Antimicrobial and Antibiofilm Activity of Silver Nanoparticles Biosynthesized by Mentha Spicata Aqueous Leaves Extract Against a. Baumannii

Hadeel Eid ibrahem[1], Abeer yousif Abd Al-Kareem[2]
[1, 2] Biology Department, College of Education for Women, University of Anbar, Iraq.
Email: Had20w4009@uoanbar.edu.iq

Abstract
Silver nanoparticles (AgNPs) have been used as antibacterial and anti-biofilm due to their different physical, chemical, and biological characteristics. Investigating silver nanoparticles' antibacterial and anti-biofilm properties was the objective of the current study. Here, we show a rapid, reliable, cost-effective, and green method for the the synthesis of AgNPs by treating silver ions with Mentha spicata aqueous leaves extract. Using the disc diffusion test, the antibacterial and anti-biofilm activities AgNPs, biofilm inhibition, in A. baumannii. Our results indicate that nanoparticles have spherical shapes and sizes.Dilutions of AgNPs (240, 120 and 60g/ml) were made. Bacteria have activated in nutrient broth for 24 hours at 37°C. The results of the study also show the effective effect of AgNPs anti-biofilm for its ability to inhibit bacterial membranes. Finally, green nanoparticles might some future function as affordable antibiotic alternatives.
Keywords: Green synthesis, Nanoparticles, Anti-Bacterial, and Anti-biofilm Plant extracts, Mentha spicata, A. baumannii

1. Introduction

Nanotechnology is a rapidly evolving field at the present time. One of the most active fields of research worldwide is investigating the possibility of generating nanoparticles utilizing different plants. [1] .The name "Nano," which translates to "dwarf," is derived from a Greek term for things that are one-billionth in size. [2] Researchers are drawn to the broad class of materials known as nanoparticles (NPs) because of their small size (1-100 nm), distinctive properties, huge surface area, heightened reactivity, simplicity in entry into the body, and numerous scientific uses. Nanoparticles made of zinc, gold, silver, copper, alginate, titanium, and magnesium have all been identified. [3, 4] Over 650 different microorganisms have been demonstrated to be inhibited by the non-toxic inorganic compound silver [6]. An environmentally friendly method of producing nanoparticles is becoming more and more necessary. Traditional physical techniques frequently produce a limited number of AgNPs while being uncomfortable and energy intensive. [5]. in green synthetic systems, biological microorganisms including bacteria, fungi (yeast), and plant extracts are employed [9]. The usage of medicinal plants by all countries throughout history makes them the oldest kind of healthcare that is currently known [10]. According to estimates from the World Health Organization, 80% of people worldwide utilize herbal medicine (WHO) [11]. In this study, silver nanoparticles have successfully synthesized using Mentha spicata aqueous leaves extract. The Lamiaceae (Labiatae) family, which includes the genus Mentha (commonly known as mints), has roughly 25–30 species. According to [13] mints are one of the most significant spices in the world. This study's objectives included creating silver nanoparticles using Mentha spicata extract and testing the nanoparticles' antibacterial efficacy.

2. Material and Methods

Isolation and identification of A. baumannii

twenty-eight A.baumannii isolates out of 200 of total isolates from different sources samples for patients attending to Ramadi Teaching General Hospital and hospital Al-Ramadi for maternity and Children, Specimens were cultured on MacConkey agar and blood agar medium incubated at 37°C for 18-24 hr. Afterward, the grown colonies were identified by the Vitek 2 compact system using a GN card depending on the instructions of the manufacturer.

Preparation of plant extract

The plant extract was taken, dried, and ground into powder The plant extract was then made by dissolving 25 g of, powder in 250 mL of solvent (water) using a Hot Plate for 2 hours. then suspension Was shaking for 1 h The extract is then filtered through medical gauze and using Whatman filter paper No.1 for The extract was filteredobtain the aqueous plant extracts [17].

Preparation of Silver Nitrate Solution

The green, production of silver nanoparticles was performed, out by dissolving (0.169g) $AgNO_3$ in (100 ml) Deionized sterile distilled water. [18].

Synthesis of AgNPs of extracts

For this experiment: 2mL of aqueous extract Mentha Spicata 10ml of 1M $AgNO_3$ aqueous solution heated at 60°C for 15 minutes in a water bath with stirring The aqueous extract was combined with an $AgNo_3$

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aqueous solution and heated for 45 minutes at 75 ° C. As the color changed to brown, AgNPs were gradually produced. AgNPs were synthesized and centrifuged. In centrifuge [19].

Characterization of the Synthesized Silver Nanoparticle

The absorption spectra using a UV-Vis spectrophotometer (CECIL7200 UV–visible spectrophotometer). X-ray diffraction (XRD) pattern using Philips PW1730.Transmission electron microscopy, (TEM).

