How to Cite:
Hashim, M. H., & Khafaji, M. H. A. (2023). Biosynthesis of silver nanoparticles using aqueous extract of Lepidium meyenii and testing their antimicrobial activity. *International Journal of Health Sciences*, *7*(S1), 2944–2953.
https://doi.org/10.53730/ijhs.v7nS1.14626

Biosynthesis of silver nanoparticles using aqueous extract of Lepidium meyenii and testing their antimicrobial activity

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***Abstract*---**Lepidium meyenii herb (maca) belongs to the cruciferous family that includes broccoli, radish, watercress, cabbage and turnip. It is also available as a dietary supplement in the form of capsules and liquid extracts. Silver nanoparticles were made using the aqueous extract of maca root for the first time locally and worldwide. As a reducing agent, the extract was utilized. Several approaches were used to diagnose silver nanoparticles, including scanning electron microscopy, atomic force microscopy, FT-IR and UV-vis spectrophotometer analyses. The diagnostic results showed the formation of nanoparticles in various shapes, with a diameter ranging from 56.11 nanometers. The optimum conditions for the bio-synthesis of silver nanoparticles were determined. The optimal conditions were pH 7, 37 °C, with a concentration of 1 mmol silver ions. These are the ideal conditions for making silver nanoparticles from L.meyenii. The antibacterial effectiveness of biosynthesized AgNP against Staphylococcus aureus was found to be very significant. This work promotes green synthesis of AgNP for a range of applications that require low toxicity and are ecologically friendly.

***Keywords*---**biosynthesis silver nanoparticles, aqueous extract, Lepidium meyenii, antimicrobial activity.

Introduction

As bacteria have attempted to establish resistance to standard antibiotics, it has become necessary to have alternatives, one of which is the use of bio-manufactured silver nanoparticles (Wang et al.,2018). Represents the relationship between raw materials and their atomic or molecular structure; raw metals have

Manuscript submitted: 09 July 2023, Manuscript revised: 18 Sept 2023, Accepted for publication: 27 Oct 2023

inert properties that change and become useful when their size is reduced to the atomic level; nanoparticles have unique physical, chemical, and biological properties that can be used in a variety of appropriate applications (Sanvicens *et al.,*2018). The use of fine particles is being developed Silver nanoparticles are used in biomedical products such as drug delivery to infected organs, cancer treatment, diagnostic devices, biosensors, and as an antibacterial (De Jong, and Borm*,* 2018). There are several methods for producing silver nanoparticles. One of these approaches is the biological method, which yields nanoparticles with minimal toxicity, small size, and the highest stability (Chen *et al.*,2016). Silver nanoparticles were physically, chemically and biologically synthesized, but these nanoparticles were not previously manufactured either locally or globally using *L. meyenii.* Scientists and scientific research are heading towards the production of these nanoparticles by non-toxic and environmentally friendly biological methods that are free from the use of toxic chemicals or harmful physical methods. A variety of biological sources exist for the manufacture of nanoparticles including plants, fungi, yeast, bacteria, etc. Single-celled and multi-organisms can produce intracellular and extracellular nanoparticles which helps harness these processes and gain control over them to reduce chemical and physical risks to health and the environment, reduce waste and prevent pollution (Karunagaran and Shampa, 2011). There are many international publications on the use of plants in the production of nanoparticles such as Pine, Persimmon, Ginkgo, Magnolia and Platanus where they have been studied for the production of nanoparticles (Nair and Pradeep 2002). The formation of nanoparticles depends on the plant extract acting as a reducing agent (Ikram**,** 2015). The study by Shankar *et al.*, (2018) showed that Capsicum frutescence helped in the biosynthesis of silver nanoparticles of spherical shape and size between (15-20) nanometers. The titanium nanoparticles were biosynthesized after 72 hours with a nanoparticle size (50 nm) from dairy products (Azhar *et al.*,2011).

