Antimicrobial and Antibiofilm Potency of Green Silver Nanoparticles Synthesized by Aqueous Garlic Extract Against Microbes Causing Otitis Media

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Abstract

Aim of study: Otitis media (OM) is highly prevalent worldwide and is the main cause of hearing loss in developing countries. Resistant microorganisms and biofilm formation have become a major health and economic problem. The aim of this study was to establish an eco-friendly method for synthesis of silver nanoparticles using aqueous garlic extract and sunlight. Moreover, detect its potential to inhibit otitis media microbes.

Methods: Basically, highly resistant and biofilm forming otitis media pathogenic isolates were determined by Kirby-Bauer disc diffusion method and their biofilm production qualitatively was tested. Then, compared their susceptibility to tetracyclin 30µg (for bacteria) and nystatin 100 µg (for fungi) alone and with different concentrations of green synthesized silver nanoparticles (AgNPs), as well as, their biofilm production. AgNPs were prepared by easy, fast, non-toxic, economically eco-friendly way, using the direct sunlight irradiation on aqueous garlic extract. Their characterization by UV- visible spectral analysis, TEM, DLS, XRD and FTIR were studied.

Results: Two multidrug resistant bacterial isolates; *Pseudomonas aeruginosa* and *Bacillus cereus* were detected as biofilm producers with black colonies on congo-red agar and three resistant fungal isolates to nystatin; *Penicillium chrysogenum*, *Aspergillus niger* and *Aspergillus flavus* were tested against green synthesized AgNPs. Highly significant ($p\text{-value} < 0.001$) antimicrobial and antibiofilm activity of biosynthesized AgNPs against tested pathogens were illustrated in this study. TEM images for *Pseudomonas aeruginosa* and *Aspergillus flavus* after treated with AgNPs, were exhibited shrinkage in the cytoplasmic materials, rupture of cell walls, precipitation of AgNPs particles inside the cells and biofilm was mostly disseminated.

Conclusion: aqueous garlic extract that used to stabilize the AgNPs may be ideal natural candidates for future studies exploring their safely use in biomedical applications especially against the repeated infections caused by multi drug resistant (MDR) and biofilm forming microbials as in otitis media.

Introduction

Otitis media (OM) resulted from combination of different pus-forming microorganisms (viruses, bacteria and fungi), coming from the nose and nasopharynx by way of the eustachian tube and aggregate at the mucous membrane lining the middle ear cleft leading to inflammation in this membrane and effusion of fluids into the middle ear due to infection (1). The extraordinary occurrence of OM in developing countries population but with high prevalence in children (second most common infection in children less than 2 years) (2) due to immaturities in their immune system (3). Chronic otitis media affect language development. In severe cases, it may lead to hearing loss (2). Moreover, it is the most common reason for antibiotic and antifungal consumption because it is well-known reappearance infection and the antibiotics enduring to be the basis for treating OM with its complications (4-6).

Unregulated and unnecessary use of antibiotics resulted in a constant decrease in their effectiveness, leading to the development of multi-drug-resistant (MDR) bacterial strains. For that reason, it is essential to search for alternative healthcare approaches to diminish the problem of bacterial infections and contaminations (7).

Several antimicrobial agents such as amoxicillin, cefotaxime, cefoxitin, tobramycin, gentamicin, tetracycline, chloramphenicol, ciprofloxacin, ofloxacin, ampicillin and erythromycin are mainly prescribed for the treatment of bacterial otitis media (8-11). Prevention of the OM is very important as the WHO has reported that hearing impairment in 42 million people (above 3 years) in the world was mainly caused by OM (2, 12).

Garlic (*Allium sativum*) is a very popular and famous plant from ancients till days all over the world. In addition, aqueous garlic extract (AGE) offers extra benefits of fabrications of highly stable, biocompatible and spherical nanoparticles with proteins and amino acids as a natural capping and stabilizing agents (13).

Silver nanoparticles (AgNPs) have global attention due to their unique properties and their numerous applications especially in biomedical such as antimicrobial ointment, medical diagnostics, biological sensing, biological control, cancer therapy, drug delivery...etc (14).

AgNPs have low toxicity to human cells, effective broad-spectrum activity against bacteria, and a lower probability to cause microorganisms resistance than conventional antibiotics (15). The generation of new efficient antimicrobial resulted from known week antibiotics with a very low concentration of AgNPs offers an advanced perspective in the medical field. However, it diminishes the long-stay costs and the side-effects of antimicrobial therapy and surgery (6).

