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Synthesis of Silver Nanoparticles using Methanolic Extract of *Salvia Officinalis* L. and its Antibacterial Activity

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Abstract

Due to its numerous applications in numerous branches of technology, medicine and other study domains, nanotechnology is regarded as a highly developed technology. The main objectives of this work are to synthesize silver nanoparticles from silver nitrate using methanolic *Salvia Officinalis L.* aerial parts extract, to characterize the synthesized nanoparticles using Ultra Violet and Fourier Transform Infrared Spectroscopies and to test its antibacterial activity compared with standard antibiotics. The manufacture of silver nanoparticles was done using methanolic extract of *Salvia officinalis L.* (mramia). Two Gram negative bacteria *E. coli* and *Pseudomonas aeruginosa* and 2 Gram positive bacteria Bacillus subtilis and *Staphylococcus aureus* were used in this study. The creation of nanoparticles is indicated by a change in color from yellow to brown. The maximum absorption was found to be at 220 nm. The spectra of S. officinalis extracts displayed a broad and strong absorbance peaks at 995.20 cm^{-1} that corresponds to Amines, 2929.67 aldehyde, 2360.17 carboxyl acid, 1691.46 aldehyde, 1456.46 is NO_2, 1382.87 ether and 1031.85 alcohol. The methanolic extract of the *S. officinalis* and the synthesized nanoparticles were tested against the studied bacteria, the results of the methanolic extracts were higher in its antibacterial activity than the activity of the synthesized silver nanoparticle and this explains the reason for using this plant in folk therapy.

Keywords: Silver • Nanoparticles • *Salvia officinalis* • Antibacterial activity • *E. coli* • *Pseudomonas aeruginosa* • Bacillus subtilis • *Staphylococcus aureus*

Introduction

Green synthesis techniques are characterised as environmentally friendly, accessible, secure and nontoxic. Nanoparticles are subatomic particles having lengths in two or three dimensions between 1 and 100 nm. The distinctive characteristics of silver nanoparticles, such as their size and form depending on the optical, electrical and magnetic properties, make them significant. Green synthesis of nanoparticles using phyto compounds as bio-reductants is attaining a greater impetus, a variety of plant materials, such as leaf extracts, fruit, bark, fruit peels, root and callus are used [1]. Stabilization of nanoparticles is claimed by the presence of terpenoids and Flavonoids in the plant extracts. Many analytical techniques, such as ultraviolet visible spectroscopy (UV-vis spectroscopy), X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), have been used to evaluate the synthesized nano materials [2,3]. The manufactured nano materials have new chemical, physical, surface and optical electronic properties, materials and chemicals with high productivity and lower energy consumption, play a role in developing innovative methods for creating new products and can solve problems which can't be solved by traditional technologies [4-8].

Gold, silver, zinc oxide, carbon and titanium dioxide nanoparticles are manufactured as much as tenfold that of other nano materials in amount due to their potential antimicrobial characteristics, deodorants, food storage containers, bandages, paints, tooth- pastes and other home appliances [9]. *Salvia officinalis L.* (Sage) family Labiatae (Lamiaceae) is a perennial shrub, *Salvia* is the largest genus of this family it includes near 900 species which cultivated in many countries due to its traditional usefulness in folk medicine and for domestic applications. *S. officinalis* is native to the Middle East and Mediterranean areas. Today's, it has been naturalized throughout the world particularly in Europe and North America. The aerial parts of *S. officinalis* used in cookery and traditional medicine. Because of its flavoring and seasoning properties, it has been used for the treatment of various kinds of disorders including seizure, ulcers, gout, rheumatism, inflammation, dizziness, tremor, paralysis, diarrhea and hyperglycemia. In traditional medicine of Europe, *S. officinalis* has been used to treat mild dyspepsia (such as heartburn and bloating), excessive sweating, age-related cognitive disorders and inflammations in the throat and skin *S. officinalis* has been used for the treatment of different kinds of disorders including seizure,

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ulcers, gout, rheumatism, inflammation, dizziness, tremor, paralysis, diarrhea, and hyperglycemia anticancer, anti-inflammatory, antinociceptive, antioxidant, antimicrobial, antimutagenic, antidementia, hypoglycemic, and hypolipidemic effects [10].

