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Use of pepper plant leaves' aqueous extract in the synthesis of silver nanoparticles and the treatment of some pathogenic microorganisms

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Original Research

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Abstract:

One of the biological techniques which can be used to create nanoparticles is the green synthesis method. The silver nitrate and the extract of the pepper leaf are employed to create silver nanoparticles. The scanning electron microscope (FE-SEM) revealed that there are findings of silver nanoparticles with diameters ranging from (23 nm) to (73 nm). X-ray diffraction analysis confirmed that a spectrum was identical to the spectrum of silver nanoparticles. The study of the ultraviolet-visible spectrum verified the existence of silver nanoparticles as well. The location of the silver surface plasmon (426 nm) has been observed carefully.
Silver nanoparticles have been used to kill a variety of bacteria due to their high efficacy in doing so. This process indicates that when the aqueous extract of the pepper plant coupled with silver nanoparticles turned out to be very successful and might be utilized as an antibiotic.

Keywords: Plant leaves'; Silver nanoparticles; *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Staphylococcus saprophyticus*

1. Introduction

Nanotechnology is the process of altering matter at a size close to the atomic level to create novel structures, materials, and gadgets [1]. An emerging area in nanotechnology and research is the synthesis of nanomaterials utilizing live organisms [2]. A cost-effective and environmentally friendly method, biosynthesis of nanoparticles is preferable to chemical and physical methods because it does not require the use of harmful chemicals, high temperatures, high pressures, or other energy-intensive processes [3]. Currently, nanoparticle synthesis involves the employment of both microbes and plants. It is quick, inexpensive, environmentally beneficial, and only requires one step to create nanoparticles using plants [4]. According to the size, shape, and disparity of the nanoparticles, the use of plants can also be appropriately scaled up for large-scale synthesis of the particles in a regulated manner. Furthermore, because nanoparticles are formed extracellularly, using plants in the process of nanoparticle production is more advantageous than using other procedures. Silver nanoparticles are particularly appealing among the nanoparticles for antibacterial sterilization [5]. In order to synthesize silver nanoparticles, numerous plants have been examined [6]. Silver nanoparticles were created using silver nitrate and pepper plant leaves.

2. Materials and methods

2.1 Preparation of the plant extract

After gathering and thoroughly cleaning the pepper plant leaves with deionized water and they left to dry for a while

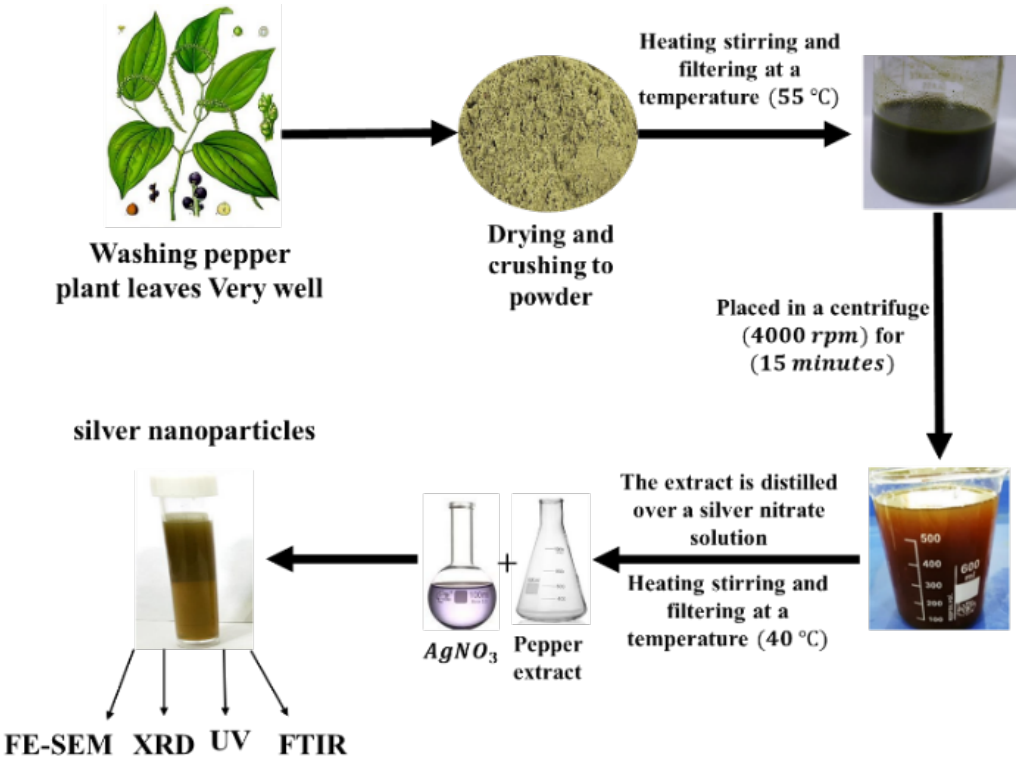

Figure 1. Steps to prepare the plant extract.

