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Original Research Article

Antioxidant and Antibacterial Activities of *Allium sativum* Ethanol Extract and Silver Nanoparticles

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ABSTRACT

The development of bacterial-fighting nanoparticles using natural materials is currently receiving a lot of attention because it is environmentally beneficial. In this paper, showed described how silver nanoparticles made from Ethanolic Allium sativum plant extract were biosynthesized. Scanning Electron Microscopy (SEM), UV-visible spectra, and Fourier Transform Infrared Spectroscopy (FTIR) were used to screen the phytochemical composition of Allium sativum ethanolic extract (ASEE) and detection of AgNPs. The ASEE particles sizes were 179.1-653.6 nm, while the AgNPs were 78.06 nm. The phytochemical screening using FTIR assay showed the presence of many active compounds with medicinal activities. Five doses of ASEE (0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL) and doses of Bio-AgNPs (0.2, 0.4, 0.6, 0.8, 1.0 mg/mL) were used to study the antibacterial activity using the of *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) were revealed to have inhibition zones of 10 and 9 mm, respectively, were observed for the ASEE, while 17 and 19 mm were recorded for the AgNPs. The Bio-AgNPs exhibits IC_{50} of 2.2 µg/mL which indicates their removal of free radicals in antioxidant activity. Scavenging activity, with the ASEE and AgNPs eliminating about 77.67 and 85.87%, respectively. Both ASEE and Bio-AgNPs posses' antioxidant by DPPH assay, and antibacterial activities

Keywords: *Allium sativum*, Silver nanoparticles, UV-is spectral, FTIR, SEM, Antibacterial, Antioxidant activity

Introduction

This study came to shed light on one of these aspects the enormous natural wealth of Iraq's environment, which aims to prepare ethanolic extract and separating a number of active ingredients from extract plant, as well as estimating the effectiveness the biological activity of the extracts against some of types bacteria using effected of extract plant in inhibiting pathogenic microbes and after combination with sliver nitrate. Nanotechnology is the creation of nanoscale particles as well as the characterization, production, and manipulation of those particles. Various plant extracts are used to create these nanoparticles, giving an easy, affordable, non-toxic way with active substances that have antibacterial qualities. [6] Particles are very different from those of materials created on a larger scale from the same source material, yet the differences are significant.[1,2] The technology involves the production, characterization, manufacturing, and modification of these nanoparticles. Furthermore, the physical and chemical properties of particles smaller than this limit result in substances with properties that differ significantly from the properties of macroscopic scales.[3,4] Nanomaterials are materials with a length of 1 to 100 nm or that are highly structured in at least one dimension.

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Nanotechnology refers to the synthesis, characterization, production, and manipulation of these particles. Incorporating or encapsulating them with suitable nanomaterials can improve their pharmacokinetic characteristics and efficacy.[5,6]

Medicinal plants form capping layers, which affect the size and structure of nanoparticles.[7,8] The medicinal plant *Allium sativum*, more often known as garlic, was used in the synthesis of silver nanoparticles (AgNPs).[9,10] The plant has medicinal properties and is used to treat a variety of diseases in most cuisines around the world. This spice is reported to have anticancer, antimicrobial, antidiabetic, hypoglycemic, hypolipidemic, antiemetic, and immunomodulatory activities.[11] *Allium sativum* contains a lot of is abundant in tannins, saponins, phenols, alkaloids, carotenoids, and flavonoids, according to phytochemical studies, and it has been shown to have significant antioxidant activities.[12] The present study was aimed at biologically synthesizing AgNPs using *A. sativum* ethanolic extract and evaluating their antibacterial and antioxidant capabilities.

Materials and Methods

Plant material

Allium sativum was obtained on March, 2022 from a popular local market (Shorga-Suk) in Baghdad/ Iraq (Figure 1). The plant material was rinsed with sterile distilled water and dried[13].

Bacterial pathogens

Escherichia coli and Staphylococcus aureus were obtained by the Department of Applied Sciences. Division of Biotechnology, University of Technology, Iraq. The isolated microorganisms were diagnosed by the VITEK-2 compact system with (GP) card for gram positive bacteria identification, (GN) card for gram negative bacteria identification.