The main objectives of this work are to synthesize silver nanoparticles from silver nitrate using methanolic plant extract of the aerial parts of *S. officinalis*, to characterize the synthesized nanoparticles using UV and FTIR and to test its antibacterial activity compared with standard antibiotics.

Materials and Methods

Plant material

Salvia officinalis plant was purchased in May 2022 from Omdurman local market Central Sudan (to which it was imported from Syria), the plant was identified and authenticated by Dr. Adam Mohammed Ahmed, Department of Botany, Omdurman Islamic University.

Bacteria

Four standard bacteria belonging to 2 Gram negative bacteria *E. coli* and *Pseudomonas aeruginosa* and 2 Gram positive bacteria Bacillus subtilis and *Staphylococcus aureus* were used in this study.

Methods

Preparation of the plant extract

100 grams of the dried aerial parts of *S. officinalis* were dried, crushed and extracted in a soxhlet device using 250 ml methanol solvent (99.9% concentration) for 12 hours, the extract was air dried and weighed. Distilled water was used to prepare several concentrations of the plant extract (100, 50, 25 and 12.5) mg/ml.

Synthesis of silver nanoparticles

10 ml of 0.1 M silver nitrate was added to 50 ml of each of the prepared ethanolic extracts in tubes, each mixture was divided into two parts one Part was heated in a water bath and the mixtures were placed in an oven of 70ºC. for an hour, for observation of color change.

Characterization of synthesized silver nanoparticles

UV- visible spectroscopy: The formation of AgNPs was confirmed by measuring its optical properties with UV-Vis spectroscopy (Shimadzu UV 1800) in the range of 200 to 800 nm. To determine the phytochemical compounds involved in the reduction of $AgNO_3$ to AgNPs as well as those involved in the stabilization of AgNPs. A solution of concentrated and diluted methanol was used.

Characterization of silver nanoparticles by fourier transform infra-red

Fourier Transform Infrared (FTIR) Spectroscopy Binding properties of AgNPs synthesized by *S. officinalis L. aqueous* extract was investigated by Fourier Transform Infrared Spectroscopy (FTIR) analysis. The FTIR measurements were taken on Bruker vertex 70 in the range of 4000-400 cmG1. Dried and powdered AgNPs were palliated with potassium bromide (KBr) (1:1 proportion). The spectra were recorded in the wave number range of 450-2500 cm-k and analyzed by subtracting the spectrum of pure KBr.

Preparation of bacterial suspensions

One ml aliquots of a 25 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37ºC for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline to produce a suspension containing about 107 CFU/ ml [11]. The suspension was stored in the refrigerator at 4º C until used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micropipette onto the surface of dried nutrient agar medium. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37ºC for 24 hours.

Testing of extracts for antibacterial activity

Using the standard Cup-Plate agar diffusion technique, the studied bacteria were examined for their susceptibility to methanolic extracts and the synthesized silver nanoparticles, four wells (9 mm in diameter) in each plate were bored in the seeded medium by using a sterile (alcoholic flamed and then cooled) cork borer and the cut discs of agar were removed and decontaminated, 0.1 ml of the different tested extract concentrations were added to the appropriate cups with a pipette and the plates were held for two hours at room temperature for diffusion of extract into agar. Subsequently, the plates were incubated at 37ºC for 24 hours. After incubation period, the diameters of the inhibition zones were measured to the nearest mm excluding well diameter. Tetramycin and Gentamycin were used as positive control for antibiotics were used as recommended by National Health Organization during period of the study. DMSO used as negative control.