Methods

Preparation of the plant extract of *L. meyenii*

L. meyenii root powder (20 g) has been boiled for 10 minutes in 100 ml of distilled water. The resultant mixture was then filtered using a standard filter and Whatman No. 1 filter paper. The extract was isolated in that way (Ezealisiji *et al.*,2018).

Preparation of stock of ($AgNO_3$)

A stock solution of silver nitrate (AgNO3) was made by dissolving 1 g of $AgNO_3$ in 100 mL deionized water and then adjusting the concentration to 10^{-3}M (Ezealisiji *et al.*,2017)

Synthesis of silver Nanoparticles (AgNPs)

The silver nanoparticles were biosynthesized By mixing 50 ml of *L. meyenii* extract with 100 ml of 1 mM $AgNO_3$ at 37 ^{o}C, After a few hours, the color changed from yellow to dark yellow. The alteration in color is due to the formation of silver nanoparticles and the reduction of Ag ions (Ezealisiji *et al.*,2017).

Characterization of silver nanoparticles
UV-Vis spectra analysis

The formation of AgNPs was confirmed by measuring the wavelength of the reaction mixture using a UV-Vis spectrum, a 2 ml quartz cuvette with a 1 cm path length and a resolution of 1nm. The samples were scanned at a rate of 500 nm/min in the 300-900 nm range. A blank reference was used to calibrate the spectrophotometer. All of the samples' UV-Vis absorption spectra were collected, and numerical data was demonstrated.

Scanning electron microscope (SEM)

The mean particle shape and diameter of nanoparticles were determined using SEM. After being sonicated with distilled water, a little drop of AgNPs solution was deposited on a glass slide and left to dry. To make the samples conductive, a thin coating of gold was applied to them.

Atomic force microscopy (AFM) analysis

AgNPs were examined using atomic force microscopy. By putting a few drops of prepared AgNPs onto a silica glass plate and allowing them to dry at room temperature in the dark, a thin film of AgNPs was formed. The AFM was used to scan the deposited film glass plate.

Fourier-transform infrared spectroscopy (FTIR)

FTIR in the range of 4000–450 cm−1 at a resolution of cm−1 was used to presume a probable interaction between the nanoparticle surface and the mixed live cell filtrate of bacteria.

Testing the anti-bacterial activity of silver nanoparticles using resazurin dye

The resazurin test is used to detect with certainty the lowest number of viable cells capable of reproducing. It determines the minimum concentration of the antagonist (silver nanoparticles) that is effective in inhibiting bacterial cell growth, the result being based on the concentration in which the resazurin dye does not appear within 24 hours. Using the standard broth dilution method, the antibacterial effectiveness of AgNPs was determined. The MIC was calculated in nutrient broth (NB) using repeated two-fold dilutions with adjusted bacterial concentration (1×10^8 CFU/ ml, 0.5 McFarland's standard) in concentrations ranging from 15 g/ml to 500 g/ml. The positive control in this study consisted of NB medium with the tested bacterial concentrations, while the negative control consisted of solely inoculated broth, with incubation times and temperatures of 24 hours and 37 degrees Celsius, respectively. The minimum inhibitory concentration (MIC) is the lowest concentration of antimicrobial drugs that visually inhibits 99 percent of bacterial growth. The visible turbidity of the tubes before and after incubation revealed the MIC (Elshikh *et al.*,2016).

Well diffusion method

The MIC of AgNPs was determined by determining the lowest concentration at which the test organisms' observable growth is inhibited. Swabs of the test organism's suspensions were placed on the culture medium. The plates were then cut with wells. AgNPs in various concentrations (500, 250, 125, 62, 31, 15.5, 7, μg/mL) were applied to the wells. To assess the inhibiting growth of AgNPs on a specific pathogen, all plates were incubated at 37°C for 24 hours. The method was repeated three times, with the average value taken into account (Elshikh *et al.*,2016).