Figure 1*: Allium sativum* plant.

Preparation of Allium sativum extract

The powder of *A. sativum* plant (50g) was extracted by boiling (300ml) at (70%) ethanol via soxholet apparatus for 7 hours. The extract was filtered by Whitman filter paper using a Buchner funnel by A rotary evaporator under vacuum at 40°C was used to filtrate the extract of *A. sativum*

, which then stored and kept in a dark refrigerator at 4°C in a glass container.[6]

Preparation of silver nanoparticles

Ten milliliters of the *A. sativum* extract were added to 90 ml $AgNO_3$ solution (0.1 M). The mixture was stirred continuously for 15 minutes using a magnetic stirrer. In order to avoid silver's natural oxidation process, the solution was stored in the dark.[5,12]

Characterization of ASEE, and ASEE-AgNPs

ASEE and silver NPs were characterized using UV-visible spectra analysis, a UV-vis spectrophotometer (UV-3000 PC, UK) was used to take consistent readings at a wavelength range of 365-540 nm. JASCO FT-IR 4100 spectrometer was used in order to carry out the Fourier transform infrared (FTIR) analysis, Spectra were collected using a transmission mode that ranged from 4000-440 cm1. The scanning electron microscope was used. All analyses were performed in the Department of Applied Science, University of Technology, and Baghdad, Iraq.

Determination of radical scavenging activity of ASEE, and ASEE-AgNPs

Antioxidant activity was measured with the stable DPPH radical scavenger in A. sativum ethanolic extract and AgNPs at three different concentrations (50, 75, and 100 µg /ml). An aliquot of 10 µl of each concentration was mixed with 490 µl of ethanol, and the amount was raised to 1000µl by adding 500 µl of DPPH solution. The solution was incubated for 30 minutes at room temperature. The DPPH residual amount was estimated based on the decrease in absorbance at 517 nm. The percentage inhibition of DPPH was calculated using the following formula.[22]

$$\text{Antioxidant activity \%} = \frac{(\text{OD control} - \text{OD sample})}{\text{OD contril}} X100$$

Where OD is the optical density.

Determination Antibacterial activity of ASEE, and ASEE-AgNPs

Antibacterial activity of ethanolic extract *Allium* sativum with silver nanoparticles. The ethanolic extract of *A. sativum* and AgNPs were tested for their ability to inhibit the growth of bacteria through the use of the agar well diffusion method.[15,16] Mueller Hinton agar was made and used according to the manufacturer's instructions. An aliquot of 0.1 ml of overnight bacterial culture (adjusted to 0.5 McFarland turbidity level) was streaked entirely on the Mueller Hinton agar using a sterile swab stick. A sterile 5 mm cork borer was used to insert five wells in the culture medium. Using a sterile micropipette, 1 g of extract was dissolved with 10% D.W, then done concentrations ranged (0.2, 0.4, 0.6, 0.8, and 1.0 µg/ml) were full put in wells. Distilled water was used as the negative control. The observed inhibitory zones were measured in mm.

Statistical analysis

All of the tests were conducted in triplicate. Data were analyzed according to analysis of prism version sigma state in two directions, using the prepared statistical program.

Results and Discussion

Scanning electron spectroscopy images of ASEE extract and the biologically synthesized AgNPs are obtained and presented in Figures 2A and B. The size and form of the AgNPs were examined, and the particles were found to be evenly distributed and generally rounded in form. The computed particle size revealed that the ASEE particles were 179.1-653.6 nm (Figure 1A) and the ASEE-AgNPs were 78.06-289.8 nm (Figure 1B). It was determined that the nanoparticles had an average size of 76.42 nanometers (nm). For the purpose of monitoring the creation of nanoparticles, the UV-visible spectroscopy approach was utilized. Before being combined with a silver nitrate solution and subjected to the effects of sunshine, the ethanolic extract of ASEE extract was yellow in color. The surface of the silver Plasmon resonance band could be obtained at a wavelength of 400 nm (Figure 3).