Results

Percentage yield of extract

The percentage yields of the methanolic extract was calculated in (Table 1) it was found to be 7% (Table 1).

Formation of silver nanoparticles

When mixing silver nitrate and plant extract in different concentrations, the color was yellow, then the color changed to brown and this indicates the formation of silver nanoparticles. The color change was the same in both cooled and heated extracts. This formation indicates that silver having the size of Nano metric range. The color of the reaction mixture changed to brown, suggesting the conversion of ionic silver (Ag+) to metallic silver (Ag). This observation is consistent with the established literature which stipulates that silver ions are reduced in the presence of plant extracts due to the reducing properties of some secondary metabolites (i.e. polyphenols, alkaloids, terpenoids, proteins, etc.) [12].

UV-Vis spectrophotometer Ultra violet visible spectroscopy (UV–vis) is the method widely used to characterize nanoparticles and to confirm the synthesis of nanoparticles, the brown color of AgNPs arises from the concomitant vibration of free electrons of the metallic silver that are in resonance with the light wave, the maximum absorption was found to be at 220 nm. Figure 1, some researchers have reported SPR peaks below 400 nm for their synthesized nanoparticles [13]. The absorbance bands below 400 nm are probably due to absorption of silver ions, complexes (Figure 1).

Characterization by fourier transform infra-red

Fourier Transform Infra-Red (FTIR) analysis was carried out and the result is depicted in Figure 2. FTIR analysis revealed that the biomolecules present in the leaf extract could be responsible for the reduction of silver ions as well as prolonged stability of nanoparticles. In this study, *S. officinalis* extract showed several spectra that indicate the complex nature of the plant

Table 1. Percentage yield of extract.

The quantity taken	Solvent name	Solvent concentration	Final output	Percentage
100	Methanol	99.9	7g	7%

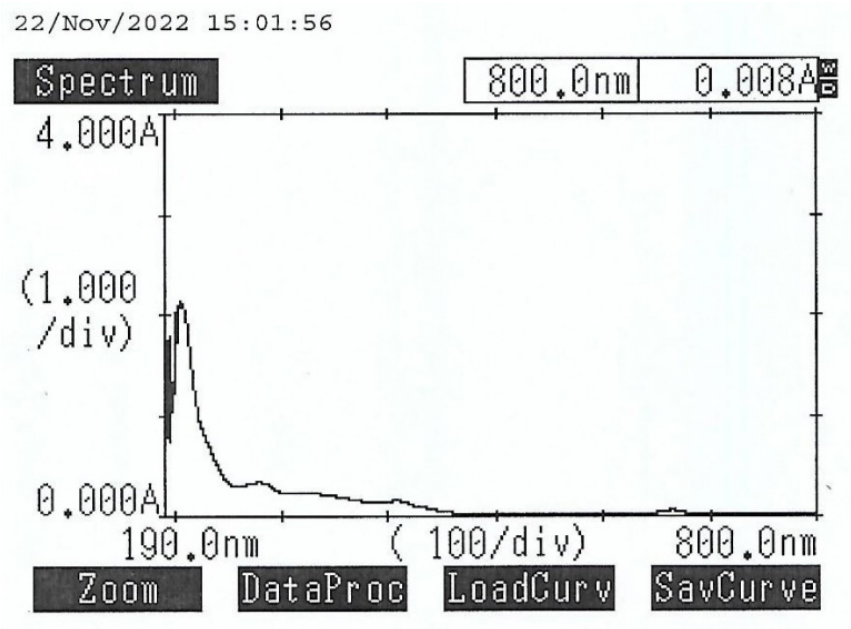

Figure 1. UV-vis spectrum of AgNPS of *S. officinalis*.