Synthesis AgNPs by chemical and physical routs have drawbacks making them unsuitable for biomedical applications. Meanwhile, green synthesis of AgNPs (using plant, enzymes or microorganism) is rapid, ease in size control, energy-efficient, facile non-toxic, environmentally conscious and friendly. In addition, to these benefits, use of plant extract for AgNPs synthesis offers a wide range of benefits over other biological synthesis methods because it is available, does not require the maintenance of cell cultures, safe in handling, and incorporates support for the large-scale synthesis of nanoparticles, presence of many metabolites act as reducing or capping/stabilizing agents (13, 16).

This study aims to synthesize the AgNPs by easy, fast and economically eco-friendly route via the aqueous extract of garlic cloves and use a very small amount of resulted AgNPs to study their influence on the susceptibility of pathogenic microorganisms isolated from OM.

Materials And Methods

Collection of Samples

All the isolates were obtained from the culture collection at Drug Microbiology Lab, Drug Radiation Research Department at (NCRRT), Cairo, Egypt. Garlic (*Allium sativum*) were purchased from local markets in Cairo, Egypt.

All the bacterial isolates were re-cultured on MacConkey and nutrient agar media. Then incubated at 37°C for 24 h. Their antimicrobial susceptibility were tested by the standard Kirby-Bauer disc diffusion method (17).

Pure colony from test organism was picked up with a sterile loop, suspended in nutrient broth and incubated at 37 °C for 2 hours. The turbidity of the suspension was adjusted to 0.5 McFarland's standard. After that, 100 µl of broth were spread on the surface of nutrient agar. Nine antibiotics with different modes of actions were chosen to perform this test; amoxicillin (25 µg), cefotaxime (30 µg), cefoxitin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), ofloxacin (5 µg), tobramycin (10 µg) and tetracycline (30 µg). The inhibition zones were measured and interpreted according to National Committee for Clinical Laboratory Standards (NCCLS).

Fungal isolates were cultured on Sabouraud dextrose broth (SDB) at 22°C - 25°C for 24 hours. Test fungi in SDB were enumerated by using serial dilution method. Final cell concentration of culture was 10⁴- 10⁵ cfu/ml.

Sterile Sabouraud dextrose Agar (SDA) at 43-45°C was prepared and poured into the Petri plates. Then the agar was allowed to solidify. 0.2 ml of fungal culture inoculum applied on each plate. Inoculum was evenly spread on agar using a glass L- rod spreader. The Petri dishes were left for one hour to allow agar surface to dry.

Antimicrobial discs were applied to the surface of the inoculated agar plate with sterile forceps and gently pressed down onto agar surface. The plates were incubated for 3-5 days at room temperature (22-25°C). The sizes of the zones of inhibition interpreted by referring to standardized chart.

Detection of biofilm production for highest resistant bacteria using Congo-red detection method

(18) described a simple qualitative method to detect biofilm production by using congo red agar (CRA) medium. Thirty-seven grams of brain heart infusion broth powder, 50g of sucrose and 15g of agar was dissolved in one liter of distilled water then autoclaved at 121°C for 15 minutes for sterilization. Eight grams of Congo red indicator powder was dissolved in one liter of distilled water then autoclaved at 121°C for 15 minutes. Then it was added to the autoclaved brain heart infusion agar with sucrose at 55°C. plates were inoculated and incubated aerobically at 37°C for 24 hours.

Isolates that produced black colonies with dry crystalline consistency were regarded as biofilm positive, while those showing pink colonies were biofilm negative. The experiment was performed in triplicate.

Green synthesis of silver nanoparticles using aqueous garlic (*Allium sativum*) extract based on sunlight irradiation (19)

For the reduction of silver ions, 25 ml of aqueous garlic extract (AGE) was added to 475 ml of 0.1 M AgNO₃ (8.5g AgNO₃ + 475ml deionized water) to give a stock solution of colloidal silver nanoparticles (AgNPs) with concentration of 100µg/ml. This reaction mixture was stirred properly and exposed to bright sunlight. Within minutes of exposure to the light the colorless solution started to change to yellow brown color indicating the formation of silver colloid. The color intensity increased with increasing time until reached after 15 minutes (Figure 1).

