

Biosynthesis and Electrochemical Characterization of Silver Nanoparticles from Leaf Extract of *Adenium obesum* and Its Application to Antibacterial Effect

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We screened plant leaf extract and biosynthesis of silver nanoparticles (AgNPs) by monitoring the conversion using electrochemistry method. In this study a simple and rapid biosynthesis of AgNPs using leaves extract of *Adenium obesum* have been expected. AgNPs can be prepared with lower amounts of leaf extract. The effect of leaf extract quantity and concentration of metal solution were also evaluated to optimize the biosynthesis route producing the metal nanoparticles. Stable AgNPs were formed by treating solution using the plant leaf extracts as reducing agents. The AgNPs were characterized by their average size, morphology, crystal nature, nanostructure dimensions and purity by various analytical techniques. Biosynthesized nanoparticles were characterized by various methods, such as atomic force microscopy (AFM), electrochemical impedance spectroscopy (EIS), UV-Vis and cyclic voltammetry (CVs). Although plant is considered as undesirable plant, but to the best of our knowledge we are the first to report its use in biosynthesizing AgNPs, which can provide a new platform to this plant making it a value added weed for nanotechnology based industries in future. This environmentally friendly method of biological AgNPs production provides rates of biosynthesis faster or comparable to those of chemical methods and can potentially be used in various human contacting areas such as cosmetics, foods, medical and antibacterial effect applications.

Keywords: silver nanoparticles (AgNPs), biosynthesis, leaves extract, *Adenium obesum*, antibacterial, electrochemistry.

1. INTRODUCTION

Science of the nanotechnology is supposed to have started by the lecture of Richard Feynman on "There is Plenty of Room at the Bottom" at the annual meeting of the American Physical Society at the

California Institute of Technology in 1959. Nanotechnology is expected to be the basis of many of the main technological innovations of the 21st century. Research and development in this field is growing rapidly throughout the world. It due to the optical, magnetic and electrical properties [1-2]. A major output of this activity is the development of new materials in the nanometer scale, including nanoparticles. These are usually defined as particulate materials with at least one dimension of less than 100 nanometers (nm) [3], even the particles could be zero dimension as in the case of quantum dots.

Silver has long been recognized as having an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes [4]. The most widely used and known applications of silver and silver nanoparticles are in the medical industry. These include topical ointments and creams containing silver to prevent infection of burns and open wounds [5]. Another widely used applications are medical devices and implants prepared with silver-impregnated polymers [6]. In addition, silver-containing consumer products such as colloidal silver gel and silver-embedded fabrics are now used in sporting equipment.

Specific surface area is relevant for catalytic reactivity and other related properties such as antimicrobial activity in silver nanoparticles (AgNPs). As specific surface area of nanoparticles is increased, their biological effectiveness can increase due to the increase in surface energy. Nanoparticles of noble metals, such as gold [7-10], silver [11-13], and platinum [14], are widely applied in products that directly come in contact with the human body, such as shampoos, soaps, detergent, shoes, cosmetic products, and toothpaste, besides medical and pharmaceutical applications.

Using plants for nanoparticle synthesis can be advantageous over other biological processes because it eliminates the elaborate process of maintaining cell cultures and can also be suitably scaled up for large-scale nanoparticle synthesis [15]. Although the biosynthesis of nanoparticles by plants such as Pear fruit [16], *Rosa rugosa* [17], *Diopyros kaki* [18], Pine, Persimmon, *Ginkgo*, *Magnolia*, *Platanus* [19] and three categories of plants: xerophyte (*Bryophyllum sp.*), mesophyte (*Cyprus sp.*) and hydrophyte (*Hydrilla sp.*) [20], has been reported, however possibilities in plant-mediated biological synthesis of nanomaterial have to be fully explore [21]. Among various metal nanoparticles, AgNPs have several effective applications as antibacterial, sensors and detectors besides their biomedical applications [22-25].