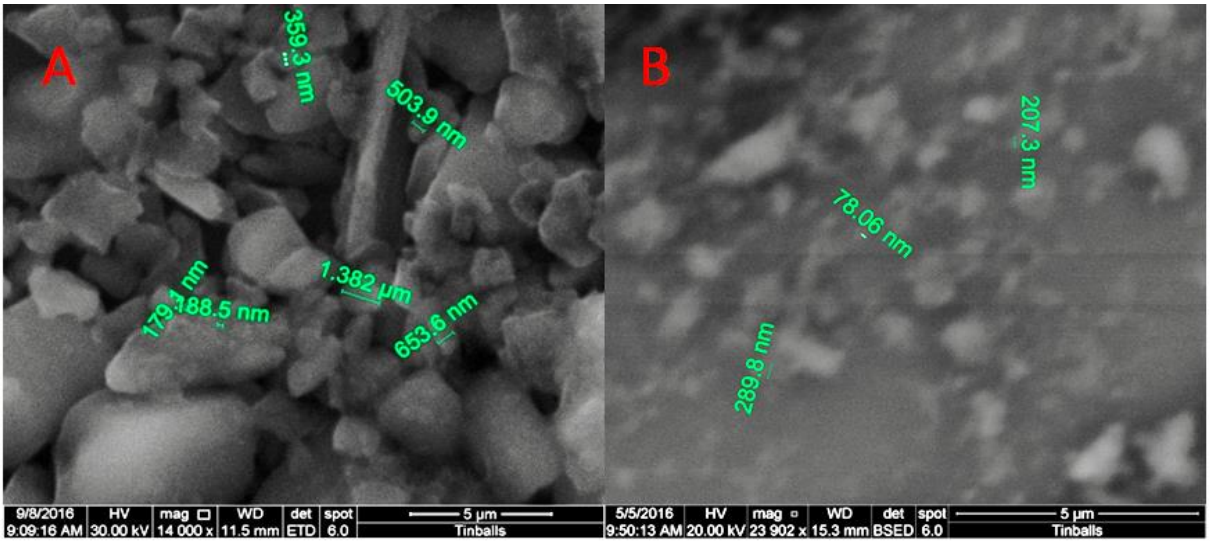

Figure 2: Characterization of test solutions by scanning electron microscope (SEM).(A)ASEE, and (B) ASEE-AgNPs.

FTIR was used to detect the functional groups in the ASEE and ASEE-AgNPs (Table 1). The ASEE extract showed bands (3600-3200 cm ^{1}) due to alcohol. It was also observed that phenol, 2939.61 cm^{-1} due to (C-H), alkanes 1651.12 cm^{-1} because (-C=C) alkynes, 1420.51 cm^{-1} due to (α -CH2) binding, 1300.07 cm^{-1} due to (S=O) sulfoxone, 1220.98 cm^{-1} due to ester carbonyl, 1070.53 cm^{-1} due to C=S thiocarbonyl, 918.22 cm^{-1} due to S-OR esters, 628.22 cm^{-1} due to C-H, 675.11 cm^{-1} due to bend = C-H alkenes, 524.00-470.65 cm^{-1} due to S-S disulfide (Table 1)..The outcome of the ASEE-AgNPs is presented both without and with bands respectively, with peaks at 1061.24, 819.77, and 777.34 due to the deletion of the silver ions.

The FTIR assay is one of the specific laboratory tests that used to identify the chemical elements in the compounds under study, in which the identification of the chemical compounds is determined by how the chemical bonds between the compounds absorb infrared radiation, as each component absorbs differently.[23] The spectral scanning of the samples was performed within the range (600-4000) cm^{-1}. The Ag^0 ions reduction to AgNPs is probably as a result of the presence of the thiocarbonyl and esters group. As seen Table1. The substantial amounts of phytochemicals found in the ASEE, as shown by the FTIR analysis, might be accountable for the biological activities that were detected in the extract.