Characterization of green synthesized AgNPs:

1. UV- visible spectral analysis:

Characterization of AgNPs was performed by UV-Visible spectrophotometer (JASCO V-560. UV-Vis. Spectrophotometer from 200-900 nm at a resolution of 1.0 nm using the control (negative) for autozero support.

1. Transmission Electron Microscope (TEM):

The morphology (shape of nanoparticles) and size of AgNPs were notified by using TEM image (JEOL electron microscopy JEM-100 CX). Drop coating AgNPs produced TEM examinations onto carbon-coated TEM grids after drying by incubation at 37.0 ±2°C in an incubator.

1. Dynamic Light Scattering (DLS):

Dynamic Light Scattering (DLS-PSS-NICOMP™ 380-ZLS particles sized system St. Barbara, California, USA) estimated average particle size of an incorporated AgNPs and size distribution. 200µl of AgNPs carried to a disposable little cuvette. Following equilibration to a temperature of 25.0 ±2°C for 2.0 min., five measures were implemented.

1. X-Ray Diffraction (XRD):

X-Ray Diffraction analysis was adjusted with the XRD-6000 lists, including outstanding austenite quantitation, crystallinity estimation, stress examination, and crystallite size/lattice strain matters. The investigation of extended X-ray diffraction models (Shimadzu apparatus) was employed Cu-Kα target, and nickel filter (Shimadzu Scientific Instruments; SSI, Japan). Working by a Cu anode at 50.0 mA and 40.0 Kv in the state of 2θ value inside 20° and 100° with a flow of 2°/min.; the intensity of the diffracted X- rays estimated as a function of the diffracted angle 2θ (20).

1. Fourier Transform Infra-Red Spectroscopy (FTIR):

FT-IR investigation was a helpful method that provides information regarding chemical functional groups remaining in the AGE. The measures were carried out following a JASCO FT-IR 3600 Infrared spectrometer by working KBr Pellet purpose. It was recorded at a resolution of 4.0cm⁻¹ in a wave number range of 400- 4000 cm⁻¹.

Antimicrobial activity of green synthesized AgNPs compared with tetracycline, nystatin and AGE against selected isolates.

Two fold serial dilutions of colloidal AgNPs were prepared from stock concentration (100µg/ml) using deionized water. Fresh bacterial suspension were adjusted to 0.5 McFarland's standard and swabbed over MHA. Sterilized Whattman no.1 filter papers, 6 mm in diameter were loaded with 10 µl of (100, 50, 25, 12.5 and 6.25 µg/ml) of synthesized colloidal AgNPs, 500mg/ml of aqueous garlic extract (AGE) also was loaded. In addition, disc of tetracycline 30 µg (TE) were used for bacterial isolates and nystatin 100 µg/m for fungal isolates to compare their influence on the sensitivity of the tested isolates. All plates were incubated at 37°C for 24 hours, followed by the measurement of the diameters of the inhibition zones.

Morphology and biofilm production of bacterial and fungal isolates before and after treatment using (TEM):

In order to obtain an electron micrograph of a bacterial and fungal specimen, the later must be subjected to a series steps, including fixation, dehydration embedding, sectioning and staining. Ultrathin sections were picked up on carbon grids and viewed on (JEOL-JEM 1010) Transmission Electron Microscope, Japan. Located at the Regional Centre for Mycology and Biotechnology- Al-Azhar University, Cairo, Egypt.

Statistical analysis

Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage. Different statistical steps were done. First, ANOVA used when comparing between more than two means. Then, LSD was used for multiple comparisons between different variables. Finally, the confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered highly significant (≤ 0.001), significant (≤ 0.05) and insignificant (> 0.05).

Results

Susceptibility test

Disc diffusion test was performed for 80 bacterial and fungal isolates from otitis media. The percent were 59% and 41%, respectively. Out of them, six types of bacteria and three fungal isolates were selected as the highly resistant isolates. Bacteria were tested against 9 antibiotics and fungi (*Penicillium chrysogenum*, *Aspergillus niger* and *Aspergillus flavus*) were considered as resistant if inhibition zone around nystatin disc is less than 13 mm.

Biofilm (slime) production by Congo red agar

Biofilm production in bacterial pathogens were determined qualitatively by Congo red agar method (CRA). Only 2 bacterial isolates (*Bacillus cereus* and *Pseudomonas aeruginosa*) gave black colored colonies on CRA as a positive indication for slime production in these isolates (Figure 2).