Adenium obesum (family Apocynaceae) commonly known as 'desert rose' primarily an ornamental plant. All parts of the plant (including the latex) contain a dangerous heart poison called adenine. The poison of this plant is used for baited meat. The pounded roots for fish poison and an arrow poison can be obtained from its seeds. An infusion from the roots kills lice. The latex is used to treat boils, septic wounds and tooth cavities. The stems powdered mixed with water kills vermin on camels. *A. obesum* has previously been reported to have cytotoxic activity [26] and anti-influenza virus [27]. The present paper deals with silver nanoparticles biosynthesis from leaf extract of *Adenium obesum*, and its electrochemical characterization and antibacterial effect.

2. EXPERIMENTAL

2.1. Materials and Apparatus

Desert Rose (*Adenium obesum*) were collected from wild during field exploration in Saudi

Arabia in Fig.1 (A). Silver nitrate (AgNO_3) was purchased from Sigma–Aldrich (St. Louis, U.S.A.). All other chemicals used were of analytical grade and used without further purification. 0.1 M pH 7.0 phosphate buffer solutions (PBS) was used as supporting electrolyte. Aqueous solutions were prepared using doubly distilled deionized water.

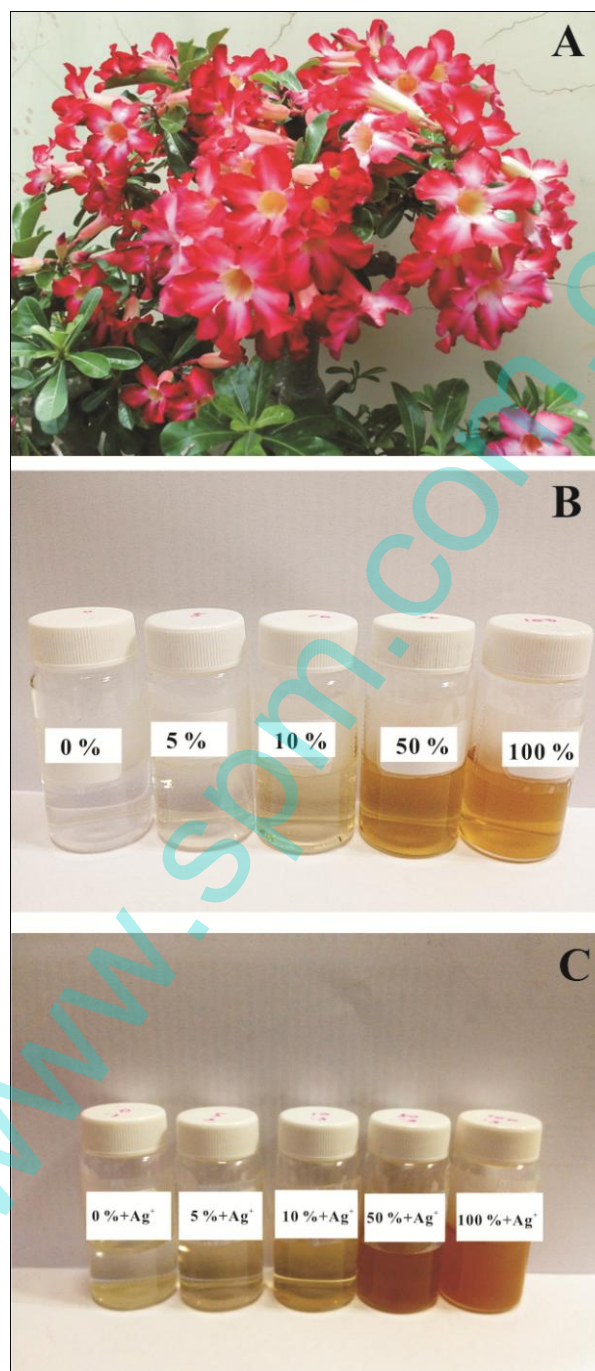


Figure 1. (A) Desert Rose (*Adenium obesum*). (B) shows different concentrations of leaf extract 0 % to 100 % in pH 7.0 PBS; (C) shows different concentrations of leaf extract 0 % to 100 % in pH 7.0 PBS containing 1 mM AgNO_3 .

