https://doi.org/10.48047/AFJBS.7.4.2025.1530-1540

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Synthesis and characterization of silver nanoparticles produced by *Origanum glandulosum* (Desf.) Ietsw. and their antibacterial activity

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Volume 7, Issue 4, Apr 2025

Received: 15 Feb 2025

Accepted: 05 Mar 2025

Published: 05 Apr 2025

doi:10.48047/AFJBS.7.4.2025.1530-1540

Abstract - The present study investigated the biosynthesis of silver nanoparticles (AgNPs) using the hydro-alcoholic extract of *Origanum glandulosum* (Desf.), and silver nitrate ($AgNO_3$) solution. The technical characterization of AgNPs has been achieved through UV-visible spectroscopy (UV-Vis), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and energy-dispersive spectrometry (EDS). Antimicrobial activity was performed by agar well diffusion method against both gram-positive and gram-negative bacteria. UV-Vis spectrum showed a peak centred at a wavelength of 462 nm. Employing the maximum magnification of our SEM, the synthesized AgNPs appeared to have a spherical shape, and showed an average of diameter size distribution that is around 62.36 nm. The EDS analysis showed that Silver (Ag) was present in these nanoparticles, which is among the clues suggesting the existence of AgNPs. The products synthesized in this process have demonstrated antibacterial activity against *Pseudomonas aeruginosa*, even though it is known to be resistant to many antibiotics. AgNPs suspension (2 mg/mL) demonstrated the most potent antibacterial effect on all tested bacterial strains than AgNPs suspension (1 mg/mL), except for *B. cereus* where both of them possess a slightly different diameter of inhibition zones.

Keywords: antibacterial activity, green synthesis, *Origanum glandulosum*, silver nanoparticles.

Introduction

Every year, a large number of deaths in the world are directly caused by antibiotic resistant infections, the replacement of a susceptible bacterial population by a resistant one is possible if the appropriate selective pressures for an antibiotic that has been prescribed are in place, this latter increases the need of a new sources of antimicrobial agents (Davey et al., 2015). The use of plant extracts for the synthesis of AgNPs, so-called green synthesis methods, is an interesting way to get AgNPs that exhibit antibacterial activity with low toxicity for eukaryotic cells

(Escárcega-González et al., 2018). For this reason, bioactive substances of the aromatic plant *Origanum glandulosum* that is an indigenous plant of western Algeria, especially very rich in essential oils and phenolic compounds, among which the flavonoid fraction is a major component (Basli et al., 2012), have been chosen for the synthesis of AgNPs. In addition to this, we preferred to use ethanol as a solvent instead of water for the extraction of polyphenols to have higher yields (López-fernández et al., 2020).

Plant extracts are not exclusive to traditional medicine, researchers have employed plant extracts for various purposes, for instance, as a green tool to prevent corrosion on steel, so that these substances are both cheap and environmentally safe (Bendahou et al., 2006), in Food Preservation (Adelakun et al., 2015), to provide an alternative treatment to antibiotic therapy (Benbelaïd et al., 2014 and Benbelaid, 2015), without forgetting that there is an increased interest over the past few years, in the use of plant extracts as a reducing and capping agent for the synthesis of nanoparticles that exhibits antibacterial properties (Das et al., 2020).

Materials and Methods

Plant materials

Aerial parts of the wild *O. glandulosum* plant (leaves, stems and flowers), were freshly harvested, from the region of Tlemcen, situated in the northwest of Algeria, botanical identification was carried out by the Laboratory of Ecology and Management of Natural Ecosystems, University of Tlemcen. The plant has been air dried, then placed in craft paper bag, and hidden away from light and moisture.

Extraction process

By means of a laboratory grinder, the plant was powdered into fine particles, 12 grams of this powder was dissolved in 50 mL of ethanol 70%, then the beaker containing the mixture was placed in the incubator shaker for 24 h at 37 °C, and 120 rpm. Afterwards, the extract was filtered and the filtration residue was subjected to a second extraction cycle under the same conditions as described above. After that the obtained filtrate was dried.

Using dry extract, a stock solution at a concentration of 7.2 mg/mL has been prepared with ethanol 70% as a solvent, then the solution was refrigerated at about 4 °C until further use.