Synthesis and Characterization of silver nanoparticles (AgNPs)

Synthesis of AgNPs were confirmed initially by changing the colorless suspension of AgNO₃ with AGE into brown after exposed to irradiated sunlight (Figure 1).

The appearance of the brown color and a sharp absorption band at 415 nm indicated the occurrence of the reaction and the formation of AgNPs. The UV/Vis spectra recorded as a function of reaction time of an aqueous garlic extract (AGE) with the silver nitrate in the presence of sunlight. Additionally, another small peak has been observed at 280 nm due to the presence of proteins in the garlic extract as shown in (Figure 3).

TEM image of synthesized AgNPs revealed the presence of polydispersed and spherical AgNPs of varying sizes in nanoscale from 3.02 to 10.89 nm with the average mean diameter of 7.33 nm as displayed in (Figure 4).

Average particle size was determined by Dynamic light scattering (DLS) method and representative size distribution graph is shown in (Figure 5), where the mean was defined as 21.72 nm.

XRD pattern to the AgNPs was presented in (Figure 6); many peaks were observed, those for AgNPs. Diffraction characteristics are showing within 2 θ (degree) as 38.13°, 44.21°, 64.47°, and 77.37° where these peaks describe the Bragg's reflections (111), (200), (220) and (311) planes in that order respectively, and these data revealed crystalline nature of AgNPs.

Fourier transform infra-red spectroscopy (FTIR) spectra were used to characterize the functional groups and the molecular interaction between the synthesized AgNPs and AGE. As shown in (Figure 7) and (Table 1), broad peak at 3320.449 cm⁻¹ assigned to O-H stretching of hydroxyl group and the peak at 2567.35 cm⁻¹ corresponds to asymmetric stretching of C-H bonds. The band at 1637.38 cm⁻¹ assigned to carbonyl and carboxylic (C=O) stretching bands of peptide linkages (stretching of amides). Peak found at 1435.84 cm⁻¹ corresponds to O-H of carboxylates. Another band at 1034.87 cm⁻¹ corresponds to SO₂ absorption of sulfones and C-N stretching vibrations of primary amines. Finally, a new peak at 983.06 cm⁻¹ was found in FT-IR spectrum of AgNPs only, which may be due to conjugation of AgNPs with –OH groups as Ag–O.

Antimicrobial activity of different concentrations of colloidal AgNPs compared with tetracycline and AGE on sensitivity of biofilm forming bacteria.

Antimicrobial activity of AgNPs were tested against two biofilm forming highly resistant bacteria (*Bacillus cereus* and *Pseudomonas aeruginosa*) in comparison with AGE and tetracycline as control where bacteria were resistant to it. The different concentrations of AgNPs had an increased inhibition zones than AGE

and tetracycline, this was considered a statistically highly significant difference where p -value<0.001 as shown in (Figure 8).

Transmission electron microscopy (TEM) for ultrastructure integrity of biofilm forming bacteria (*Pseudomonas aeruginosa*):

The isolated *Pseudomonas aeruginosa*, which is considered as a highly pathogenic bacteria, was treated with AgNPs.

The ultrastructure study showed the cells of the untreated *Pseudomonas aeruginosa*, where the protoplast can be differentiated into a cytoplasmic region, which is rich in ribonucleic acid, distributed throughout the whole cytoplasm, and chromatic region contain some ribonucleic acid of the cell. The cell wall looks rigid, intact and retaining its structural integrity. The robust biofilm was clearly illustrated as shown in Figure 9 (A) with different magnification power. Meanwhile, Figure 9 (B) illustrated the same isolate treated with colloidal AgNPs. Intensive corrugation of the surface layers accompanied by shrinkage in the cytoplasmic materials and rupture of cell walls were occurred. Other cells showed complete loss of nuclear and cytoplasmic materials and precipitation of AgNPs inside the cells. Biofilm was mostly disseminated as shown.

Antimicrobial activity of different concentrations of colloidal AgNPs compared with nystatin and AGE on sensitivity of resistant fungal isolates.

Antimicrobial activity of AgNPs were tested against three resistant fungal isolates (*Aspergillus niger*, *Aspergillus flavus* and *Penicillium chrysogenum*) in comparison with AGE and nystatin as a control. The results showed that the colloidal AgNPs at higher concentrations (100, 50 and 25 µg/ml) had an increased inhibition zones than AGE and nystatin, this was considered a statistically highly significant p -value<0.001 as shown in (Figure 10).