2.2. Apparatus

Cyclic voltammetry (CVs) were performed in an analytical system model CHI-1205A potentiostat. A conventional three-electrode cell assembly consisting of an Ag/AgCl reference electrode and a Pt wire counter electrode were used for the electrochemical measurements. The working electrode was glassy carbon electrode (GCE; area 0.07 cm^2). Indium tin oxide (ITO) ($7 \text{ } \Omega/\text{cm}^2$) was purchased from Merck Display Technologies (MDT) Ltd (Taiwan). In these experiments, all the potentials have been reported versus the Ag/AgCl reference electrode. The morphological characterizations of the films were examined by atomic force microscopy (AFM) (Being Nano-Instruments CSPM5000). Electrochemical impedance spectroscopy (EIS) measurements were performed using an IM6ex Zahner instrument (Kroach, Germany). The UV-visible absorption spectra were checked by using a U3300 Spectrophotometer (HITACHI). Fresh cultures for the experiments were made by 3M™ Petrifilm™. All the solutions were purged with high purity nitrogen gas for about 20 min before performing electrochemical experiments. Also, a continuous flow of nitrogen over the aqueous solution was maintained during measurements. All the experiments were carried out at room temperature ($\approx 25^\circ\text{C}$).

3. RESULTS AND DISCUSSIONS

3.1. Preparation of leaf extract from *Adenium obesum*

Plant leaves of *Adenium obesum* were collected, washed several times with ultra pure water to remove the dust and dried for 2 days at room temperature. The plant leaf extract solution was prepared by taking 5 g of thoroughly washed and finely cut leaves in 400 ml of pH 7.0 PBS and then boiling the mixture for 5 min before finally decanting it. The leaf extract concentrations were also varied between 0 % to 100 % by volume in pH 7.0 PBS (Fig. 1 (B)). They were stored at 4°C and used within a week. 10 mL of leaf extract was added to 190 mL of 1 mM aqueous AgNO_3 solution for reduction of Ag^+ ions. The concentrations of AgNO_3 solution and leaf extract were also varied at 0.1 to 1 mM and 0 to 100% by volume (Fig. 1 (C)), respectively. The effects of temperature on the synthesis rate and particle size/shape of the prepared silver nanoparticles (AgNPs) were studied by carrying out the reaction in a water bath at 95°C with reflux. The AgNPs solution thus obtained was purified by repeated centrifugation at 15,000 rpm for 20 min followed by redispersion of the pellet in deionized water. The leaf extract concentrations were also varied between 0 % and 5 % by volume in pH 7.0 PBS. Different concentrations of leaf extract containing 0.1 and 1 mM AgNO_3 . We observed external of the color variation by different concentrations of leaf extract. Obviously color change to explain that the plant leaf extract as reducing agents. Stable AgNPs were formed by treating solution using the plant leaf extract as reducing agents. With leaf extract, it was reported that they are believed to be the surface active molecules stabilizing the nanoparticles and reaction of the metal ions is possibly facilitated by reducing sugars and/or terpenoids present in the leaf.

3.2. Morphological characterization of Silver nanoparticles

Successfully biosynthesized nanoplates were observed, elegantly assembled with AgNPs. The AFM is one of the foremost tools for imaging, measuring, and manipulating matter at the nanoscale. Prior to modification, ITO surfaces were cleaned and ultrasonicated in acetone–water mixture for 15 min and then dried. Nanostructure size, crystal nature and morphologies were characterized by AFM. Biosynthesized of AgNPs at different leaf extract quantities were observed in Fig. 2. Different conditions of leaf extract (A) 0 % and (B) 5 %, The 5 % of leaf extract containing (C) 0.1 mM and (D) 1 mM AgNO_3 . With an increase AgNO_3 concentrations, AgNPs possess different shape and particle size of both the synthesized metal nanoparticles.

