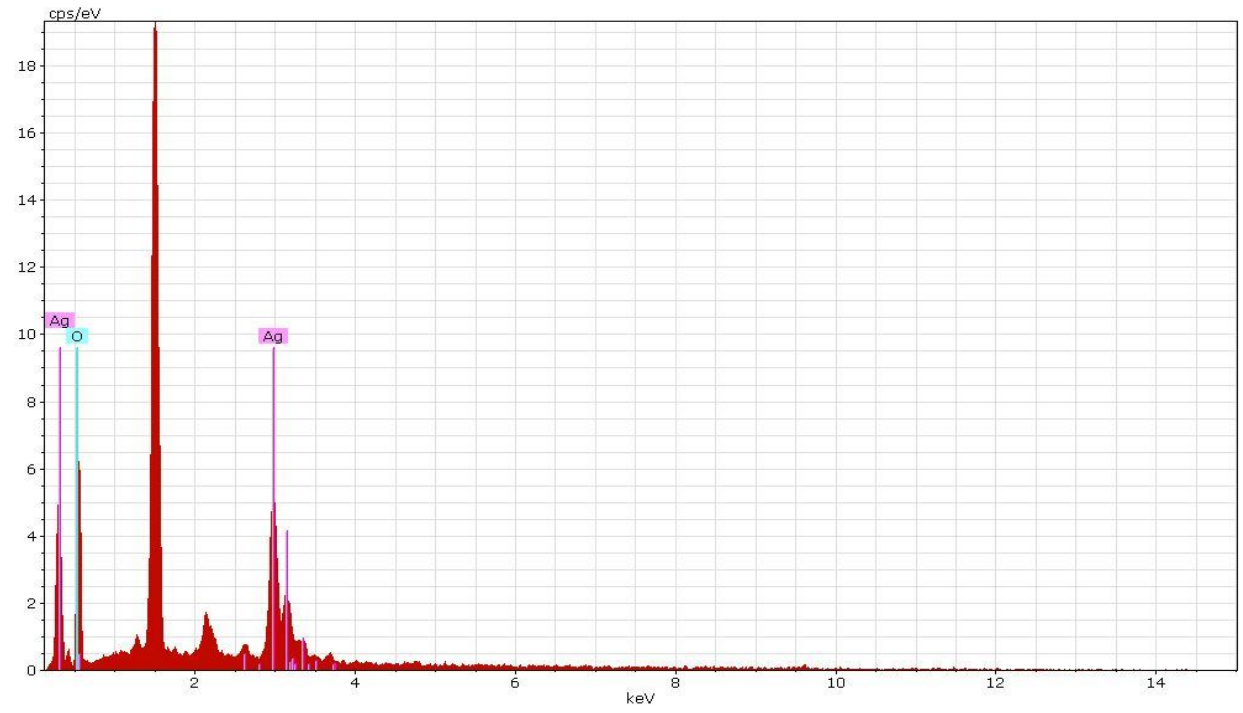


Fig. 3: SEM analysis of Ag nanomolecules sample.

Application Note
Company / Department



Spectrum: Acquisition

Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (1 Sigma) [wt.%]
Oxygen	K-series	19.76	47.22	85.78	3.69
Silver	L-series	22.09	52.78	14.22	0.81
	Total:	41.86	100.00	100.00	

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Fig. 4: Energy dispersive-x-ray spectroscopy analysis of Ag nanoparticles obtained by extract of the Ziziphus, appear strong signals of the silver and medium signal of Oxygen, the optical the weight percentage of silver (52.78%) and oxygen (47.22%).

were medium and weaker respectively (Fig. 4). The EDS was used for elemental composition of silver and other metals in nanoparticles mixture obtained by plant extracts (Strasser *et al*., 2010). Interaction of the X-ray excitation and a sample of silver nanoparticles reflect the unique atomic structure which giving a unique models of the peaks on its electromagnetic emission spectrum (Goldstein *et al*., 2003).

Antibacterial activity

The activity of Ag nanomolecules towards different

Table 1: Mean of Inhibition zones in diameter (mm) of clinical bacteria.

Pathogenic Bacteria	0.1 mg/ml	0.2 mg/ml	0.4 mg/ml	0.6 mg/ml	0.8 mg/ml	1 mg/ml
Staphylococcus aureus	8	12	17	20	22	24
Streptococcus aureus	7	13	15	18	20	22
Escherichia coli	0	0	0	8	11	14
Klebsiella pneumoniae	0	0	0	5	9	11

clinical G^+ (*Staphylococcus aureus*, *Streptococcus aureus*) in addition G^- (*Escherichia coli* and *Klebsiella pneumoniae*) bacteria was identified. Generally, inhibition zones diameters around wells showed linearly increasing with increase of the concentration for gram-positive bacteria. The highest inhibition (24 mm) noticed in *Staphylococcus aureus* at 1mg/ ml while *Escherichia coli* and *klebsiella pneumoniae* appeared resistance at 0.1, 0.2 and 0.4 mg/ml. In other hand the resistance of gram-negative bacteria decreased gradually with other concentration especially at 0.8 and 1 mg/ml. (Table 1 and Fig. 5). The lipopolysaccharide in outer membrane shields and underlying peptidoglycan of the cell wall which may be play important role in resistance of gram-negative bacteria in spite of the gram-positive has thick layer of peptidoglycan in outer surface. In addition, silver metals perhaps interfered and caused damage to nucleic acids, proteins and enzymes of bacteria at different effected concentrations. The obtained results of the bacterial inhibition donate ability to utilizing of silver nanoparticles in medical fields.

Conclusions

Our study concludes capability of the Ziziphus extract of the leaves to reduce of $AgNO_3$ into Ag nanomolecules which have antibacterial activity.

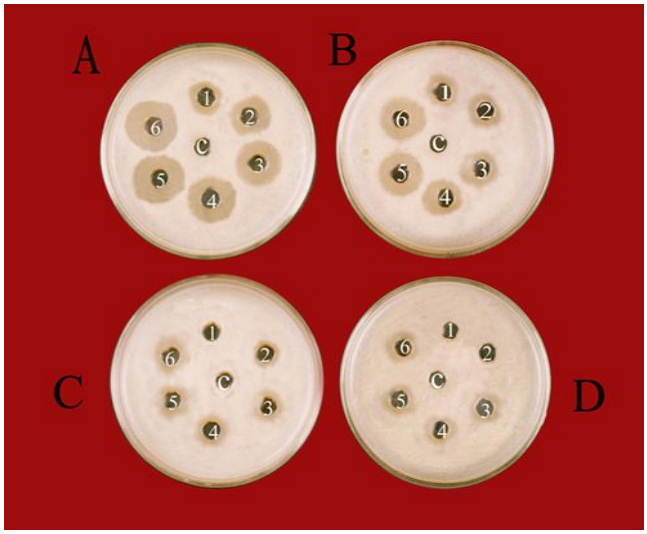

Fig. 5: Inhibition zones in diameter (mm) of clinical bacteria, A, *Staphylococcus aureus* B, *Streptococcus aureus* C, *Escherichia coli* and D, *Klebsiella pneumoniae*. 1-6, concentration from 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg/ml respectively C, control

Conflicts of Interest

An author declares have no competing interest relevant to this article.

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