before being ground and turned out to be powder. The next process by adding 5 g of powdered pepper leaves to 100 mL of deionized water at a temperature of 55 °C for 30 mints, the suspended blots were removed by using filter paper and the extract is mixed with water by using a rapid centrifuge at (4000 rpm) for 15 minutes [7].

2.2 Preparation of silver nitrate solution

By combining (0.2 g) with (100 mL) from distilled water, the silver nitrate was created without being exposure to light.

2.3 Preparation of the nanomaterial

The pepper plant's aqueous extract was distilled using a burette onto silver nitrate while the silver nitrate was heated to a temperature of (40 °C), creating the nanomaterial. This process takes place until the color of the solutions changes and gives another wavelength, which means that a substance with a different wavelength is formed for mixed materials. After completing the drip process and preparing the nanomaterial, the resulting concentration was considered the original concentration, as it will be diluted using distilled water in different proportions. Tests were carried out for the resulting material and will be discussed in the results (Fig. 1, and 2) [8].

3. Laboratory tests of samples

3.1 Perform a field emission scanning electron microscopes examination (FE-SEM)

The samples obtained were examined using a (FE-SEM), and images of the prepared nanomaterials were obtained with sizes ranging from (23 nm) to (73 nm). These sizes appeared spherical, which means that silver particles were obtained within the nanoscale limits, as shown in the figure. That is, the apparent nanosized sizes when using this method were better than the physical methods, as larger nanosized sizes appeared [9]. The size of the crystals can be measured through the Scherrer's equation $D = K\lambda/\beta \sin\theta$ to measure the size of the crystals that appeared where K is the Scherrer constant, λ is the wavelength of light used for the diffraction, β the "full width at half maximum" of the sharp peaks, and θ the angle measured Fig. 3 [10].

3.2 Characterization of silver nanoparticles

The samples were subjected to an X-ray diffraction analysis, which is one of the methods for assessing the material's quality by obtaining a spectrum between intensity and angle. The obtained results demonstrated that it is inside the silver nanoparticles' curves and were matched with the overall results that were obtained as depicted in the figure. The results also corresponded with the search in the source Fig. 4 [11].

3.3 Fourier-transform infrared spectroscopy (FTIR)

The presence of functional groups was confirmed by Fourier-transform infrared spectroscopy (FT-IR) in the solution of biosynthesized Ag NPs. The absorption bands are visible in (FT-IR) spectra in ranges between 3433.29 and 590.22 cm^{-1}. Also significant absorption peaks can be seen in the spectra at cm1 3433, 1654.92, 1624.06, 1500.62, 1361.74, 1307.74, 864.11, and 713.66 cm^{-1}. These peaks demonstrate how various functional groups present in the solution, such as alcohols, carboxylic acids, amides, alkynes, alkanes,

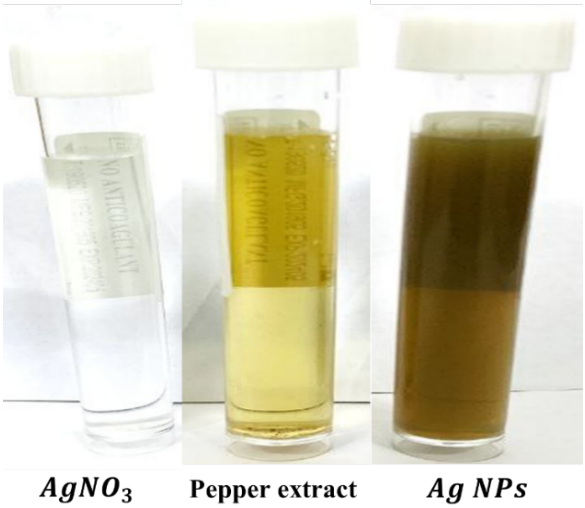

Figure 2. The difference between silver nitrate, plant extract and the resulting nanomaterial.