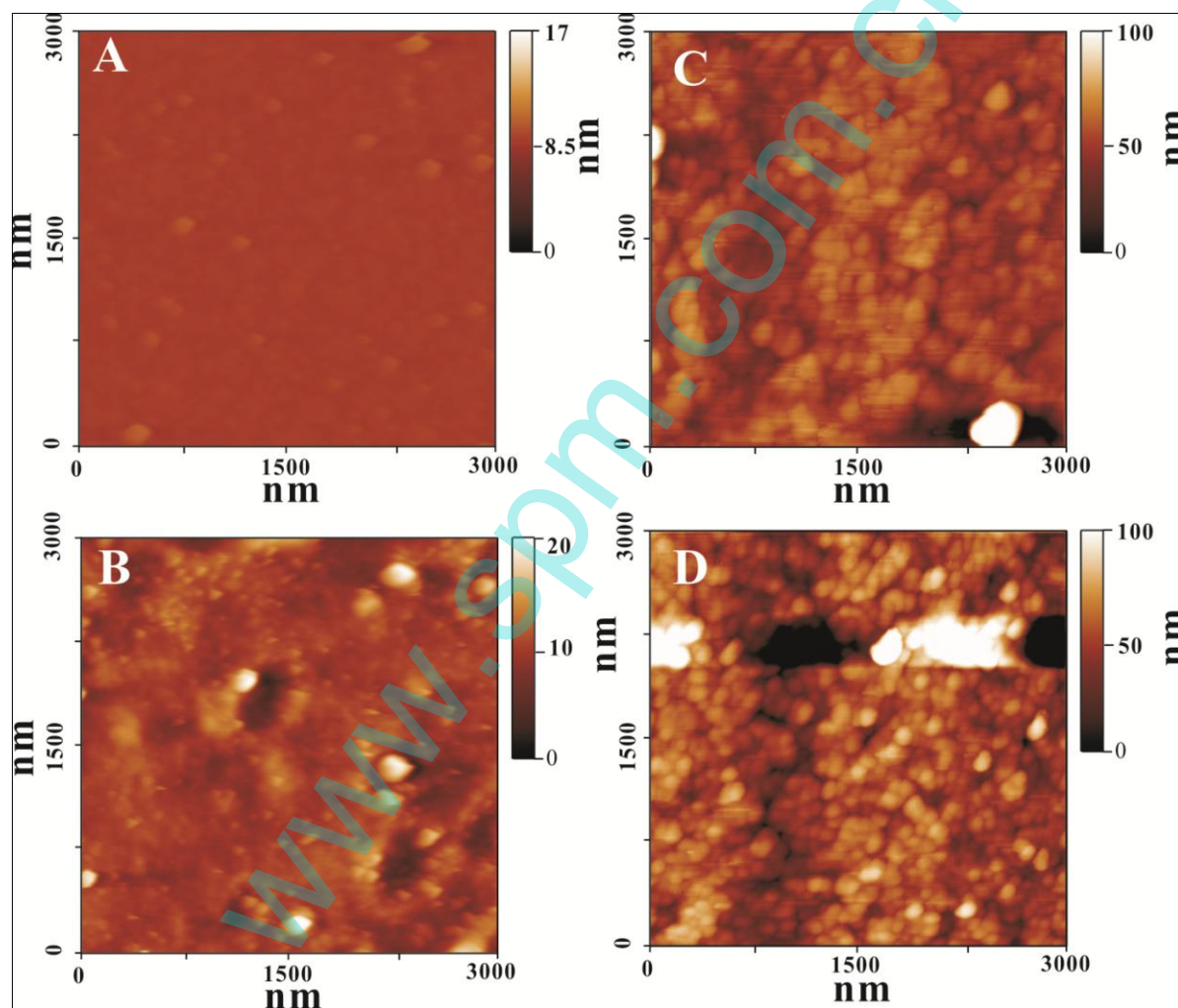


Figure 2. AFM of AgNPs from leaf extract (A) 0 % and (B) 5 % in pH 7.0 PBS. The 5 % leaf extract in pH 7.0 PBS containing (C) 0.1 mM and (D) 1mM AgNO_3 .