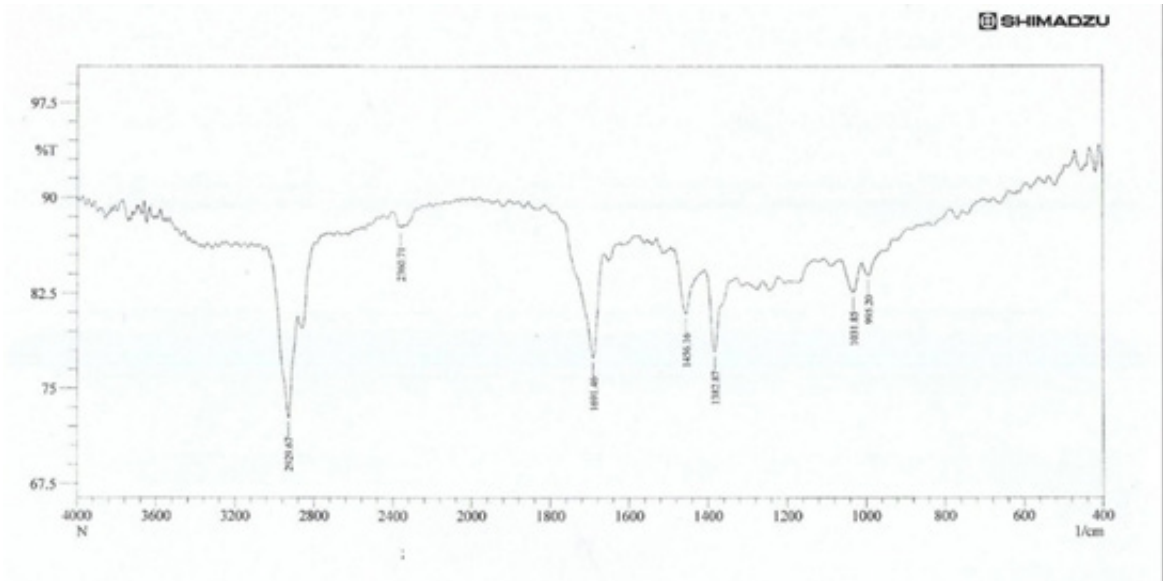

Figure 2. FITR spectrum of AgNPS of *S. officinalis*.

extract and our findings corroborate with the previous results. The spectra of *S. officinalis* extracts displayed a broad and strong absorbance peaks 995.20 cm^{-1} that corresponds to Amines, 2929.67 aldehyde, 2360.17 carboxyl acid, 1691.46 aldehyde, 1456.46 is NO_2, 1382.87 ether and 1031.85 alcohol (Figure 2).

Antibacterial activity

***E. coli*:** Different concentrations of the plant extract gave results ranging from 100 mg/ml to 12.5 mg/ml. The higher the concentration, the higher the inhibition zone against *E. coli* bacteria, as well as for nanoparticles before and after heating (Table 2 and Figuer 3). The results of the extract dissolved in the DMSO solution had a higher effect than the nanoparticles (Table 2 and Figure 3).

***P. aeruginosa*:** Different concentrations of the extract gives different results, but the concentration of 25 mg/ml had the highest effect, followed by a concentration of 100. As for nanoparticles, the effect was according to the concentration from higher to lower. Nanoparticles after heating gave higher results than nanoparticles before heating (Table 3 and Figure 4). Gram-negative bacteria *P. aeruginosa* had the highest zone of inhibition compared with other tested Gram-positive bacteria, Same line of observation was also recorded by Mie R, et al. [14] who found that silver nanoparticles have relatively higher antibacterial activity against Gram-negative bacteria than Gram-positive bacteria, which might be attributed to the presence of porins and the thinner peptidoglycan layer (Table 3 and Figure 4).

***S. aureus*:** The extract gives higher results than the nanoparticles; the higher concentration of the extract gave higher results, and the effect against bacteria was graded with the concentration gradient from higher to lower. Nanoparticles before heating (Table 4 and Figure 5) gave higher yields than particles after heating, and the effect was gradual from higher to lower (Table 4 and Figure 5).