Results

Biosynthesis of Silver Nanoparticles (Ag NPs)

To biosynthesize silver nanoparticles, 50 mL of *L. meyenii* extract was mixed with 100 mL 10^{-3}M $AgNO_3$ at 37 °C. After several hours the color altered from light yellow to dark. The color change indicates the process of Ag reduction and formation of silver nanoparticles. as shown in Figure (1).

Figure(1): Color change due to the reduction of metal ions and the formation of silver nanoparticles.

Uv-vis spectrophotometer

The color change of the mixture from yellow to dark yellow after the incubation period is an initial detection of the biosynthesis of silver nanoparticles in the reaction vessel; the measurement with a (UV-vis spectrophotometer) is the next step for characterizing the (AgNPs), and the results obtained in this application showed that the biosynthesis of AgNPs gave the highest absorbance at the wavelength of 380 nm, as shown in Figure (2).

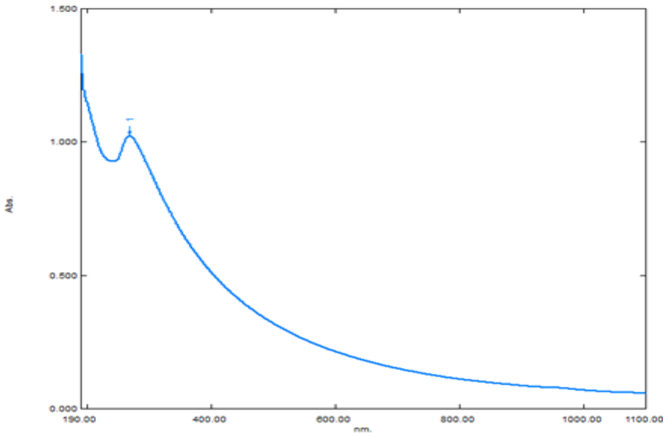

Figure (2): Spectrophotometric measurement of the visible and ultraviolet rays of silver nanoparticles biosynthesized by L. meyenii

Atomic force microscopy (AFM)

Atomic force microscopy was employed as a confirmation tool to assess AgNP biosynthesis by revealing their mean diameter and shape in two and three dimensions. As indicated in Table (1) and Figure 1(3 a,b,c), the silver particles produced by *L. meyenii* have an average diameter of 56.11 nm.