Fig. (A) and (B) shows without AgNO_3 treating has smooth surface, on AgNPs biosynthesized. Fig. (C) 5 % leaf extract with 0.1 mM AgNO_3 condition shows synthesized of AgNPs, we observed the existence of nanostructures in obvious manner with the average size range of 75.2 nm. By AFM

section analysis, the other amplitude parameters such like roughness average (sa) was found as 1.63 nm. The root mean square roughness was found as 2.29 nm. Fig. (D) 5 % leaf extract with 1 mM AgNO_3 condition shows best biosynthesized of AgNPs, the average size range of 55.7 nm. By AFM section analysis, the other amplitude parameters such like roughness average (sa) was found as 0.32 nm. The root mean square roughness was found as 0.44 nm. As the AgNO_3 concentrations increased, the AgNPs biosynthesis increased. We also investigated the effects of reaction conditions such as reaction temperature, leaf extract concentration and reaction time on biosynthesis rate and particle size of the AgNPs. The stability of particles was evaluated at different concentrations of AgNO_3 without any additional stabilizing chemicals.

3.3. Electrochemical impedance spectra (EIS) of Au nanoparticles

Electrochemical Impedance Spectroscopy (EIS) or ac impedance methods have seen tremendous increase in popularity in recent years. In theory, any intrinsic property that influences the conductivity of a nanoparticles/solution interface can be examined by impedance measurements. The ratio of the Laplace transforms of potential and current, $E(s)/i(s)$ is expressed in the units of resistance, Ω , and is called *impedance*, $Z(s)$. The inverse of impedance is called *admittance*. They are *transfer functions* which transform one signal, e.g. applied voltage, into another, e.g. current. Both are called *immittances* [28-32].

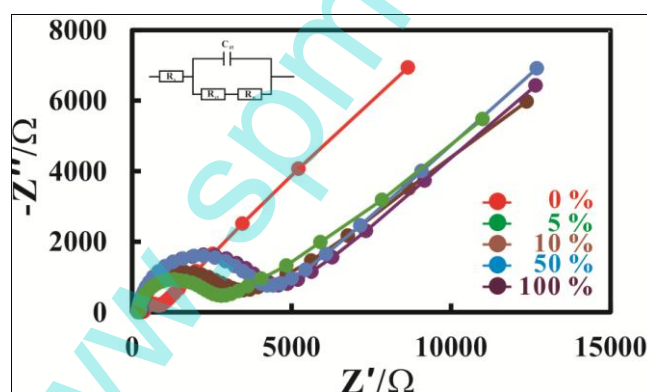


Figure 3. Electrochemical impedance spectroscopy (EIS) of 0 % to 100 % of leaf extract in the presence pH 7.0 PBS of equimolar 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$. The insert displayed the equivalent circuit (Randles model) was used to fit Nyquist diagrams.

The result may be represented graphically using two types of plots: *complex plane* (also known as *Nyquist plots*) and *Bode plots*. In order to simplify the calculations of impedances, the result obtained for the periodic perturbation of an electrical circuit may be represented using complex notation. In the the system impedance, $Z(j\omega)$ and the real and imaginary parts of the impedance are: $Z' = R$ and $Z'' = -1/\omega C$, respectively [33-37]. The complex plane plot is a plot of Z'' versus Z' , that is, the imaginary versus the real components, plotted for various frequencies. Impedance methods are based upon the well-established theory of electronic AC circuit analysis with both instrumentation and data