The DPPH radical scavenging capabilities of the ASEE and ASEE-AgNPs at increasing concentrations (50, 75, and 100 µg/ml) are shown in Figures 4 and 5, respectively. Each test solution demonstrated excellent DPPH radical scavenging capabilities, though their effects were lower than that of ascorbic acid. The antioxidant activity of the ASEE, and ASEE-AgNPs dropped with a decrease in concentration. The test solutions demonstrated high antioxidant activity, eliminating about 77.67% of the ASEE extract and 85.87% of the ASEE-AgNPs. In a separate experiment, it was discovered that total phenol and flavonoid levels, in addition to DPPH radical scavenging capacity, have a positive correlation with one another. This discovery was consistent with what has been discussed and reported in the past.[13,14] It was found that this studied plant contains many effective chemical compounds modules of *A. salivum* obtained by FTIR (tannin, flavonoids, and phenols), antioxidant action was sought. The antioxidant action observed in the ASEE extract could be linked to the high phytochemical content such as tannin, flavonoids, and phenols obtained by the FTIR analysis.

Table 1 and Figure 6 showed the antibacterial effect of ASEE extract and ASEE-AgNPs on the growth of the test bacteria. To suppress the growth of *S. aureus* and *E. coli*, the ethanolic extract of *A. sativum* (ASEE) produced zones of inhibition measuring 10 and 9 mm, respectively. Meanwhile, the AgNPs showed the greatest inhibition zones of 17 mm against *S. aureus* and 19 mm against *E. coli*. The phytochemical components of the plant, which include saponins, anthraquinones, tannins, paleobotanies, alkaloids, and flavonoids, are responsible for the plant's antibacterial activities.

Nanomaterials exhibited effective antibacterial activity against the test microorganisms. The biological activity of AgNPs may be connected to the strong affinity of Ag^+ for thiols, which disrupts enzyme functions required for nutrition intake and cellular energy production/storage, ultimately killing the bacterium.[15,16,20] The majority of AgNPs obtained from medicinal plants have been shown to have strong antibacterial activities, in particular drug-resistant human pathogens and microorganisms that cause food deterioration.[17,18] This discovery lends credence to findings from earlier studies indicating that the majority of plant extract-AgNPs showed enhanced antibacterial activity throughout a broad spectrum.[19] This shows that NPs derived from *A. sativum* extracts may be effective antibacterial agents. Environmentally friendly nanoparticles' antimicrobial effectiveness has been connected to numerous characteristics, including their small size and surface area.

They make it possible to connect with the cells of bacteria through increasing membrane permeability and cell death in bacteria and fungus, leading to cell death and alterations in cell membrane permeability. In addition, because AgNPs attach themselves to the outer membrane of cells, bacteria are able to be penetrated by them, which disrupts the function of the cells.

Table 1: The functional groups present in the FTIR analysis for ASEE, and ASEE-AgNPs

Bond type	Functional group	Wavenumber (cm^{-1})
O–H	alcohol	3600-3200 cm ^{1}
C–H	phenol	2939.61 cm^{-1}
(-C=C)	alkanes,	1651.12 cm^{-1}
(S=O)	sulfoxone	1300.07 cm^{-1}
(α -CH2)		1420.51 cm^{-1}
	ester carbonyl	1220.98 cm^{-1}
C=S	thiocarbonyl	1070.53 cm^{-1}
S-OR	esters	918.22 cm^{-1}
C-H	C-Istretching	524.22 cm^{-1}
C-H	alkenes	675.11cm^{-1}
S-S disulfide	S-S disulfide	524.00-470.65cm^{-1}

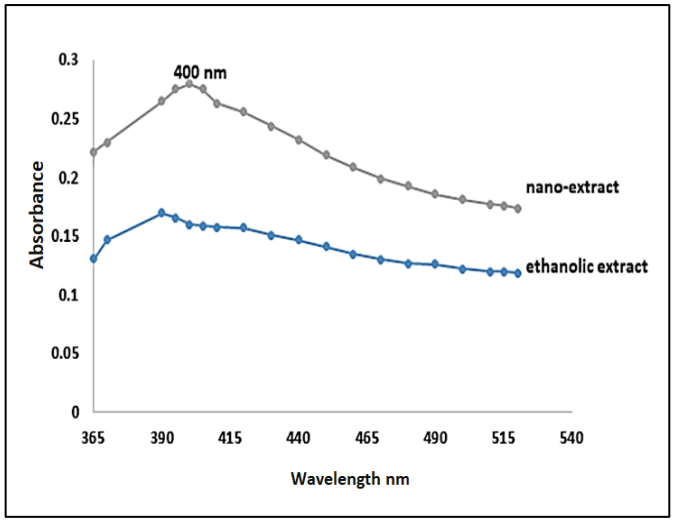

Figure 3: Spectrum peak of ASEE, and ASEE-AgNPs.

