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Phyto-fabrication, characteristics and anti-candidal effects of silver nanoparticles from leaves of Ziziphus mauritiana Lam

Rusol M. Al-Bahrani[1], Sura Muayad Abdel Majeed[1], Mustafa Nadhim Owaid[2,3,*], Abdullah B. Mohammed[1], Duha A. Rheem[1]

1 Department of Biology, College of Science, University of Baghdad, Jadriya, Baghdad 10071, Iraq
2 Department of Heet Education, General Directorate of Education in Anbar, Ministry of Education, Hit, Anbar 31007, Iraq.
3 Department of Ecology, College of Applied Sciences, University of Anbar, Hit, Anbar 31007, Iraq.

ABSTRACT

In this work, silver nanoparticles (AgNPs) were biosynthesized from leaves of *Ziziphus mauritiana* Lam. jujube plant in Iraq and tested against fungal pathogens. Extract of leaves of *Z. mauritiana* mixed with 10^{-3} M $AgNO_3$ exposed to slight sunlight for 3 days. Characterization of AgNPs was done using UV-visible spectroscopy, SPM (scanning probe microscopy) and atomic force microscopy (AFM). The change of solution color from pale brown to dark brown and the exhibited maximum peak at 445 nm accepted as an indicator to biosynthesized AgNPs. Aqueous extract of *Ziziphus mauritiana* is considered as biological reduced and stabilized agent for Ag^+ to Ag^o. AFM showed the formation of irregular shapes of AgNPs. The biosynthesized silver nanoparticles have an average of diameter of 67.19. The biosynthesized AgNPs from *Z. mauritiana* leaves were tested as nano-drugs against four human pathogenic fungi. The highest concentration 100% of AgNPs has 25 mm inhibition zone against *Candida krusei*. These nanoparticles were found to be useful to reduce Candidiasis.

Keywords: Pharmaceutical, AgNPs, *Candida* sp., green nanotechnology, Jujube, biosynthesis.

INTRODUCTION

Nanoparticles are the most fundamental component in the fabrication of nanostructures. A nanoparticle is bigger than an atom or a simple molecule that is governed by quantum mechanics[1]. Currently, most of applications of silver nanoparticles are antibacterial and antifungal agents, anticancer nano-drugs[2] and in the drug delivery[3]. The biosynthesis is considered of the best ways to reduce

*Corresponding author: Mustafa Nadhim Owaid, e-mail: mustafanowaid@gmail.com
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metal ions to atoms and produce nanoparticles which are prepared by the safe and eco-friendly way using microorganisms and extracts of the plant[4]. Recently, many plants were used to synthesize potent AgNPs from *Cassia fistula fruit*[5], *Bryophyllum pinnatum leaf extract*[6] and *Limonia acidissima* L. leaf [7] against various fungal and bacterial species.

Few recent studies, started in 2014, were achieved to synthesize silver and gold nanoparticles using barks, and fruits of *Ziziphus* spp. and tested against some human pathogenic bacteria[8-10]. While Divband *et al.*[11] used the produced nanoparticles from *Z. spina christi* fly ash to remove Lead (Pb^{+2}) from aqueous solutions. In contrast, many studies were achieved in vitro to test bioactivity of crude extracts of this plant against bacteria, molds, and yeasts. Also, Alcoholic and aqueous extracts of the leaves showed the highest inhibition activity toward *Candida albicans*[12] compared with bacterial strains[13] because Ziziphus plant leaves have many biologically active compounds like phenols, saponins, and alkaloids[14]. Furthermore, saponin extract of *Z. spina-christi* has clear antibacterial activity[15]. Leaves of *Ziziphus* sp. has an antimicrobial effect[12,13,15], thus it was used in the treatment of wounds, diarrhea, and gonorrhea[16].