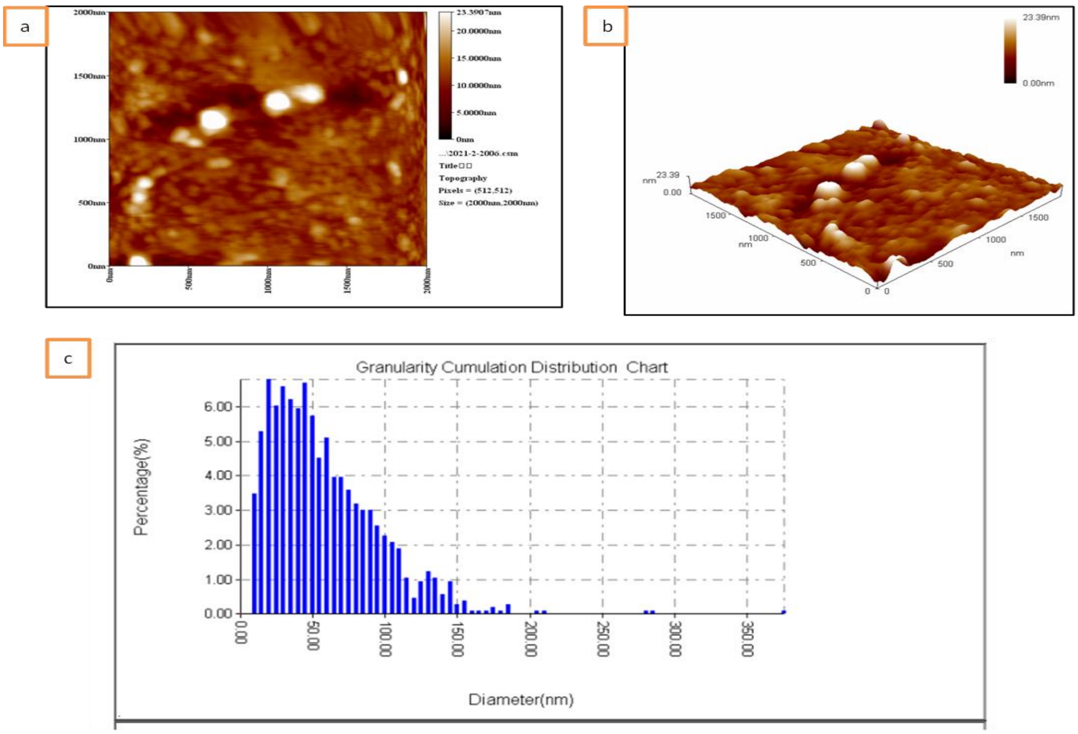

Figure 3: Silver particles biosynthesized by L. meyenii examined under an atomic force microscope (a) two-dimensional image (b) three-dimensional image (c) size distribution diagram of the synthesized particles

Fourier transform infrared (FTIR) analysis

FTIR analysis was achieved to determine the potential effective aggregates of silver ion-reducing molecules produced by *L. meyenii* Figure (4).

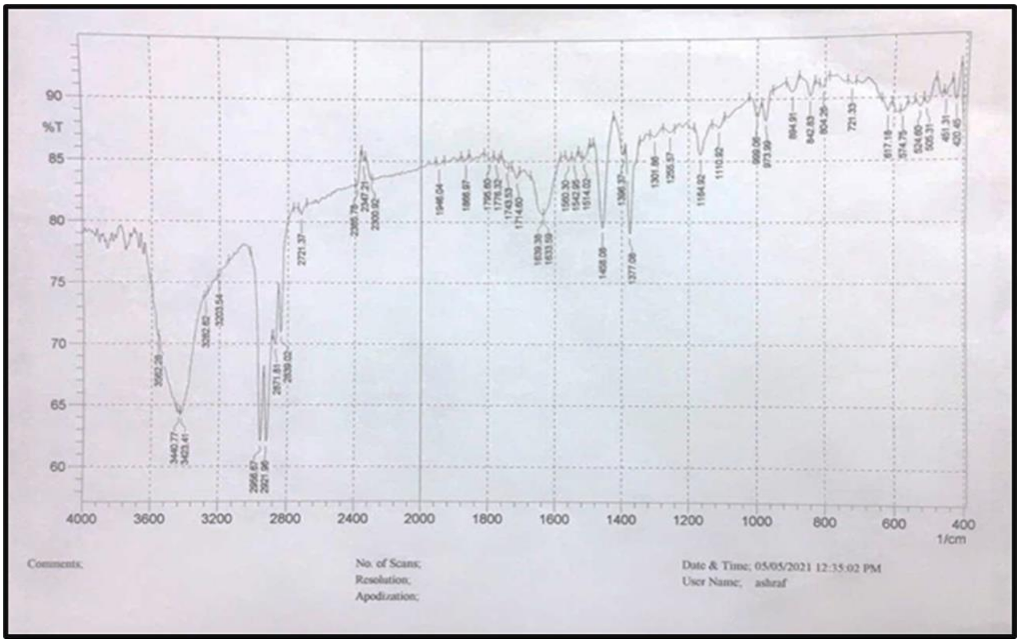

Figure (4): FTIR analysis of silver nanoparticles biosynthesized by L. meyenii

Zeta potential is a measurement

The size of the charges was established by measuring the effective electric charge on the surface of the nanoparticles. When nanoparticles have a net surface charge, the charge is investigated by concentrating opposite charge ions near the nanoparticle surface Figure (5).