Transmission electron microscopy (TEM) for ultrastructure integrity of fungal isolate (*Aspergillus flavus*):

The isolated *Aspergillus flavus*, was treated with AgNPs. The ultrastructure study showed the cells of the untreated *Aspergillus flavus* where micrograph exhibits normal / untreated hypha with typical cell wall, cell membrane and organelles as shown in figure 11(A).

TEM results of *Aspergillus flavus* treated with AgNPs are shown in the same figure (B). Severe damage and disappearance of cell wall and cell membrane can be observed, empty spaces found inside the cells due to degradation of the organelles. Also, observed fragmentation of mitochondria and shrinkage of cell membrane.

Discussion

Otitis media is highly prevalent worldwide and in many cases it may degrade the quality of life (2). Therefore, it is important to catch associated pathogens and find a safe antimicrobial and antibiofilm

treatment. Bacteria cause over 50% of the otitis media cases. Occasionally, fungi or other pathogens such as viruses may cause it (21, 22). Formation of biofilm accelerates bacterial infections and diminish the efficacy of anti-microbial therapy. Leading to prolonged or inappropriate antibiotic use and causing emergence of antibiotic resistance which is considered as a big therapeutic problem(11).

In this study, *Pseudomonas aeruginosa* and *Bacillus cereus* out of 12 multidrug resistant isolates were biofilm forming bacteria. Other study in Egypt (10) found that *Pseudomonas aeruginosa* was predominant isolate from chronic suppurative otitis media Moreover, in nearby developing country as Nigeria, (23) reported that *Pseudomonas aeruginosa* was the most commonly isolated bacteria from otitis media and was biofilm forming bacteria followed by *S. aureus*.

Many efforts have been put to develop environment friendly protocols for the production of metal nanoparticles. In work done by (19), they had exploited aqueous garlic extract and sunlight irradiation for facile and fast synthesis of silver nanoparticles. Das *et al.*, (24) reported that sunlight and biosurfactant plays an important role as a catalyst in the synthesis of AgNPs.

UV-visible spectroscopy is preliminary technique for the characterization of AgNPs. It is an indirect method generally used to examine the bio-reduction of AgNPs from aqueous AgNO₃ solution (25). The reduction of AgNO₃ to AgNPs on addition of AGE was confirmed by observing color change, due to the reduction of Ag⁺ to Ag⁰ due to the presence of various types of biomolecules in the AGE. These results corroborate the findings of (19) for synthesis of AgNPs. Saranyaadevi *et al.* (26) reported that when the plant extract was added into the AgNO₃ solution, the pale yellow color was obtained. After 20 minutes, the color changes to dark reddish brown. The other peak at 367 nm ..represent the AGE. As reported by (27) that the same peaks at 320 nm was for Magnolia leaves water extract in sterile distilled water.

The results of TEM examination of biosynthesized AgNPs were in harmony with (19) who reported the presence of polydispersed and spherical nanoparticles of varying sizes.

It was observed that the particle size obtained from DLS measurement was obviously larger than TEM results, because DLS measures the hydrodynamic radius which take into consideration the native protein on the surface of AgNPs as well.

The XRD outline obviously displayed that the silver nanoparticles formed by the reduction of Ag⁺ ions by AGE are crystal-like in nature and these results are consistent with (20). The presence of structural peaks in XRD patterns and average crystalline size clearly illustrates that the bio-synthesized AgNPs were nanocrystalline in nature.

The FT-IR results were in consistence with (19) who explained the bio-functional group interaction between the AGE and AgNPs. Von White *et al.*, (28) found that, primary non-structural sugars extracted from garlic function as both reducing and stabilizing agents.

These infectious diseases have not only occurred in developing countries with low levels of hygiene and sanitation, but have also been recognized in developed and undeveloped countries (2, 29).

Silver has a greater affinity to react with sulfur or phosphorus-containing biomolecules in the cell; therefore, sulfur-containing proteins in the membrane or inside cells and phosphorus containing elements like DNA are likely to be preferential sites for binding AgNPs (29).