Jujube, *Ziziphus mauritian*a, is distributing in western and southern Iraq under dry conditions[17]. The genus *Ziziphus* sp. belongs to the evergreen herbal plants and to Rhamnaceae family[18]. Generally, many recent studies investigated antifungal activity of Ag-NPs against *Candida* sp., dermatophytic and plant pathogenic fungi[19]. From literatures, no tests against yeast infections were applied or studied by silver or gold nanoparticles of *Ziziphus* spp. This work is considering the first achievement for using the biosynthesis eco-friendly silver nanoparticles from *Ziziphus mauritiana* Jujube plant in the treatment of Candidiasis (yeasts infections) *in vitro*.

METHODOLOGY

Fungal Isolates

Four *Candida species, C. krusei, C. zeylanoides, C. albicans* and *C. guillermondii,* were obtained from Central Public Health Laboratory, Medical City Hospital, Baghdad, Iraq, which were isolated from mouths of patients. These isolates were maintained using Sabroud Dextrose Agar and used to detect bioactivity test of silver nanoparticles synthesized from *Ziziphus mauritiana* Lam. Leaves toward the fungal pathogens.

Preparing aqueous extract of Ziziphus mauritiana Lam. leaves

Leaves of *Z. mauritiana* Lam. were collected from its tree in Baghdad, washed with distilled water, dried at room temperature at 25±2 °C, and grinded by the blender. About 200 g of the powder was put in a clean glass bottle, added 2 liters of distilled water 1:10 (w/v), and heated for 1 hour at 60 °C. After the cooling, the aqueous extract was filtered using Whatman No. 1 filter paper. This solution was considered as a crude extract solution and stored at 2 °C until use.

Green synthesis and Characteristics of Ag nanoparticles

The required weight of $AgNO_3$ was dissolved in D.W. and then 5 ml of 10^{-3} M $AgNO_3$ solution was mixed with 45 ml of aqueous extract of *Z. mauritiana* leaves and put in 250 ml-volumetric flask. In another flask 5 ml D.W. was added to 45 ml of the aqueous extract as control. The two flasks exposed to slight sunlight at 26±2 °C for 3 days. When putting $AgNO_3$ solution with aqueous extract of Z. mauritiana leaves, the changing in color was observed that indicated formation of AgNPs. Characterization of AgNPs was done using UV-visible spectroscopy, SPM (scanning probe microscopy) and AFM (atomic force electron microscopy).

Anti-candidal activity of AgNPs

Silver Nanoparticles were applied against fungi by determination zone of inhibition *in vitro*. Anti-fungal activity of the biosynthesized AgNPs was investigated against some *Candida species (C. krusei, C. zeylanoides, C. albicans and C. guillermondii)* by well diffusion method using Sabouraud Dextrose Agar (SDA). The density of the yeast inoculum was adjusted to 105 cfu/ml. One hundred microliters (100 µl) of the yeast inoculum was added on SDA medium and spread using a sterile cotton swab even distribution of the inoculum. A sterile cork was used to make 9 mm wells into the plate, added AgNPs solution and left at cooling place for 30 min to allow absorption of excess fluid. Of AgNPs concentrations 25, 50, 75 and 100%, only 50 µl per well were poured into the wells of the plate. The control well was studied with 50 µl/well crude sample in each concentration (25, 50, 75 and 100%) for comparison purpose. These Petri dishes were then incubated at 35 °C for 18 hours and examined for measuring zone of inhibition.

RESULTS AND DISCUSSION

Characterization of silver nanoparticles

The changing color of the mix (aqueous extract plus $AgNO_3$ solution) takes place from pale brown to dark brown color, while no color change is observed in the aqueous extract without $AgNO_3$ solution because of the role of Z. mauritiana Lam. leaves extract in the mix as a reductant and stabilizer agent. The change in color from pale brown to dark brown indicates biosynthesis of AgNPs as mentioned by Kredy15. The formation of AgNPs by using *Z. mauritiana* Lam. leaves extract is confirmed by UV-Vis spectroscopy after 27 hr under slight sunlight at 26±2 °C. The highest UV-visible peak is 445 nm at absorption 0.925 that confirms the for-

mation of silver nanoparticles due to excitation of surface plasmon vibrations in silver nanoparticles. These results of color and UV-Vis spectrum agree with many recent studies for biosynthesis of AgNPs from plants and fungi extracts[9,20].