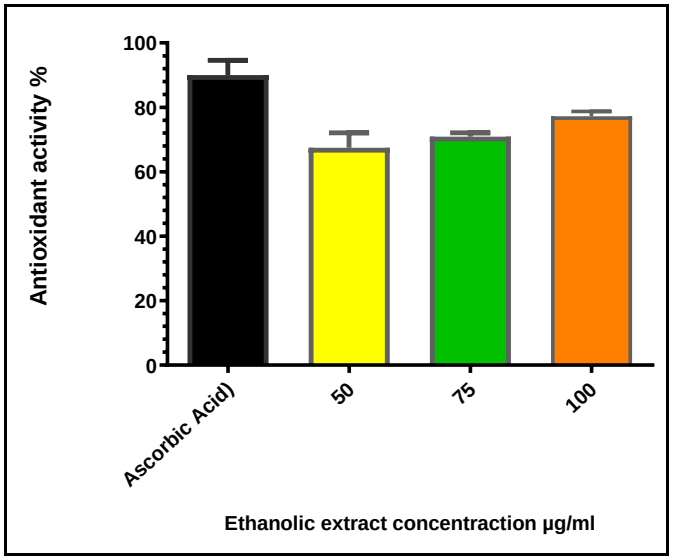

Figure 4: Antioxidant activity of ASEE.

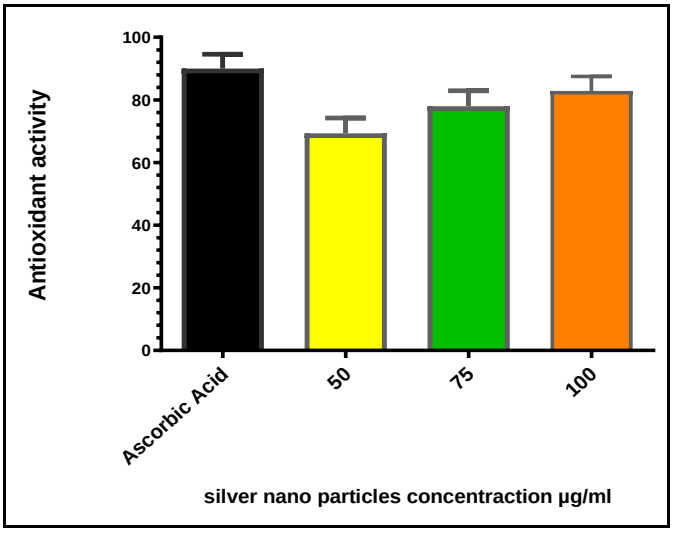

Figure 5: Antioxidant activity of ASEE-AgNPs

The mechanism of action involves a number of different processes, including the exchange of AgNPs with amino acids and enzymes, interaction with amino acids (especially to the -SH group), and formation of reactive oxygen species (ROS).[5,18] There are many studies to find alternative drugs to kill pathogenic bacteria or inhibit some of virulence factors. [20-24]

The antibacterial activity could also be linked to the fact that cells have largely sulfur and phosphorous, which are smooth sources, and DNA is mostly sulfur and phosphorous. AgNPs can act on these sensitive sources and destroy DNA, causing cell death. [25-26.]