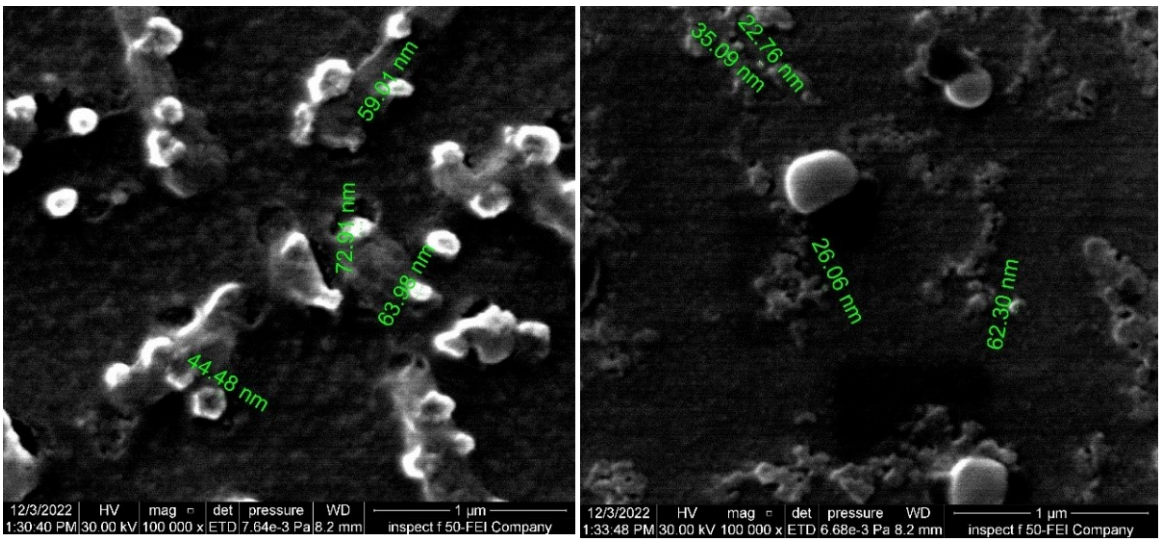

Figure 3. Show the size of nanoparticles under (FE-SEM).

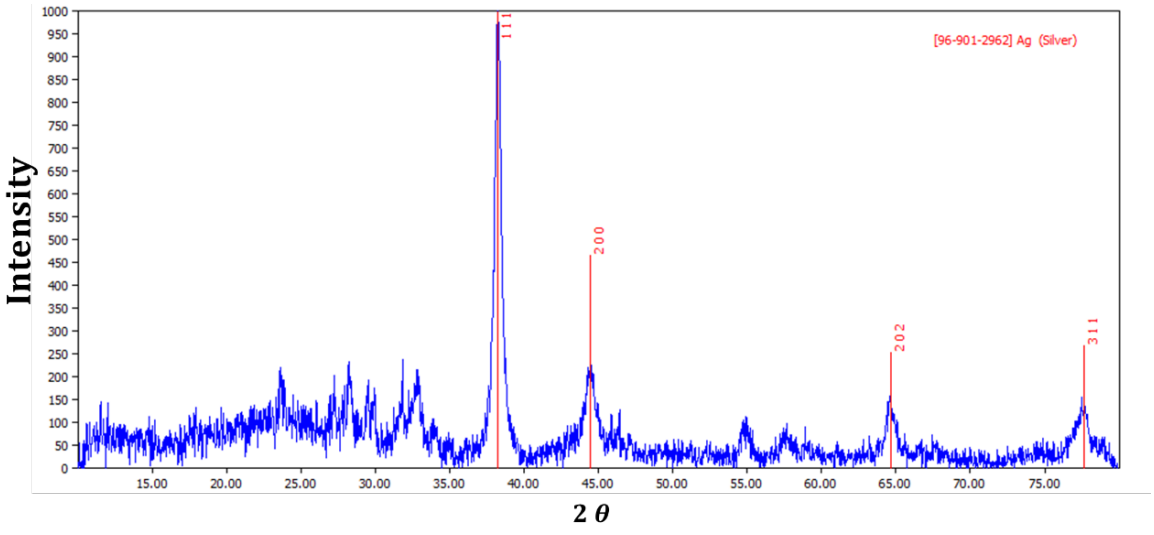

Figure 4. X-ray diffraction of silver nanoparticles.