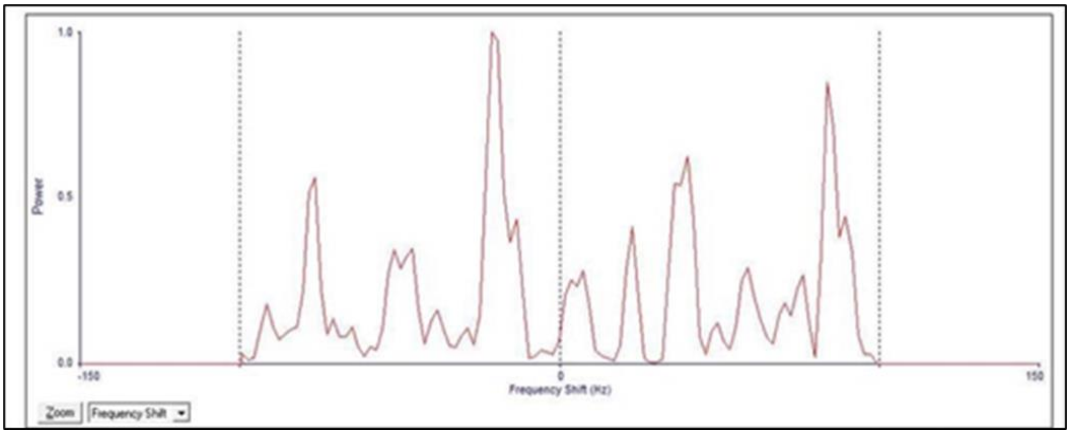

Figure (5): Zeta potential analysis of silver nanoparticles biosynthesized by L. meyenii

Scanning Electron Microscope (SEM)

The biosynthesized AgNPs were inspected under a scanning electron microscope (SEM) to determine their predicted size and morphology, and the results verified that AgNPs had formed. The production of nano-sized AgNPs was observed in SEM images. The morphology of the nanoparticles was irregular with size range of 56 nm as shown in Figure (6) that also verifies the AFM results .