analysis techniques being analogous. The fundamental approach of EIS is the application of a spectrum of small-amplitude sinusoidal voltage excitations to interrogate the system of interest and the measurement of that systems' response. It will provide a way to monitor the H^+ process and long-term performance of red cabbage extract structures and could also provide a simple stability [36]. Fig. 3 shows the results of EIS for different concentrations of leaf extract in the presence pH 7.0 PBS of equimolar 5 mM $[Fe(CN)_6]^{3-/4-}$. The 0 % (red line) exhibited almost a straight line with a very small depressed semicircle arc ($R_{et} = 720 (Z'/\Omega)$) represents the characteristics of diffusion limited electron-transfer process on the electrode surface. On the same conditions, the 5 % (green line) shows like a depressed semicircle arc ($R_{et} = 2820 (Z'/\Omega)$) clearly indicated the higher electron transfer resistance behavior comparing with 0 %. The 10 % (brown line), 50 % (blue line) and 100 % (purple line) show dispersed semicircle arc ($R_{et} = 3310 (Z'/\Omega)$), ($R_{et} = 4430 (Z'/\Omega)$) and ($R_{et} = 4435 (Z'/\Omega)$). The insert displayed the equivalent circuit (Randles model) was used to fit Nyquist diagrams. It constitutes a distributed element which can only be approximated by an infinite series of simple electrical elements. EIS for different concentrations of leaf extract in the presence pH 7.0 PBS of equimolar 5 mM $[Fe(CN)_6]^{3-/4-}$ containing 1 mM $AgNO_3$. All groups of resistance in each conditions reveals that the value increased. Only the 5 % leaf extract containing 1 mM $AgNO_3$ decreased to arc ($R_{et} = 2320 (Z'/\Omega)$), provided to biosynthesized of AgNPs. The results showed individual resistance value in different concentrations conditions as in Table 1.

Table 1. EIS of different concentrations conditions.

Group	0%	5%	10%	50%	100%
A (Z'/Ω)	720	2820	3310	4430	4435
□ B(Z'/Ω) □	781	2320	4580	5840	5846

A: EIS of 0 % to 100 % leaf extract in the presence pH 7.0 PBS of equimolar 5 mM $[Fe(CN)_6]^{3-/4-}$.
 B: EIS of 0 % to 100 % leaf extract in the presence pH 7.0 PBS of equimolar 5 mM $[Fe(CN)_6]^{3-/4-}$ containing 1 mM $AgNO_3$.

3.4. Cyclic voltammetry of Silver nanoparticles

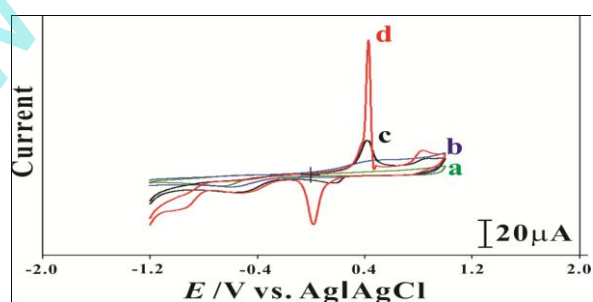


Figure 4. Cyclic voltammograms of (a) 0 % and (b) 5 % of leaf extract in the presence pH 7.0 PBS, The 5 % leaf extract in pH 7.0 PBS containing (c) 0.1 mM and (d) 1mM $AgNO_3$. The corresponding CVs have been obtained at 100 mV s^{-1} scan rate in the potential range of -1.2 to 1.0 V.