In this study, the results showed that colloidal AgNPs had an antimicrobial activity against biofilm forming bacteria. The different concentrations of colloidal silver nanoparticles had an increased inhibition zones when compared with antibiotics and aqueous garlic extract. These results were in concordance with (19) who found that the zones of inhibition increased when increasing concentration of AgNPs. In addition, the results showed similarity with (24) who reported that antimicrobial activity increase with increase in AgNPs concentration. It had been observed by (30) that the AgNPs showed high antibacterial activity against Gram-positive and Gram-negative bacteria. This activity may be due to the size and the large surface area of these AgNPs, which facilitate reaching easily to the nuclear content of bacteria.

The bactericidal activity was attributed to the silver cations escaped from AgNPs causing changes in the membrane structure of bacteria, which in turn led to increasing membrane permeability of the bacteria and lastly cell death (30)

In the present study, colloidal AgNPs at higher concentrations had an increased inhibition zones against the tested fungal isolates than nystatin and AGE. These results were in harmony with (31) who found that photosynthesized AgNPs exhibited higher antifungal activity.

The ultrastructure study using the transmission electron microscope was carried out for the microbial isolate *Pseudomonas aeruginosa* treated with aqueous garlic extract (AGE). The results in this study were in consistence with (32), who found that exposure to organo-sulfur compounds extracted from garlic resulted in morphological damage, such as loss of the structural integrity of the cell wall, cell membrane and intracellular matrix. Also cell deformation, breakage of cell walls and membranes, condensation of cellular material, and the presence of significant amounts of cytoplasmic material and membrane fragments were observed in the damaged cells of the bacteria.

The results of *Pseudomonas aeruginosa* treated with AgNPs were in conformity with (33), who reported that AgNPs are observed to be located in the membrane of the bacteria. The changes in morphology presented in the membrane of the bacteria, as well as the possible damage caused by the nanoparticles reacting with the DNA, will affect the bacteria in processes such as the respiratory chain and cell division, finally causing cell death.

The results of TEM analysis on *Aspergillus flavus* treated with AgNPs proved the ability of it to damage the cell wall, cell membrane and the cytoplasmic components, causing shrinkage and disruption. These results matches with (34) who found that AgNPs damaged the fungal cell wall and cell membrane, penetrated inside the cells, damaged the organelles including mitochondria and ribosome and caused condensation and margination of chromatin, a marker of apoptic cell death.

Several studies have reported that the electrostatic attraction between the negatively charged cell membrane of microorganisms, including bacteria, viruses and fungi; and the positively charged nanoparticles is crucial for the antimicrobial mode of these particles (35).

Conclusions

Changes in OM microbial flora and biofilm formation lead to antibiotic weakness and occurrence of multi-drug resistant pathogens. Routine sensitivity screening for OM isolates must be done with qualitative test of biofilm formation. Find an alternative / or enhance already exist antimicrobial/ antibiofilm is necessary for public health. In this study fast, cost effective and ecofriendly green synthesized silver nanoparticles by using aqueous garlic extract (AGE) and sunlight have been demonstrated. The biosynthesized AgNPs have shown extremely good *in vitro* antimicrobial and antibiofilm activities. The AGE-stabilized nanoparticles may be ideal candidates for future studies exploring their use in biomedical applications.

Declarations

- Ethics approval and consent to participate:

Not applicable.

- Consent for publication:

Not applicable.

- Availability of data and material:

Please contact author for data requests.

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- Authors' contributions:

EM performed collection of samples, detection of biofilm production for highest resistant bacteria using Congo-red detection method and synthesis of silver nanoparticles.

AH participated in the design of the study.

HE conceived of the study, and participated in its design and coordination. In addition, wrote the main manuscript.

HN Assist in research work and helped to draft the manuscript.

RK performed the statistical analysis.

All authors read and approved the final manuscript.

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- Competing interests:

The authors declare that they have no competing interests.

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Tables

Table 1. FTIR spectrum of AGE and AgNPs synthesized by AGE and sunlight, and its corresponding assignment.

AGE	AGE-AgNPs	Assignment
3299.25	3320.49	Stretching vibrations of hydroxyl group (O-H bond).
2388.61	2567.35	CH ₂ asymmetric stretching.
1638.26	1637.38	Assigned to carbonyl and carboxylic (C=O) Stretching bands of peptide linkages.
1447.09	1435.8	Corresponding to O-H of carboxylate.
1369.37	1366.56	C-H bending of CH ₂ or OH bending.
1131.08	1034.87	Attributed to SO ₂ absorption of sulfones and C-N stretching vibrations of primary amines.
□□□□□□	983.06	New peak, which may be due to the conjugation of AgNPs with -OH group as Ag-O.