***B. subtilis*:** The extract gives higher zones than the nanoparticles concentration of 100 mg/ml and 25 zone shows the highest effect, followed by a concentration of 12.5 mg/ml then 50 mg/ml zone at an extract concentration of 25 synthesized nano particles followed by 12.5 with an equal lower effect of 100 and 50. Heated extract shows the highest effect at the lowest concentration and the effect was graded in reverse gradient from the lowest concentration giving the greatest effect on Ba bacteria to the highest concentration giving the least effect (Table 5 and Figure 6).

Table 2. Inhibition zones in cm (*E. coli*).

Concentration	Methanolic extract	Silver nanoparticle before heating	Silver nanoparticle after heating
100 Mg/ML	15	13	13
50 Mg/ML	13	12.5	12.5
25 Mg/ML	12	12	12
12.5 Mg/ML	12	12	12

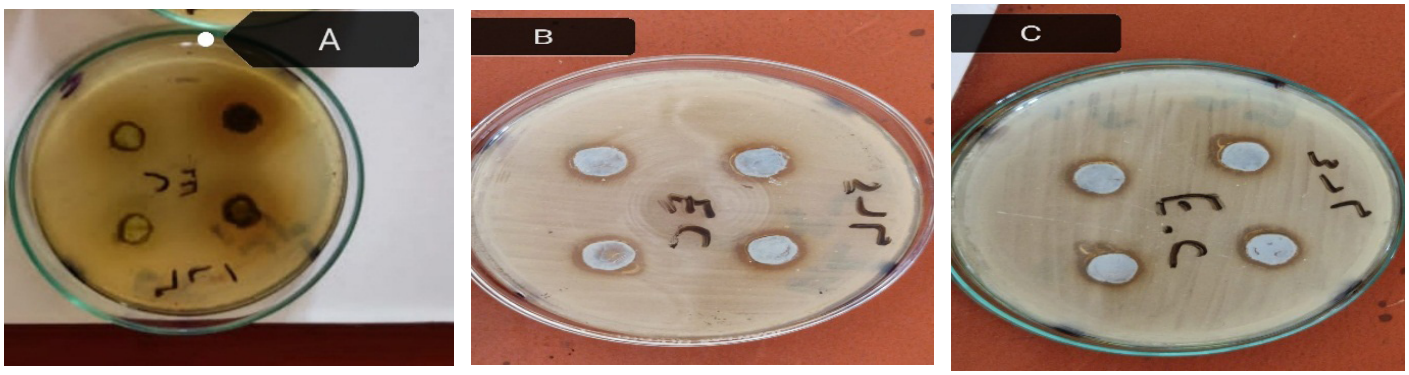

Figure 3. Inhibition zones of **(A)** Methanolic extract, **(B)** Silver nanoparticle before heating and **(C)** Silver nanoparticle after heating *E. coli.*

Table 3. Inhibition zones (*P. aeruginosa*).

Concentration	Methanolic extract	Silver nanoparticle before heating	Silver nanoparticle after heating
100 Mg/ML	16	15.5	16
50 Mg/ML	15	14	15.5
25 Mg/ML	19	13	15
12.5 Mg/ML	13	12	15