Surface topography and size of the sample film were investigated using Granularity Cumulation distribution chart by scanning probe microscopy (SPM) and atomic force electron microscopy (AFM) images. Also, AFM images exhibited the histogram of the percentage of AgNPs as a function of the grain size as in Figure 1. The average particle size determined from SPM is approx. 67.19 nm, and the diameters of 50 nm (≤10%), 65 nm (≤50%), 85 nm (≤90%) and 105 nm (≤2.3) as shown in Table 1 and Figure 1. The range of diameters is from 55 to 105 nm and this is an evidence for silver nanoparticles biosynthesis from *Z. mauritiana* leaf extract. AFM of the surface morphology of the film gives a good indicator for the formation of AgNPs. Of SPM imager surface roughness analysis (Figure 2), average of roughness is 51 nm, core roughness depth is 176 nm and the reduced valley depth is 34 nm. The surface topography of the AgNPs exhibited in Figures 2A (lateral view) and 2B (3D view) and it is clear that Ag nanoparticles are irregular in shape, having a cover of the organic shell[21] and are aggregated as in table1. These results agree with preparations of gold nanoparticles (AuNPs) from extract of *Z. mauritiana* leaves with range from 20 to 40 nm[22].

Table 1. Granularity Cumulation distribution, volume and average of diameters of AgNPs

Diameter (nm)<	55	60	65	70	75	80	85	90	95	100	105
Volume (%)	2.3	29.41	27.06	14.12	11.76	1.18	2.35	3.53	3.53	2.35	2.35
Cumulation (%)	2.35	31.76	58.82	72.94	84.71	85.88	88.24	91.76	95.29	97.65	100

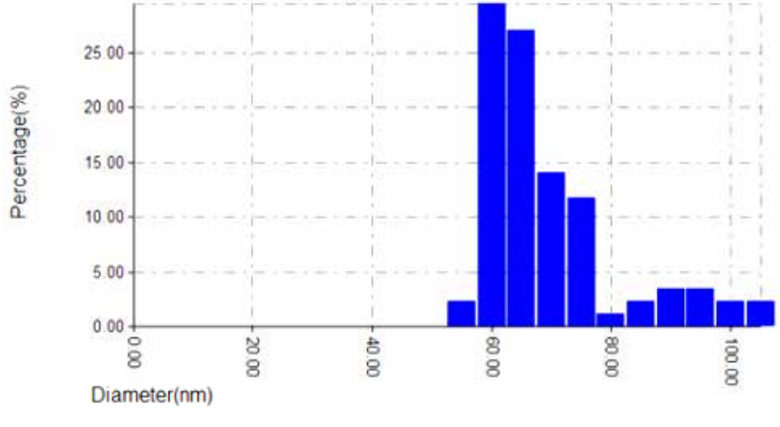
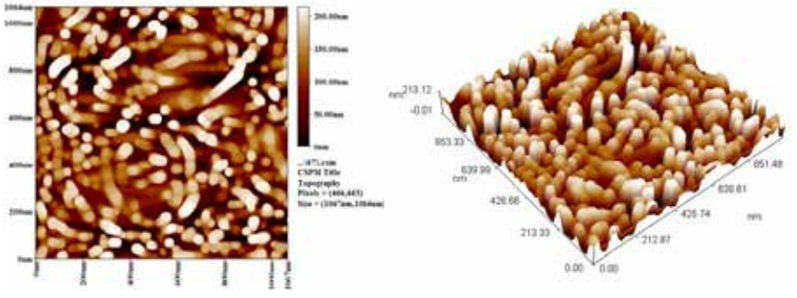