AgNPs synthesis

Erlenmeyer flask was used to blend 10 mL of $AgNO_3$ 0.1 M and 5 mL stock solution of *O. glandulosum* extract, then the mixture has been covered with aluminium foil like protection against light to avoid silver nitrate's photo activation (Valli and Vaseeharan, 2012), and placed on a magnetic hotplate stirrer for 1 h at 75 °C, and 300 rpm, using magnetic stirring bar. When this experiment was over, the solution shifted from Yellowish to dark brown, which confirm the formation of AgNPs.

The resulting solution has been introduced in a 15 mL corning tube and centrifuged twice at 2700 rpm for 30 min, then the supernatant has been removed and the precipitate that contains the silver nanoparticles was dried. By using distilled water as a dispersive medium, various concentrations of AgNPs have been prepared for different analysis.

Technical characterization of AgNPs

UV-Vis absorption spectra of the Ag-NPs suspension of 0.25 mg/mL, was carried out at a wavelength range of 200-800 nm using a spectrophotometer (Hach Lange DR 5000, USA). The functional groups present in the *O. glandulosum* extract and the synthesized AgNPs were analysed by FT-IR Spectrometer (PerkinElmer spectrum two, USA), at the wavenumber region (4000 – 450 cm^{-1}), a solid sample has been crushed in the presence of KBr, then compressed to form a fine pellet. Samples for scanning electron microscopy (SEM) (ZEISS EVO 15, Germany) and energy-dispersive spectrometry (EDS) that is integrated in the SEM, were dispersed by the use of an ultrasonic cleaner (Model: WUC – D06H, DAIHAN Scientific, Korea), then analysed (suspension of 0.5 mg/mL) to determine the shape of the AgNPs, SEM images were used in order to measure the diameter of our nanoparticles and to estimate their size distribution, employing the image processing software ImageJ (National Institutes of Health, USA), the EDS was utilized to determine the elements present in the sample.

Bacterial strains

This study was performed with the following bacterial strains: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 43300), *Enterococcus faecalis* (ATCC 29212), and *Bacillus cereus* (ATCC 11778), which were obtained from laboratory veterinary regional of Tlemcen, Algeria, and they were conserved on a nutrient agar slant at 4°C.

Antibacterial activity of AgNPs

The antimicrobial activity was tested by agar well diffusion method against both gram-positive and gram-negative bacteria listed above, using two different concentrations of AgNPs (1; 2 mg/mL), and antibiotic discs of ofloxacin 5 µg as a positive control. In the current study several instructions cited by Matuschek et al. (2014) was followed with a modification, 90 mm diameter Petri dishes previously filled with Mueller-Hinton agar with a depth around 4.25 mm were used, wells with a diameter around 5.5 mm were applied onto the agar using Pasteur pipettes, and were filled with 60 µL of the test solution, the bacterial suspensions were prepared using sterile saline (0.85% NaCl w/v in water), then compared with the 0.5 McFarland turbidity standard, at the end of the test, the plates were incubated for 18 hours at 35 °C, after that the diameters of the zones of inhibition were measured.

Statistical analysis

LibreOffice Calc version 7.1, and GeoGebra Classique version 6.0.775.0 were used to perform statistical analysis, results are presented as means ± SD. Paired t-test was carried out using the RStudio software version 4.1.0.

Results

AgNPs physicochemical characterization

The UV-Vis spectrum displayed peak centred at a wavelength of 462 nm (Figure 1). The FTIR spectra of the synthesized AgNPs showed major absorption bands at: 3423, 2919, 1614, 1511, and 1384 cm^{-1}, while the extract of *O. glandulosum* displayed major absorption peaks at: 3400, 2928, 1606, and 1522 cm^{-1} (Figure 2). Using the maximum magnification (x5 000 000) of our SEM, the synthesized AgNPs appeared to have a spherical shape (Figure 3), and showed a diameter size distribution that is in the range of 33 to 106 nm with an average of 62.36 ± 12.33 nm, while the most of them have a diameter size that varies from 47 to 75 nm (Figure 4). The analysis of EDS revealed the presence of Ag and other elements like Silicon (Si), Oxygen (O) and Carbon (C) (Figure 5).

AgNPs antibacterial activity

AgNPs synthesized by the extract of *O. glandulosum* (Desf.) found to possess antibacterial properties, both of the concentrations tested of AgNPs demonstrated the highest antibacterial activity against *P. aeruginosa* (13.00 ± 0.41), (14.75 ± 0.65), and the lowest antimicrobial effects against *B. cereus* (9.75 ± 0.50), (9.75 ± 0.29) (Table 1).