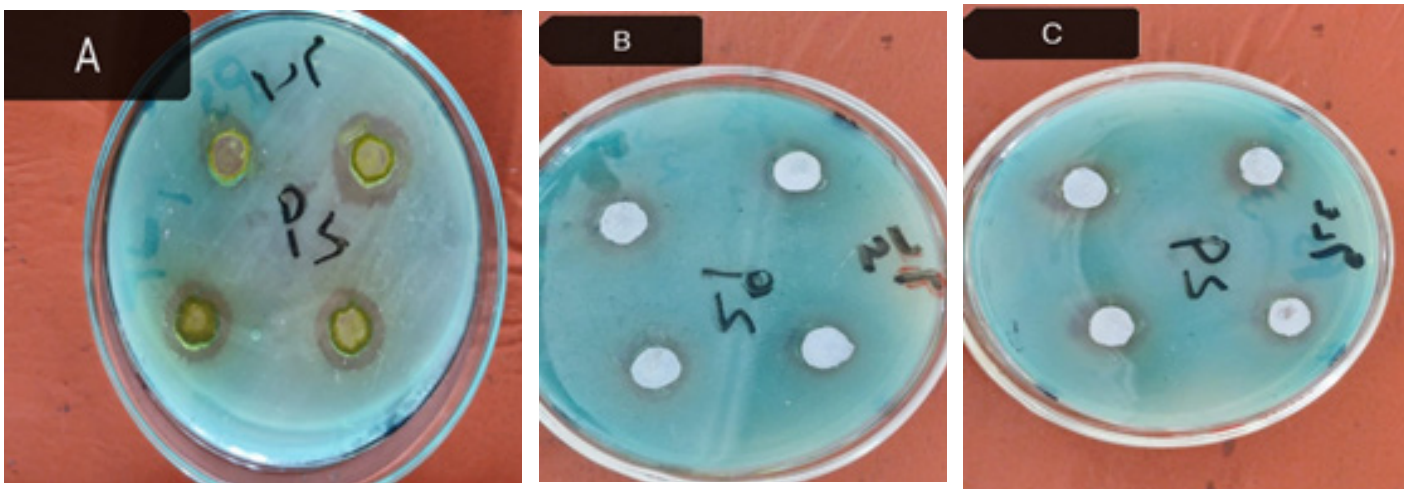

Figure 4. Inhibition zones of **(A)** Methanolic extract, **(B)** Silver nanoparticle before heating and **(C)** Silver nanoparticle after heating *P. aeruginosa.*

Table 4. Inhibition zone in cm (*S. aureus*).

100Mg/ML	Methanolic extract	Silver nanoparticle before heating	Silver nanoparticle after heating
Concentration	23	17	14
50 Mg/ML	23	15	14
25 Mg/ML	22.5	14	13.5
12.5 Mg/ML	20	13.5	13

Inhibition zones standard

The results of the synthesized nanoparticles and methanolic extract show better results than the standard against P. S. It also gave similar results against *E. coli* and less effect against B. S bacteria, although it is less, but it has high results, as the diameter of the zone reached up to 25 (Table 6 and Figure 7).

In this study, the synthesized silver nanoparticles of the methanolic extracts are less effective than the normal methanolic extracts, whereas in other study [15] *aqueous* silver nanoparticle extracts from *S. officinalis* are found to be more effective against *Klebsiella pneumonia, Pseudomonas aeruginosa* and *Staphylococcus aureus* than the normal *aqueous* extract.