In light of the information that has been presented thus far, determining whether or not Nanoparticles are effective against a diverse collection of pathogenic bacteria, viruses, and parasites was an absolute necessity. Recommended applied molecular technique such as PCR to get accurate results related to alternative antibiotics against pathogens. However, PCR was employed in different areas. [27-45]

Conclusion

The findings of this study revealed that *Allium sativum* extract can be used to successfully synthesize AgNPs and improve their efficiency and stability. The biologically synthesized AgNPs have significant antibacterial and antioxidant properties. Due to these properties, AgNPs are effectively employed in the field of medicine to prevent infectious and non-infectious diseases.

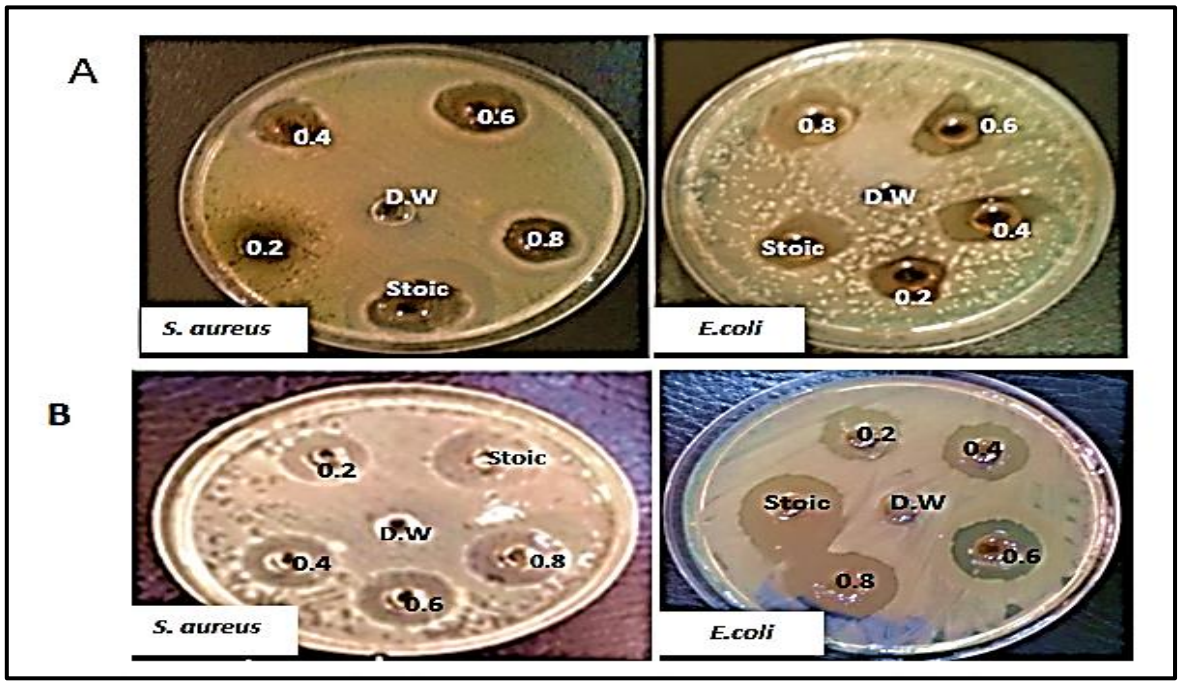

Figure 6: Antibacterial activity of (A) ASEE, and (B) ASEE-AgNPs.

Table 2: Antibacterial activity of *Allium sativum* ethanolic extract and *Allium sativum*-AgNPs

Microorganisms	**Ethanolic extract of *Allium sativum* zone (mm)**					
	Control	**0.2 µg/ml**	**0.4 µg/ml**	**0.6 µg/ml**	**0.8 µg/ml**	**1µg/ml**
S.aures	5 mm	7 mm	8 mm	9 mm	10 mm	10 mm
E.coli	5 mm	6 mm	7 mm	8 mm	8 mm	9 mm
Microorganisms	***Allium sativum* - AgNPs zone (mm)**					
	Control	**0.2 µg/ml**	**0.4 µg/ml**	**0.6 µg/ml**	**0.8 µg/ml**	**1µg/ml**
S.aures	5 mm	10 mm	11 mm	13 mm	15 mm	17 mm
E.coli	5 mm	9 mm	10 mm	12 mm	17 mm	19 mm

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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