Determination of minimum inhibitory concentration of Silver nanoparticles

Using the method of CLSI (2021). Briefly, serial concentrations MIC of AgNPs was defined of AgNPs (240, 120, 60, 30, and 15 µg/ml) were prepared. After that, the lowest concentration prevents bacterial growth which is considered MIC.

Serial Dilution

Stock solutions with concentrations of 120, 60 µg/mL, and 240 µg/mL, were transferred to two sterile tubes in a rack. 2mL of normal saline is contained in each tube. After adding two ml of stock solution to the first tube and thoroughly mixing it, that 2ml was mixed and transferred from the first tube to the second tube In this case, the first tube contains 120 g/mL, whereas the second tube contains 60 µg/mL.to determine antimicrobial susceptibility.

Anti-bacterial efficacy of AgNPs by agar well method

The antibacterial activity of was determined using the agar well diffusion method. Biosynthesized AgNPs [23].

a) cultured of A. baumannii sub cultured were in tryptone soya broth Individually, pure at 37°C for 18 h.

b) In Mueller-Hinton Agar About twenty ml of medium,of was poured in Petri dishes, separately and for each, the isolate was uniformly swabbed on dishes, with a sterile cotton swab.

c) Using a sterile corn borer, Wells (6mm) diameter was made in each of these plates

d) About 100 µl of different concentrations of biosynthesized AgNPs were added in the sterile syringe to different wells. By the clear zone of inhibition, the bactericidal efficacy was defined, around the loaded well after plates incubation overnight at 37 °C.

Anti-biofilm activity of silver nanoparticles by using micro-titer plate Method

[24] Developed the following method for estimating biofilm formation

a) A bacterial culture was modified overnight (in tryptone soy broth) to be compatible with the No. 0.5 McFarland standard.

b) Inoculated with previously prepared bacterial suspension (step a), tryptone soy broth with sub-MIC of AgNPs was incubated at 37°C for 24 h.

c) A total of 200 l of the culture obtained in step a was placed in triplicate into polystyrene microtiter plates in the vertical rows of the plate for each isolate as a control. (Strong, Moderate, and Weak).

d) A culture volume of 200 l that contains the sub-MIC, The AgNPs concentration (made in step b) was put into three sub-MIC wells all plates were incubated for 24 h at 37°C.

e) Following that, biofilm production was assessed using the crystal violet assay (CV) assay, and optical density at 630 nm was obtained using a microtiter plate reader after dimethyl sulphoxide was used to dissolve the insoluble purple formazan (DMSO).

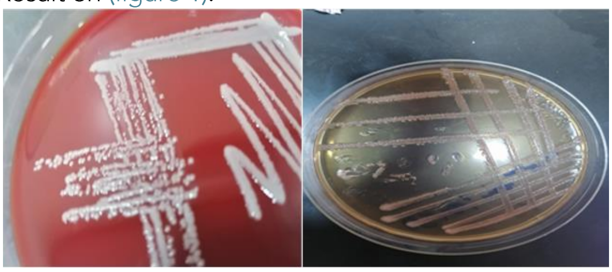
f) The percentage of bacterial adhesion inhibition achieved using [25].

$$\% \text{ of inhibition of biofilm formation} = 1 - \frac{\text{O.D of treatment}}{\text{O.D of control}} \times 100$$

3. Results and Discussion

Isolation and Identification of A. baumannii

Using MacConkey agar, A. baumannii isolates showed as small, round, pale, lactose non fermenter colonies, however on blood agar, they presented as opaque, creamy, non-hemolytic colonies with a fish smell. at a fast rate All A. baumannii isolates that demonstrated the ability to Growth at 44oC were positive.this degree of temperature This test was used to identify A. baumannii from other A. baumannii species.able to grow at this temperature) from other Acinetobacter species that are able to grow at this temperature .At this temperature, microorganisms are unable to grow. Result on (figure 1).



A B

Figure (1): Identification of A. baumannii on culture media: A – Blood Agar - B- MacConkey agar

Synthesis of AgNPs

UV-Vis Spectrophotometer analysis

In the nanoparticle preparation reaction, the hue changed to brown, indicating the development of silver nanoparticles. The existence of silver nanoparticles with Plasmon resonance with a range between (400 to 550) nanometers was discovered using UV-Visible spectroscopy (Figure 2). In the spectral pattern, there appears to be a strong band. because of the stimulation of localized surface plasmons, which causes substantial light dispersion when an electric field is applied at a certain angle wavelength at which resonance is placed [20].[21]

Biosynthesis and Characterization of Silver Nanoparticle [22].