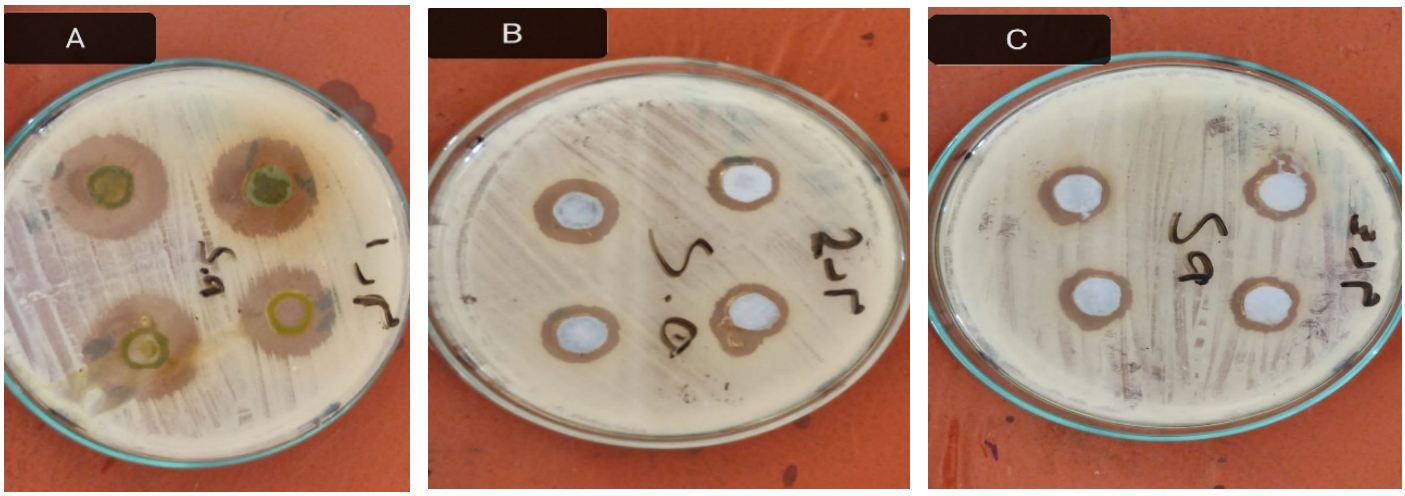

Figure 5. Inhibition zones of **(A)** Methanolic extract, **(B)** Silver nanoparticle before heating and **(C)** Silver nanoparticle after heating *S. aureus*.

Table 5. Inhibition zones in cm (*B. subtilis*).

Concentration	Methanolic extract	Silver nanoparticle before heating	Silver nanoparticle after heating
100 Mg/ML	25	15	14
50 Mg/ML	20	15	14
25 Mg/ML	25	17	15
12.5 Mg/ML	22	16.3	15.6