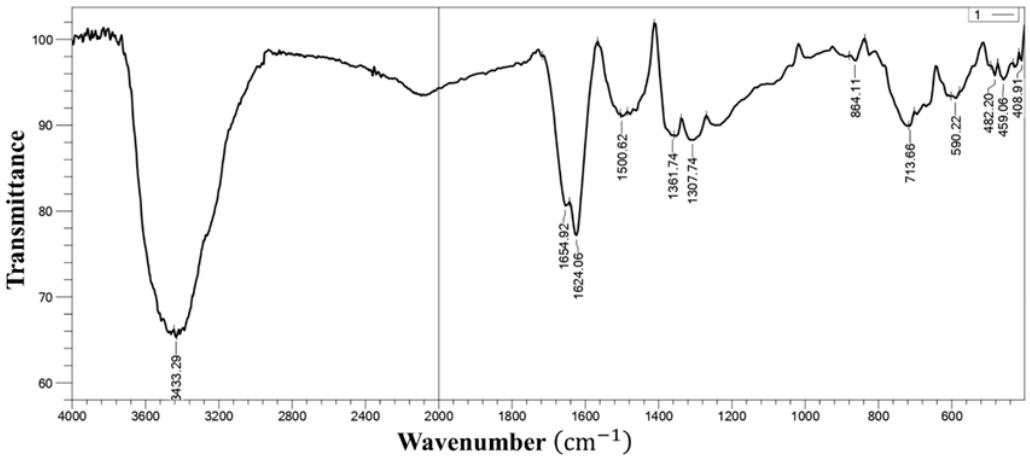

Figure 5. FT-IR absorbance spectra of biosynthesized Ag NPs assisted by aqueous extract (pepper plant leaves).

alkyl amines, halogen, and cycloalkanes, have served as capping and stabilizing agents Fig. 5 [12].
An examination (FTIR) was carried out for the obtained samples (the plant extract and the obtained nanomaterials). We note that the absorbance of the prepared nanomaterials increased significantly compared to the plant extract Fig. 6.

3.4 Ultraviolet examination (UV)

The study of the ultraviolet-visible spectrum verified the existence of silver nanoparticles as well. The UV-visible spectra are displayed in Figure 7. The location of the silver surface Plasmon (426 nm) has been carefully observed [13].

3.5 Preparation of concentrations

The nanomaterial was diluted using distilled water in different proportions, as shown in the Table 1. The table below shows the concentrations that will be used to treat several types of bacteria.
After preparing these concentrations, they were placed in the ultrasonic device for the purpose of separating the nanoparticles from each other and spreading them in the solution more and to give more effectiveness.
Also, for the purpose of the success of the experiment, filter papers were used, which were perforated, and then suitable amounts of them were placed in each tube and placed in the ultrasonic device again for the purpose of saturating them with the solution.

4. Results and discussion

Several types of pathogenic bacteria were selected for the purpose of treating them with the nanomaterial that was synthesized. The types that were used are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus* (Fig. 8).
This type of bacteria was treated with the previously prepared concentrations, and the results were obtained as shown in the figure below.
The highest concentration of bacterial inhibition was at the concentration (3 & 4) and the lowest concentration was when using the plant extract only.
All concentrations were effective in killing bacteria, as we notice that when the concentration increases, the effectiveness of the nanomaterial used in killing bacteria increases.
The Table 2 shows the diameter of the inhibition zone in relation to the concentration used in each type.
From the above diagram we notice that the greatest effect was at the concentration (4) where the inhibition zone ranged from (19 mm) to (22 mm) in which the nanomaterial

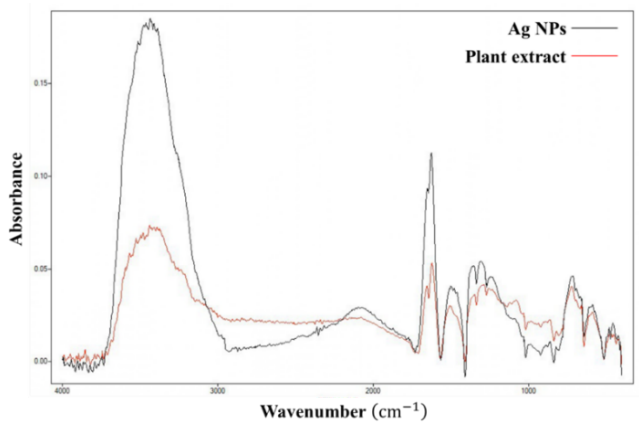

Figure 6. Diagram showing the absorbance of the plant extract compared to the nanomaterials.