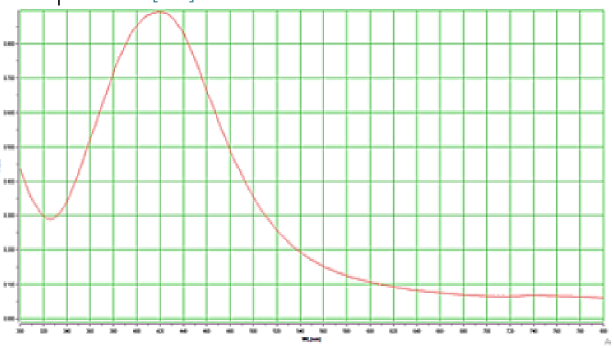


Fig. (2): UV-Visible spectrum of nano- Mentha Spicata

XRD analysis

The X-ray diffraction (XRD) patterns of Ag nanoparticles produced on glass substrate at different pulse counts are shown in Figure (3). as shown by the X-ray diffraction patterns, which exactly match the typical patterns (JCPDS 89-3722) [28] .The diffraction peaks indexed to (111) , (111) and (311) planes were observed, confirming the AgNPs face centered cubic (fcc) showed that peaks at 2ϴ = 32.25o, 38.15o and 77.25o respectively which observed.

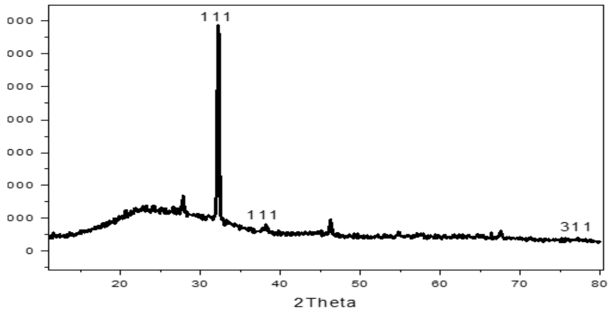


Fig. 3: XRD of silver nanoparticles

Transmission Electron Microscope analysis (TEM)

TEM was used to analyze the size, shape, and size distribution of AgNPs for structural characterization. According to the current TEM analysis, the majority of the produced AgNPs were inert and had a small particle size. (Figure 4) shows a (TEM) image of silver nanoparticles (AgNPs) prepared from an aqueous extract, as nanoscale particles of mostly spherical shapes were obtained, and the sizes of these particles ranged between (2.47 nm - 19.78 nm) at a rate of The average size of the nano-diameters is (9.9 nm) as shown in the (figure 5).

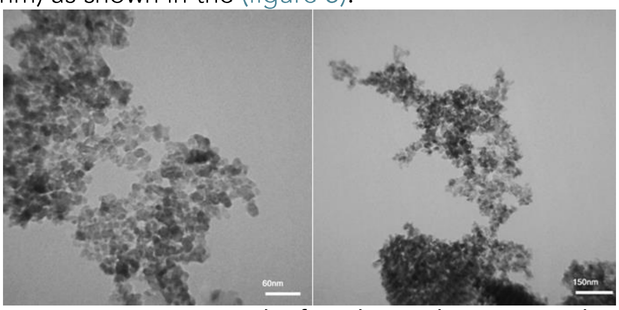


Fig. 4: TEM micrograph of synthesized Nano- Mentha spicata extract

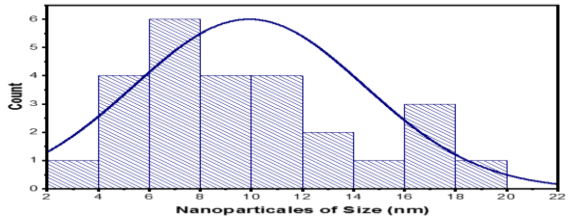


Fig. 5: Average diameters of silver nanoparticles by TEM

Anti-bacterial activity of silver nanoparticles against a. baumannii isolates

In this study,well-agar diffusion methods were performed to determine the efficacy of silver nanoparticles on A.baumannii isolates isolated from clinical cases, as these isolates were resistant to ceftriaxone. Silver nanoparticles showed antibacterial activity by measuring the inhibition area around the wells of AgNPs, and the result showed that aqueous Mentha spicata extracts significantly inhibited the growth of Gram-negative bacteria in this investigation when mixed with silver nitrate solution. The plant extract was also used as a control agent (it did not give any inhibition against A .baumannii) as in (Figure 7). The results showed that the aqueous extracts of Mentha spicata for silver nanoparticles had different bacterial inhibition activity (Figure 6). The highest inhibition results at a concentration of 240 were (26) for fungus A .baumannii. As shown in (Table 1) and (Figure 8) for the inhibition diameters for the concentrations. According to [13] AgNPs prefer to target the respiratory chain of bacteria, which promotes cell growth and results in cell death [14]. Besides, [15] found that the gradual production of lipopolysaccharides and membrane proteins during metal depletion results in the formation of asymmetrically shaped pits in the external membrane and alters membrane permeability. Additionally, both inside and outside of the cell membrane, silver nanoparticles can interact with sulfur-containing amino acids, decreasing the survival of bacterial cells. Additionally, silver nanoparticles may connect with phosphorus molecules in DNA, inhibiting DNA replication, or with proteins containing sulfur, increasing enzyme activity [16].