Cyclic voltammetry or CVs is a type of potentiodynamic electrochemical measurement. CVs takes the experiment a step further than linear sweep voltammetry which ends when it reaches a set potential. When cyclic voltammetry reaches a set potential, the working electrode's potential ramp is inverted. This inversion can happen multiple times during a single experiment. The current at the working electrode is plotted versus the applied voltage to give the cyclic voltammogram trace. CVs is generally used to study the electrochemical properties of an analyte in solution. Glassy carbon electrode was polished with 0.05 μm alumina on Buehler felt pads and then ultrasonically cleaned for about a minute in water. Finally, the electrode was washed thoroughly with double distilled water and dried at room temperature. After that electrode was immersed in different concentrations of leaf extract (a) 0 % and (b) 5 % in pH 7.0 PBS, The 5 % of leaf extract containing (c) 0.1 mM and (d) 1 mM AgNO_3 from -1.2 V to 1.0 V, scan rate 100 mVs^{-1} in Fig 4. Curve (a) 0 % of leaf extract has peak current $7.91 \mu\text{A}$ at -0.64 V. With increase concentrations of leaf extract to 5 % (curve b), reversible peak current increased occur at -0.57 V and 0.49 V. Curve (c) 5 % of leaf extract containing 0.1 mM AgNO_3 shows new peak current at 0.17 V and 0.425 V. Containing 1 mM AgNO_3 of leaf extract shows higher current was $37.17 \mu\text{A}$ and $101.4 \mu\text{A}$ at 0.02 V and 0.43 V as curve (d). We supposed that characterization of Ag peak [39-41]. The 5 % concentrations of leaf extract have been already proved to be suitable to biosynthesized AgNPs present in this paper. Here we have biosynthesized AgNPs by using leaf extract. In this work, a single-step biosynthetic route for producing AgNPs using leaf extract. The preparation of nanostructured AgNPs provides an environmentally friendly option, as compared to currently available chemical and/or physical methods. The process for the biosynthesis of AgNPs is rapid, novel and ecofriendly.

3.5. UV-visible absorption spectra of Silver nanoparticles

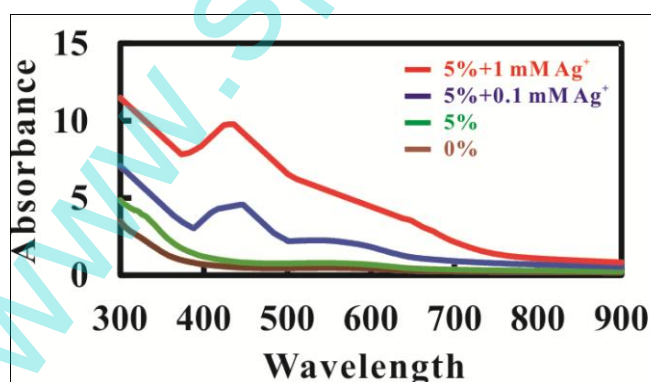


Figure 5. UV-visible absorption spectra of 0 % (brown line) and 5 % (green line) of leaf extract in the presence pH 7.0 PBS, The 5 % leaf extract in pH 7.0 PBS containing 0.1 mM (blue line) and 1mM (red line) AgNO_3 .

Recent results with leaf extract indicated that the functional groups played a reducing and controlling role during the formation of silver nanoparticles in the solutions, and that the structure of the leaf extract changed after reaction with silver ions. Reduction of the Ag^+ ion to silver nanoparticles

during exposure to the plant leaf extracts could be detected by the color change. Formation of the AgNPs were confirmed by surface plasmon spectra using UV–Vis spectrophotometer. The absorption spectra at different leaf extract quantities and metal concentrations were measured spectrophotometer in 300–900 nm range. Naturally biosynthesized AgNPs of diameters (25–100 nm) gave sharp peaks in the visible region of the electromagnetic spectrum. Fig. 5 shows the UV–vis spectra recorded from the aqueous solution of 0.1 mM and 1 mM AgNO_3 as a function of the reaction time using leaf broth at 95 °C, 5 min. The maximum absorbance was observed to occur at ca. 440 - 460 nm, and the intensity steadily increased to saturation as a function of the reaction time. The 0 % (brown line) of leaf extract shows flat absorb the wavelength in the NIR region, no significant peak was found. The 5 % (green line) of leaf extract has peak at 575 nm, and the intensity of 0.75. The 5 % of leaf extract containing 0.1 mM AgNO_3 (blue line) and 1 mM AgNO_3 (red line) shows peak at 460 nm and 446 nm, the intensity of 4.4 and 9.6, proved leaf extracts as reducing agents for biosynthesis AgNPs. In case of AgNPs, absorbance and sharpness increases by higher AgNO_3 concentration.