Figure 1. Histogram of particle size distribution of the biosynthesized silver nanoparticles



a **b**

Figure 2. AFM of the biosynthesized silver nanoparticles

Antifungal activity of the synthesized silver nanoparticles

The biosynthesized AgNPs from *Ziziphus mauritiana* leaves were tested as a nano-drug against human pathogenic fungi which were isolated from patients' mouth in the Medical City Hospital in Baghdad. The possible anti-fungal activities of the biosynthesized AgNPs and the aqueous extract were examined toward four pathogenic fungi, *Candida sp., (C. krusei, C. zeylanoides, C. albicans and C. guillermondii)* on SDA medium by well diffusion method (Figures 3 and 4). The aqueous extract of *Z. mauritiana* at concentration of 100% showed a slight inhibitory effect toward some *Candida species,* while concentrations of 25%, 50%, 75% were not exhibited any inhibitory effects except with the final two concentrations (57% and 50%) as shown in Figure 3. However, this figure showed the bioactivity of silver nanoparticles against *Candida species* is increasing with the increase of AgNPs concentrations. The higher inhibitory effect (zone of inhibition) is 25 mm against *Candida krusei* by the concentration 100% of AgNPs compared with 20 mm by the plant extract alone (100%), followed 24 and 18 mm by the same pathogen in case the concentrations 75% and 50% respectively. Furthermore, the lower concentration of $AgNO_3$ (25%) was inhibited *C. krusei* (12 mm) while the concentration of extract 25% did not have any inhibitory effect as shown in Figures 3B and 4. Generally, the concentrations of extract 25%-75% did not inhibit other species *C. zeylanoides, C. albicans and C. guillermondii* (Figure 3A). The zone of inhibition of AgNPs is 18 mm when using the concentrations 50% and 100% in case *C. krusei and C. zeylanoides,* respectively. From another hand, the zone of inhibition of *C. albicans* and *C. guillermondii* is 16 mm when using the dose 100% (AgNPs). While the lower inhibitory effect is reached 12 mm by the concentration 25% for all *Candida* species (Figure 3B). The results of the zone of inhibition in this study agree with biomedical applications of the biosynthesized AgNPs from this plant against pathogenic bacteria.

Some researchers have reported that the positive charge on the silver ion is critical for its antibacterial and antifungal activities through the electrostatic attractions between the negative charge of the cell membrane of microbes and the positive charge of AgNPs[23,24]. Generally, AgNPs have a moderate inhibitory effect against *Candida* infections that agrees with results of zone of inhibition of AgNPs against *Candida* spp. by Owaid et al.[25]. The bioactivity of *Ziziphus* sp. leaves may be return to their antibacterial and antifungal effects in the crude form as mentioned by many studies[12,13,15] especially toward *Candida* yeasts[12] because *Ziziphus* plant leaves have phenols, saponins, and alkaloids[14]. Also, the role of these AgNPs are considered as antifungal agents, maybe return to the presence of plant bioorganic capping material upon the AgNPs which enables them to observe enhanced anticandidal activity[26]. All these results are agreeing with the recent works[8,9,16,22] but this study is considered the first test to investigate activity of the biosynthesis AgNPs from leaves of *Ziziphus mauritiana* Lam. against native *Candida* spp.

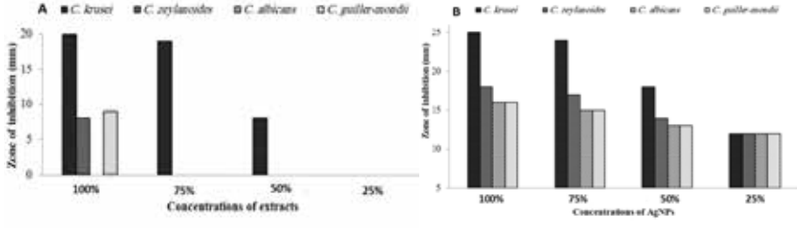


Figure 3. Zone of inhibition of aqueous extract of Jujube leaves (A) and its silver nanoparticles (B) against *Candida* species.