Table 1: Mean of inhibition zone of AgNPs on Muller Hinton agar		
No.	Con.	A. baumannii
1	240 µg/ml	26 mm
2	120 µg/ml	20.1 mm
3	60 µg/ml	17 mm

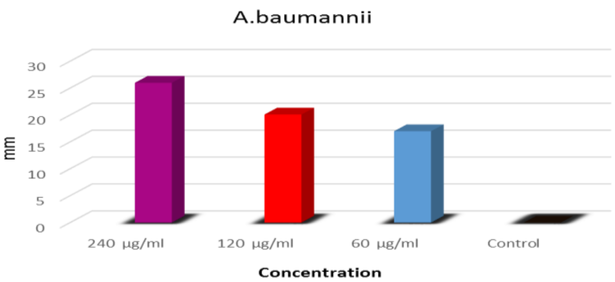


Fig. 6: Inhibitory activity of the nano aqueous extract of M. spicata leaf against A.baumannii

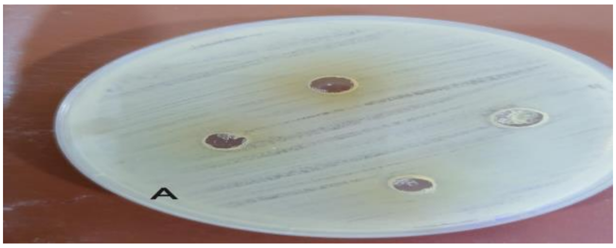


Fig. 7: control elements: Botanical extract of M. spicata

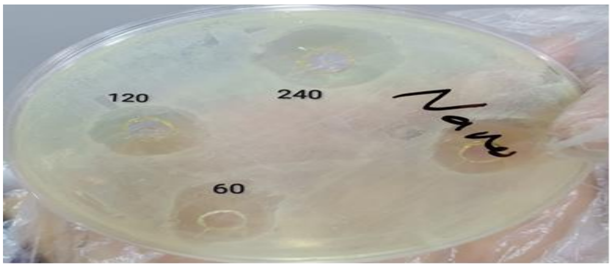


Fig. 8: Antibacterial activity of AgNPs: A. baumannii by well diffusion method.

Antibiofilm activity of AgNPs against biofilm-producing A.baumannii isolates

In this method, crystal violet and were used to determine the anti-biofilm activity of Silver nanoparticles by using AgNPs (SupMIC). The results of these tests showed the optical density of wells that contain AgNPs (SupMIC) Approach to the optical density of the wells that do not contain bacterial isolates. This indicates that prevented the growth of bacteria and thus the absence of a biofilm in these wells. The effectiveness of biofilm inhibition of A.baumannii as shown in (table 2) and (figure 9).

Biofilms are microbial aggregations encased in an extracellular polymeric substance (EPS) matrix that adheres to one another and/or to a surface [26].

Table (2): AgNPs anti-biofilm activity against A.baumannii				
NO.	Bacterial type	O.D. of untreated	O.D. of 0.5MIC	%biofilm Inhibition
1	A.baumanii	0.499	0.077	84.56 %
2	A.baumanii	0.613	0.087	85.80 %
3	A.baumanii	0.357	0.119	66.66 %

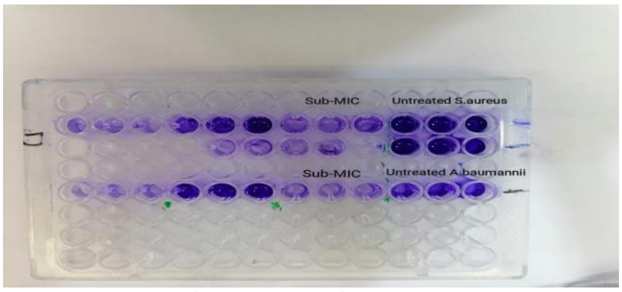


Fig. 9: Antibiofilm activity of AgNPs synthesized by aqueous leaf extract of M. spicata

4. Conclusion

Make use of the plant extracts of mint spicata leaves. As a reducing agent in the synthesis of green silver nanoparticles, it is a simple and environmentally friendly technology that can be used as an antibacterial and effective treatment in a promising future.

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