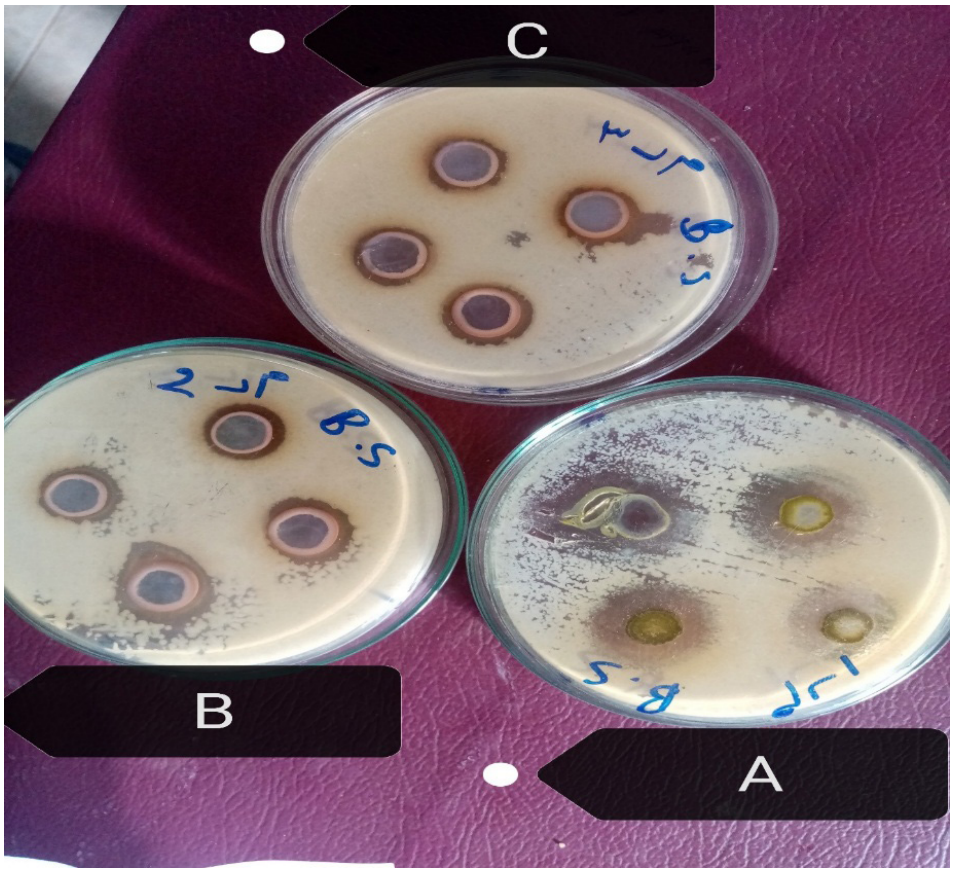

Figure 6. Inhibition zones of **(A)** Methanolic extract, **(B)** Silver nanoparticle before heating and **(C)** Silver nanoparticle after heating *P. aeruginosa*.

Table 6. Inhibition zones (standard).

Bacteria	Gentamycin	Tetracyclin
E.coli	18	24
B. subtilis	30	32
P. aeruginosa	20	12

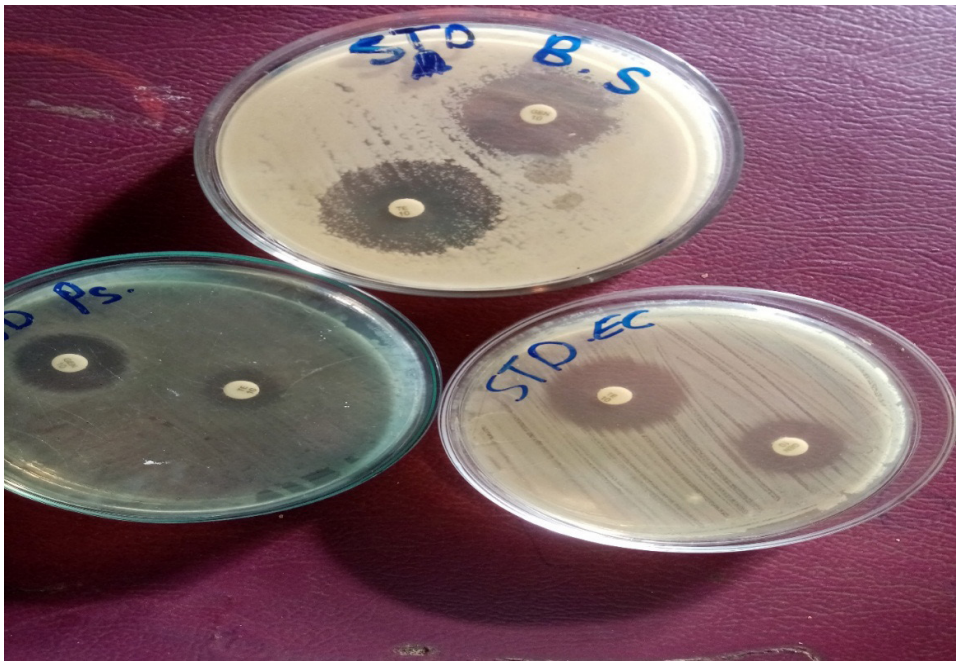

Figure 7. Inhibition zones of standard.

Conclusion

The formation of nanoparticles was indicated by color change, by measuring the wavelength with a UV device and the functional groups contained in these particles *via* the FTIR device. The extract of the *S. officinalis* plant and the synthesized nanoparticles were tested against bacteria, it showed high activity against bacteria and the results of the extract were higher and this explains the reason for using this plant in folk therapy.

Authors' Contributions

Elnour was the one who came up with the concept with the help of Ahmed and Eltahir, Elnour and Elamin drafted the manuscript. The results of the paper were considered by all writers and they all contributed to the final form of the manuscript. The final version of the text was co-authored by all writers.

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Conflict of Interests

None.

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