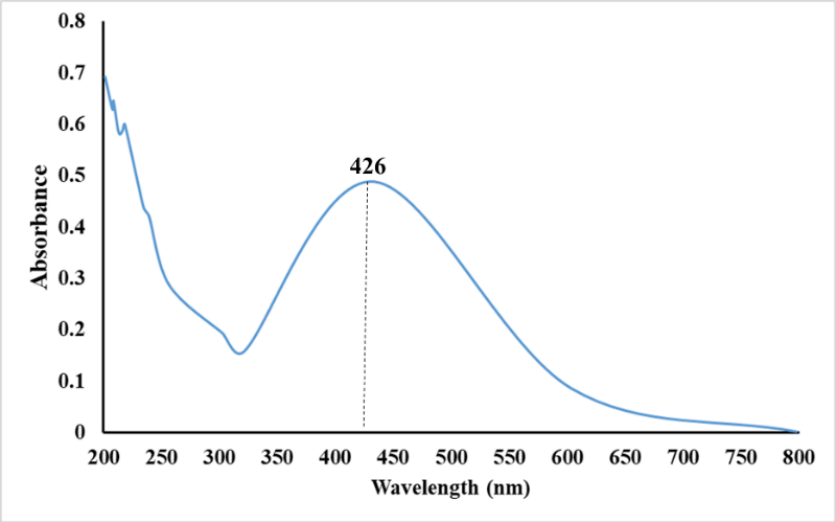

Figure 7. UV-Visible spectra of Silver nanoparticles synthesized from pepper plant leaves' aqueous extract.

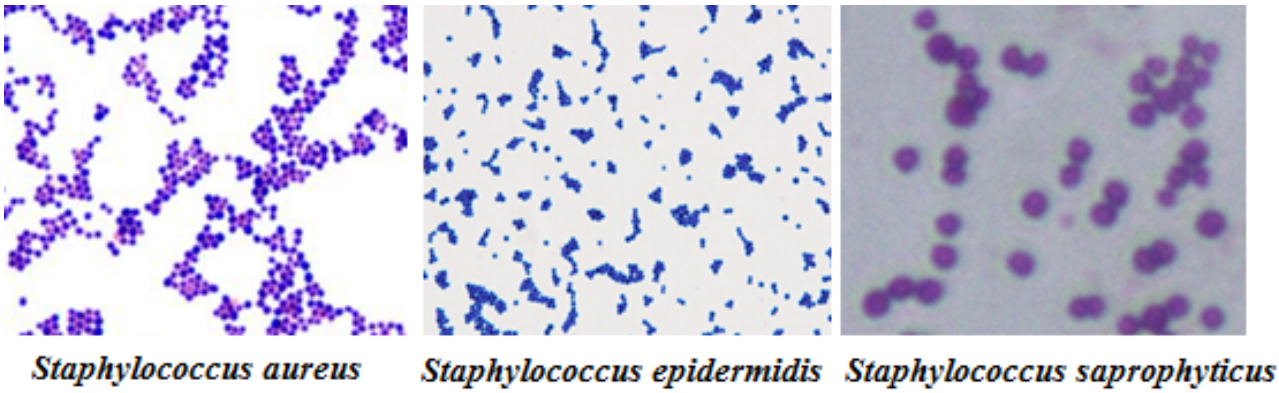

Figure 8. Bacteria under an electron microscope.

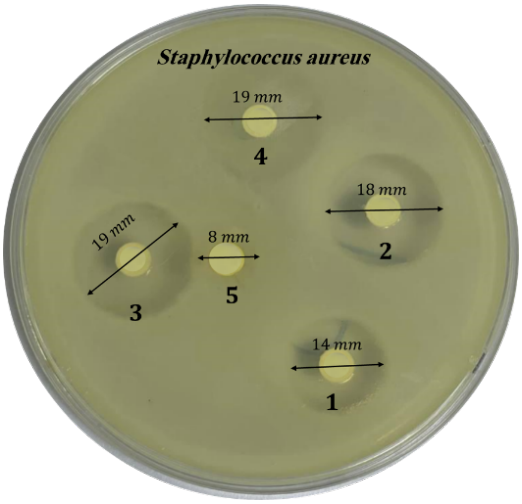

Figure 9. The concentrations of silver nanoparticles and aqueous extract of pepper leaves and their effect on bacteria (*Staphylococcus aureus*) are shown.

was used with the aqueous extract of the pepper plant. The least inhibition appeared when using pepper plant extract alone.