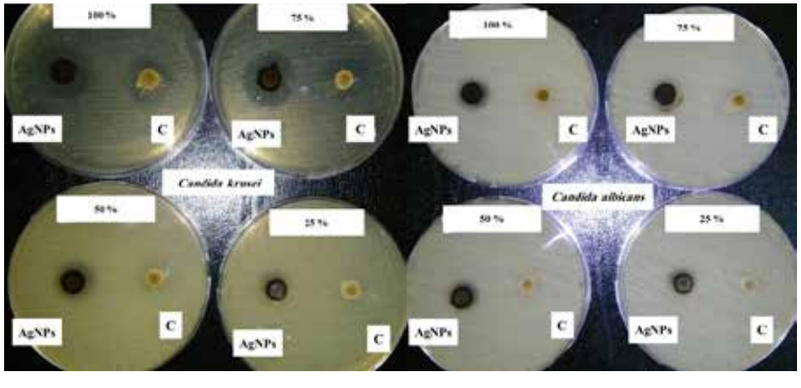


Figure 4. Zone of inhibition of Ag nanoparticles and aqueous extracts of Jujube (C) against *Candida* spp.

CONCLUSION

In this investigation, silver nanoparticles were synthesized in irregular shapes from leaves of *Ziziphus mauritiana* jujube tree in Iraq. The best absorption peak was located at 445 nm which established by using UV-Visible spectrum after change of color of the mix from pale brown to dark brown. The silver nanoparticles of *Z. mauritiana* leaves have average of diameter 67.19 nm with ranging from 55 nm to 105 nm. The biosynthesized AgNPs were used as a nano-drug against human pathogenic fungi (Candida sp.) which were isolated from patients' mouth in the Medical City Hospital in Baghdad. The higher anticandidal activity showed against *Candida krusei* with zone of inhibition 25 mm in case AgNPs concentration 100 % compared with zone of inhibition 20 mm by the plant extract alone (100%), followed 24 and 18 mm by the same pathogen in case the concentrations of AgNPs 75% and 50% respectively. The lower sensitivity of AgNPs was 12 mm recorded for all *Candida* species at the concentration 25%.

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A new spectrophotometric method for the determination of gabapentin using chromotropic acid

Olayemi M. Adegbolagun[1], Olusegun E. Thomas[1], Elizabeth O. Aiyenale[1], Olajire A. Adegoke[1]*

1 University of Ibadan, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ibadan, Nigeria.

ABSTRACT

The purpose was to develop a colorimetric method for determining gabapentin.

The method was based on the diazo coupling reaction between diazotized gabapentin and chromotropic acid. The method was validated using ICH guidelines before its application to generic brands of gabapentin.

Coupling reaction generated an orange azo adduct whose absorbance was linearly correlated with concentration in the range of 1-6 µg/mL at 470 nm. The method was accurate and precise with recovery range of 97.6-103.1%; intra- and inter-day precisions (%RSD) were less than 0.65% and showed no statistical difference when compared with reference method in the analysis of the dosage forms. The 3D optimization of the adduct revealed an E-type configuration around the azo linkage which would contribute to its stability.

The new method can serve as a reliable alternative to the official method for the routine analysis of gabapentin in bulk and dosage forms.

Keywords: Gabapentin, colorimetric analysis, chromotropic acid, diazo coupling reaction.

INTRODUCTION

Epilepsy is a neurological disorder that is associated with a deficiency in gamma-aminobutyric acid (GABA) receptors in the microgyric cortex of the brain[1]. With an estimated 4 to 10 persons per 1000 people in the general population with active epilepsy i.e. continuing seizures or need for treatment, epilepsy is the fourth most common neurological disorder after migraine, stroke and Alzheimer's disease [2, 3]. Of this world-wide incidence, 80% of those with the disorder live in low- and middle-income countries where the incidence is prob-

*Corresponding author: Olajire A. Adegoke, e-mail: ao.adegoke@mail.ui.edu.ng
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