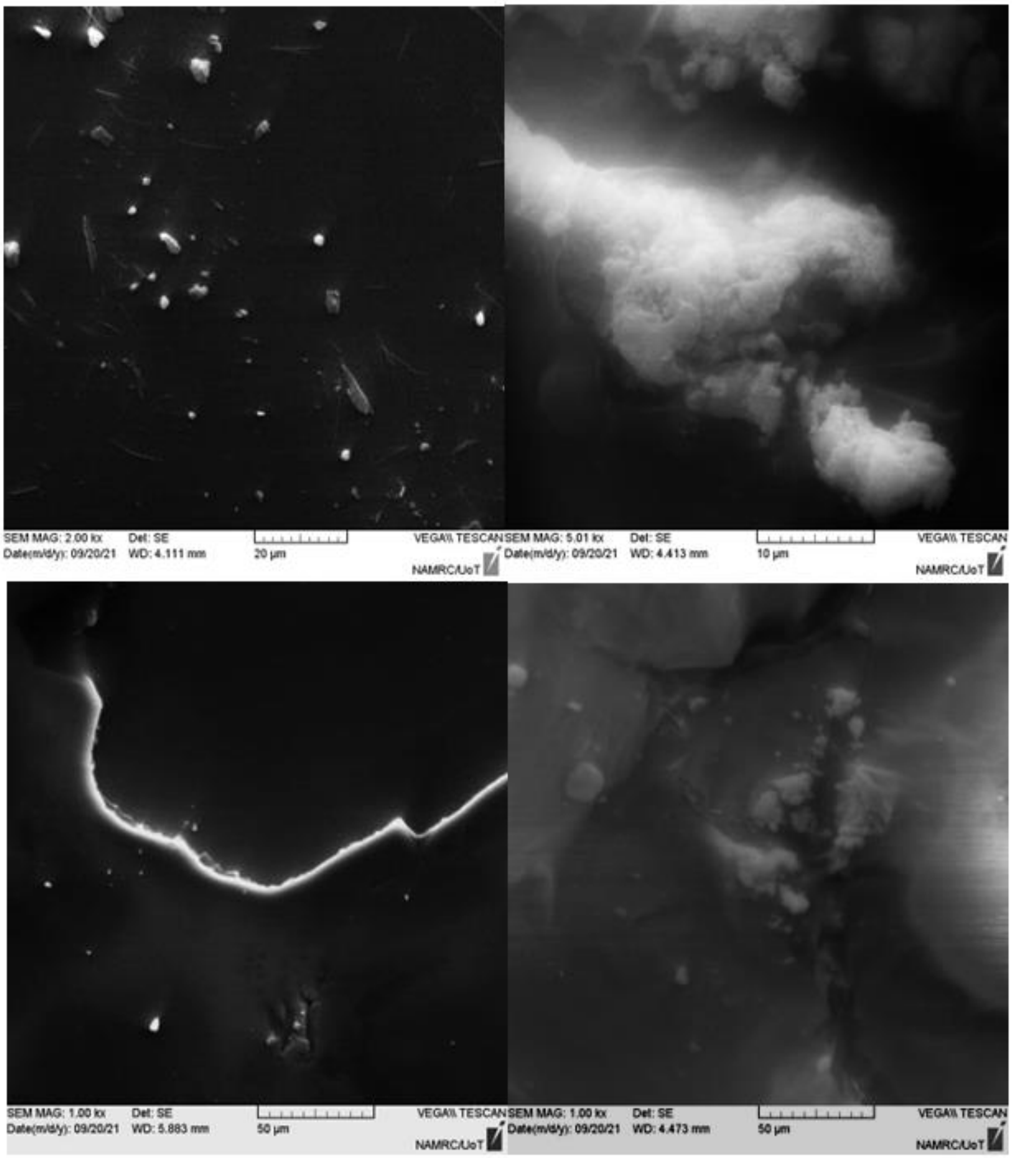

Figure 6: SEM images showing the morphology of the AgNPs synthesized using of L. meyenii (a: nanoparticles; b and c: biofilm d: biofilm treated with nanoparticles

Testing the anti-bacterial activity of silver nanoparticles using resazurin dye

The resazurin assay relies on oxidizing molecules present in the active cells that turn the dye from blue to pink and thus, the conversion of the dye is an indicator of cell growth (Elshikh *et al.*,2016). The results showed that silver nanoparticles are effective in inhibiting the growth of staphylococcus bacteria with lowest MIC 15 µg as shown in Figure (7).

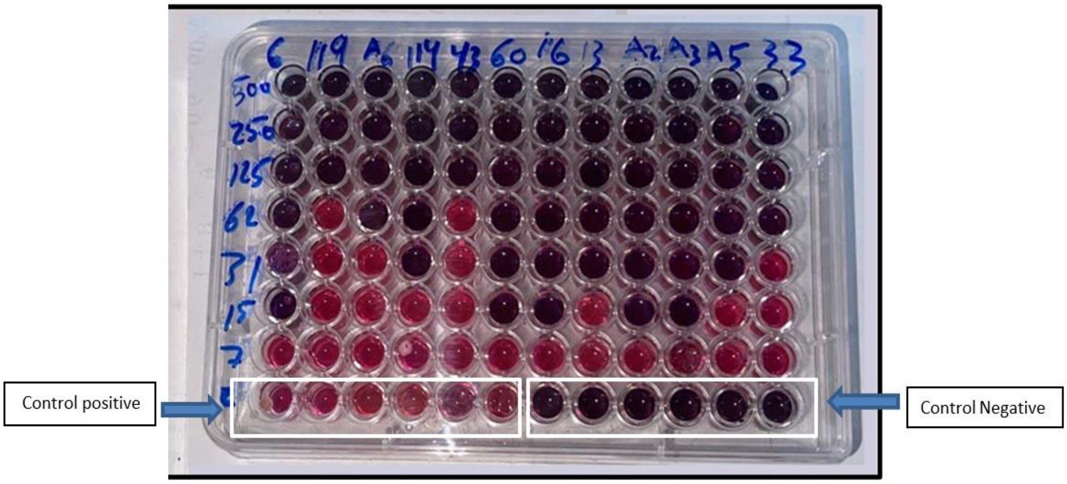

Figure (7): Testing the anti-bacterial activity of biosynthetic silver nanoparticles using resazurin dye