5. Conclusion

From the results obtained, we note that the nanomaterial demonstrated clear effectiveness in inhibiting bacteria, and the effectiveness of inhibition increased when adding the

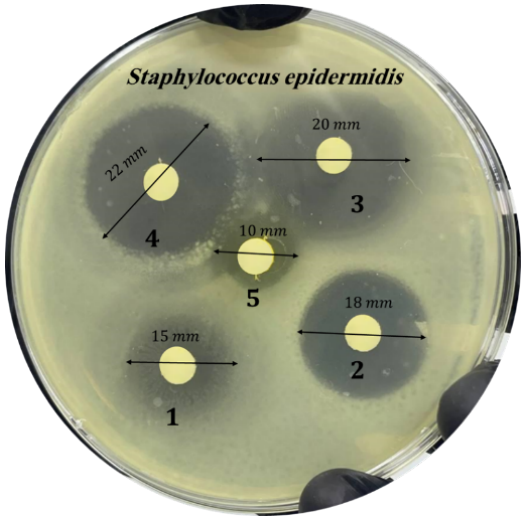

Figure 10. The concentrations of silver nanoparticles and aqueous extract of pepper leaves and their effect on bacteria (*Staphylococcus epidermidis*) are shown.

Figure 11. The concentrations of silver nanoparticles and aqueous extract of pepper leaves and their effect on bacteria (*Staphylococcus saprophyticus*) are shown.

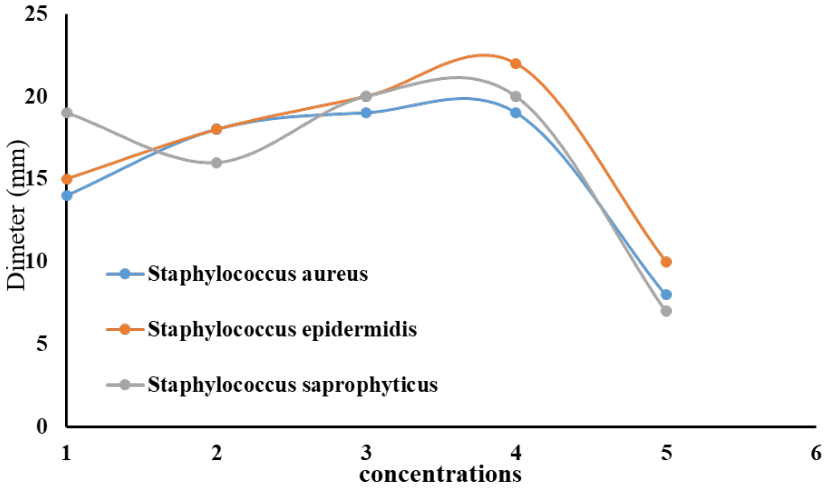

Figure 12. Inhibition zones and concentrations used for each species.

Table 1. Dilution ratios of Nano silver with aqueous extract of pepper plant.

No.	Nano-silver (mL)	Distilled water (mL)	Dilution ratio (%)	Plant extract (mL)
1	1	10	10	–
2	2	10	20	–
3	3	10	30	–
4	2	10	20	10
5	–	–	–	10

Table 2. Inhibition zones for each type of bacteria using different concentrations.

	Inhibition zone (mm)		
No.	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Staphylococcus saprophyticus*
1	14	15	19
2	18	18	16
3	19	20	20
4	19	22	20
5	8	10	7

aqueous extract of pepper leaves with Nano silver. The manufactured nanomaterial can be used to inhibit the activity of several types of bacteria.
The manufactured silver nanoparticles can be applied to areas where bacteria are expected to be found and utilized to destroy a variety of germs that could cause diseases in humans.

Ethical approval
This manuscript does not report on or involve the use of any animal or human data or tissue. So the ethical approval is not applicable.

Authors Contributions
All the authors have participated sufficiently in the intellectual content, conception and design of this work or the analysis and interpretation of the data (when applicable), as well as the writing of the manuscript.

Availability of data and materials
The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interests
The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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