AGE = Aqueous garlic extract. AGE-AgNPs = Aqueous garlic extract with silver nanoparticles.

Figures

Figure 1. Green synthesis of silver nanoparticles (AgNPs). AgNO₃ suspension and aqueous garlic extract (AGE) were exposed to sunlight irradiation. Changing in color is clearly observed after 15 minutes. Indicating the presence of colloidal AgNPs.

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Figure 2. A- Formation of black colored colonies on Congo red agar plate indicating positive biofilm (slime) production, B- pink colored colonies indicating inability to produce biofilm (slime).

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Figure 3. UV-Visible spectrum of AgNPs synthesized by irradiating 0.1M AgNO₃ in the presence of AGE under sunlight for 15 mins.

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Figure 4. TEM image and statistical analysis of synthesized AgNPs produced by fast, safe, economically eco-friend way using aqueous garlic extract and irradiated sunlight for 15 minutes

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Figure 5. Particle size distribution by DLS of AgNPs synthesized by AGE in sunlight.

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Figure 6. X-Ray Diffraction pattern of synthesized AgNPs.

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X-Ray Diffraction pattern of synthesized AgNPs.

Figure 7. FTIR spectra of AGE-AgNPs and AGE alone.

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FTIR spectra of AGE-AgNPs and AGE alone.

Figure 8. Antimicrobial activity of different concentrations of biosynthesized colloidal AgNPs (µg/ml) compared with both; tetracycline disc (30µg) and aqueous garlic extract (AGE) on sensitivity of biofilm forming bacteria; *Bacillus cereus* (A) and *Pseudomonas aeruginosa* (B).

**= highly significant (p-value < 0.001)

Figure 8

Antimicrobial activity of different concentrations of biosynthesized colloidal AgNPs (µg/ml) compared with both; tetracycline disc (30µg) and aqueous garlic extract (AGE) on sensitivity of biofilm forming bacteria; *Bacillus cereus* (A) and *Pseudomonas aeruginosa* (B). **= highly significant (p-value < 0.001)

Figure 9. TEM images of *Pseudomonas aeruginosa* (A) without any treatment, where the cells appear normal with intact cell wall, cytoplasmic components are normal without any disruption and robust biofilm. (B) treated with AgNPs, where the cells showed intensive corrugation of the surface layers, shrinkage in the cytoplasmic materials, rupture of cell walls, precipitation of AgNPs particles inside the cells and biofilm was mostly disseminated

Figure 9

TEM images of *Pseudomonas aeruginosa* (A) without any treatment, where the cells appear normal with intact cell wall, cytoplasmic components are normal without any disruption and robust biofilm. (B) treated with AgNPs, where the cells showed intensive corrugation of the surface layers, shrinkage in the cytoplasmic materials, rupture of cell walls, precipitation of AgNPs particles inside the cells and biofilm was mostly disseminated

Figure 10. Antimicrobial activity of different concentrations of biosynthesized colloidal AgNPs (µg/ml) compared with nystatin disc and (100 µg) and aqueous garlic extract (AGE) on sensitivity of of resistant fungal isolates; Penicillium chrysogenum (A), Aspergillus niger (B) and Aspergillus flavus (C).

**= highly significant (p-value < 0.001)

*= Significant (p-value < 0.05)

Figure 10

Antimicrobial activity of different concentrations of biosynthesized colloidal AgNPs (µg/ml) compared with nystatin disc and (100 µg) and aqueous garlic extract (AGE) on sensitivity of of resistant fungal isolates; *Penicillium chrysogenum* (A), *Aspergillus niger* (B) and *Aspergillus flavus* (C). **= highly significant (p-value < 0.001) *= Significant (p-value < 0.05)

Figure 11. TEM studies section of *Aspergillus flavus*, (A) a typical untreated hypha was with normal cell wall, cell membrane and organelles. (B) after treated with AgNPs. The cell wall was vague and ruptured. Shrinkage of cell membrane and degradation of organelles. Empty space inside the cell and fragmentation of mitochondria

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TEM studies section of *Aspergillus flavus*, (A) a typical untreated hypha was with normal cell wall, cell membrane and organelles. (B) after treated with AgNPs. The cell wall was vague and ruptured. Shrinkage of cell membrane and degradation of organelles. Empty space inside the cell and fragmentation of mitochondria