3.6. Biosynthesis of AgNPs for antibacterial effect

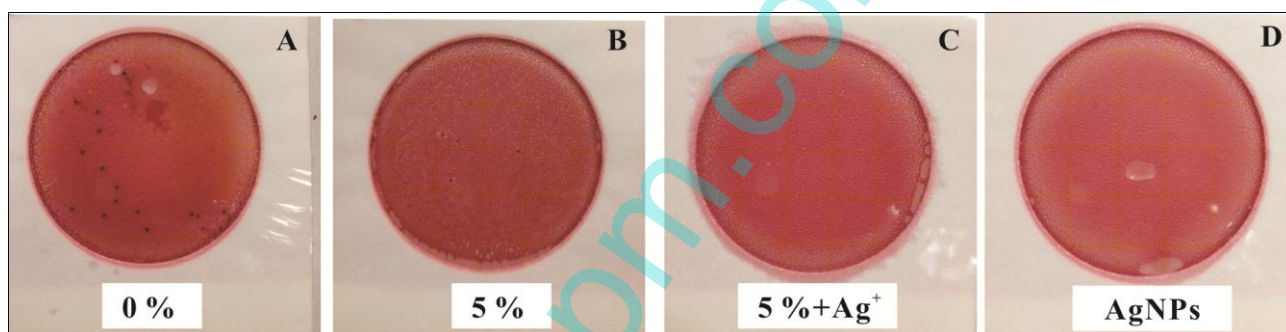


Figure 6. Antibacterial effect of (A) 0 % and (B) 5 % of leaf extract in the presence pH 7.0 PBS, The 5 % leaf extract in pH 7.0 PBS containing (C) 1 mM AgNO_3 and (D) pure AgNPs.

Food borne pathogens, wild *Escherichia coli* were obtained from the raw meat. Fresh cultures for the experiments were made by 3M™ Petrifilm™ broth with incubation at 35 °C for 48 h containing 0 % and 5% leaf extract, 5% leaf extract with 1 mM AgNO_3 and pure AgNPs (from Sigma–Aldrich 20-10 nm) in Fig.6. In these experiments, found 0 % and 5% leaf extract groups obtained 200 and 40 colony-forming units (CFU)/g. The 5% leaf extract with 1 mM AgNO_3 and AgNPs groups shows antibacterial effect.

4. CONCLUSIONS

In this paper we have reported the biosynthesis of silver nanoparticles (AgNPs) by reduction of silver nitrate (AgNO_3) from leaf extract of Desert Rose (*Adenium obesum*). The properties of prepared nanoparticles were characterized by AFM, EIS and CVs. From AFM, we observed the existence of nanostructures in obvious manner with the average size range of 55.7 nm to 75.2 nm. EIS results that

the 5 % leaf extract containing 1 mM AgNO₃ decreased to arc ($R_{et} = 2320 (Z/\Omega)$), provided to biosynthesized of AgNPs. CVs of 5 % leaf extract containing 1 mM AgNO₃ shows higher current was 37.17 μ A and 101.4 μ A at 0.02 V and 0.43 V. We supposed that characterization of Ag peak. Formation of the AgNPs were confirmed by surface plasmon spectra using UV–Vis spectrophotometer and absorbance peaks at 446 and 460 nm. In these experiments, found 0 % and 5% leaf extract groups obtained 200 and 40 colony-forming units (CFU)/g. The 5% leaf extract with 1 mM AgNO₃ and AgNPs groups shows antibacterial effect. The process for the biosynthesis of AgNPs is simple, novel and ecofriendly.

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