Testing the anti-bacterial activity of AgNPs by well diffusion method

The efficacy of biosynthesized silver nanoparticles was tested by well diffusion (Balouiri *et al.*,2016). It showed a high efficacy in inhibiting the growth of staphylococcus bacteria as shown in Figure (8).

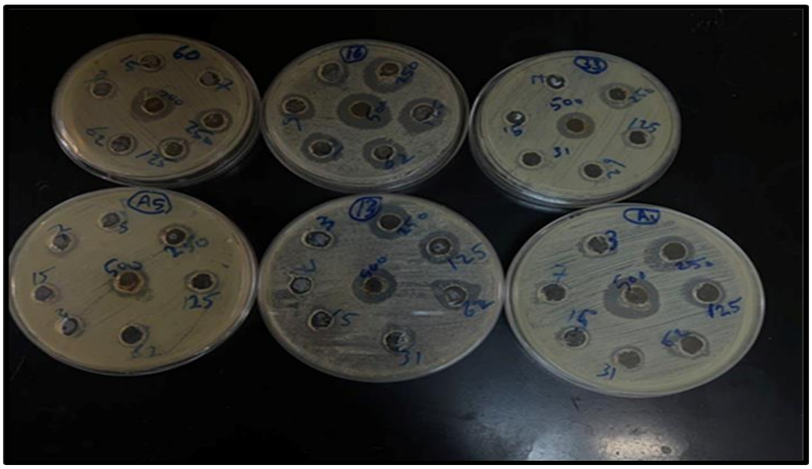

Figure (8): Testing the anti-bacterial efficacy of biosynthetic silver nanoparticles using the well diffusion method

Discussion

The current work clearly shows that AgNPs have great antibacterial action against gram positive organisms of S. aureus, and other investigations have been conducted to demonstrate AgNPs' antimicrobial efficacy. Similar findings have previously been reported, with MIC values of AgNPs against *S. aureus* ranging from 15.625 to 125 µg/ml (Larayetan *et al.*, 2019). Mostafa *et al.,* (2015) showed that AgNPs effective against *S. aureus* and *P. aeruginosa* at 2–4 µg/mL. While Gad *et al.* (2021) recorded that AgNPs prepared by green synthesis method were effective against *S. aureus* and at concentration ranged from 48 to 56 µg/ml. Differences in biological components involved in AgNP reduction and stabilization are expected to have some influence on reported antibacterial action. The mechanism of microorganism growth suppression by AgNPs has been proposed: AgNPs enter the cell walls of bacteria, causing degradation in the plasma membrane, which leads to bacterial cell death. (Vu *et al.*,2018). AgNPs exhibit antibacterial effects by causing membrane instability and the inactivation of respiratory chain dehydrogenases, as well as the creation of ROS, which inhibits cell respiration and growth (Xu *et al.*,2021). The use of biologically synthesized AgNPs represents a suitable and alternative strategy for treatment of infections caused by MDR bacteria in dairy animals. In addition, AgNP-based antimicrobial agents can overcome the limitations of conventional antimicrobial agents (Yun'an *et al.*,2021).

Conclusion

For the first time in the world, a simple biological process of silver nanoparticles (AgNPs) production was reported in this study using *L.meyenii*. AFM, SEM, and FTIR measurements revealed that the biosynthesized AgNPs were irregularly shaped with a size of 56.11 nm. The optimal conditions for L-AgNPs biosynthesis were 37°C, pH 7, and 1mM $AgNO_3$

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