Table 1. The bacterial activity of synthesized silver nanoparticle.

Bacterial strains	Diameter of inhibition zones (mm)		
	AgNPs 1 mg/mL	AgNPs 2 mg/mL	Ofloxacin 5 µg
Staphylococcus aureus	12.50 ± 0.57	13.00 ± 0.41	20.50 ± 0.58

Pseudomonas aeruginosa	13.00 ± 0.41	14.75 ± 0.65	16.25 ± 0.96
Enterococcus faecalis	9.88 ± 0.25	10.00 ± 0.41	0.00 ± 0.00
Bacillus cereus	9.75 ± 0.50	9.75 ± 0.29	11.00 ± 0.00
Escherichia coli	11.25 ± 0.29	11.88 ± 0.25	18.67 ± 0.29

Discussion

Physicochemical characterization of AgNPs

The peak obtained by the UV-Vis spectrum (Figure 1), might be corresponded to surface plasmon resonance (SPR) of silver nanoparticles (Logeswari et al., 2015). The characteristic peaks of the FTIR spectra (Figure 2), that were observed in both samples are as follows, a noticeable peaks at 3423 – 3400 cm^{-1} due to (O–H) stretch in alcohols, peaks at 2919 – 2928 cm^{-1} corresponding to the stretching of (C–H) in alkanes, peaks at 1614 – 1606 cm^{-1} due to (N–H) bending in amines, peaks at 1511 – 1522 cm^{-1} which correspond to (C=C) stretch in aromatic compounds, conversely the peak that was found only in the AgNPs sample was located at 1384 cm^{-1}, the latter can be attributed to (NO_2) stretch in nitro compounds.

The Ag is present in the synthesized nanoparticles as revealed by EDS analysis, which is among the indications suggesting that Ag-NPs exist (Figure 5). The presence of Si and O can correspond to the Silicon dioxide (SiO_2), because the substrate used for the SEM/EDS analysis is made up of glass, while C and O can be attributed to the organic molecules present within the ethanolic solution of *O. glandulosum* extract, that had been blended with $AgNO_3$ solution during the synthesis process of the AgNPs.

Antibacterial activity of AgNPs

The suspension of AgNPs 2 mg/mL showed the most effective antibacterial action against all of the bacterial strains tested than the suspension of AgNPs 1 mg/mL, except for *B. cereus* where both of them possess a diameter of inhibition zones slightly different, this difference may be due to the experimental protocol followed. Although the paired-t test revealed a significant difference in antimicrobial activity between the two concentrations of AgNPs tested (p = 0.04387), it can be seen in the (Figure 6), that there's not much difference between them except for *P. aeruginosa*, moreover, the p-value is very close to 5%.

P. aeruginosa is a microorganism that has crucial clinical importance, it is an opportunistic pathogen, frequently involved in nosocomial infections (Mishra and Agrawal, 2012), in addition to this, it is intrinsically resistant to most antibiotics used to treat infections that are more tractable (Davey et al., 2015). Even if there are a large number of essential oils that inhibit the development of *P. aeruginosa*, for example, those mentioned by Tariq et al. (2019), there are also many essential oils that are ineffective against this bacterial species (Murbach Teles Andrade et al., 2014), moreover, in this study, the AgNPs synthesized in this process have shown the highest antibacterial activity against *P. aeruginosa* (Table 1), which underline their importance.

Our results are in agreement with those of Escárcega-González et al. (2018) which noted that the AgNPs produced by *Acacia rigidula* extract exhibit antimicrobial effects in both Gram-negative and Gram-positive bacteria.

Conclusions

In brief, silver nanoparticles biosynthesized with *Origanum glandulosum* (Desf.) aerial part extract, found to possess antibacterial properties, in addition to this they can hinder the growth of *Pseudomonas aeruginosa* that has a reputation for being resistant to many antibiotics, that is why the green synthesis could be a promising method for producing nanoparticles with bactericidal properties.

Acknowledgments

The authors acknowledge the General Direction of Research and Development DGRSDT Algeria, for the financial assistance; The Laboratory of Natural and Bioactive Substances (LASNABIO), and Laboratory of Technology of Separation and Purification (LTSP) of the university of Tlemcen for the technical support.

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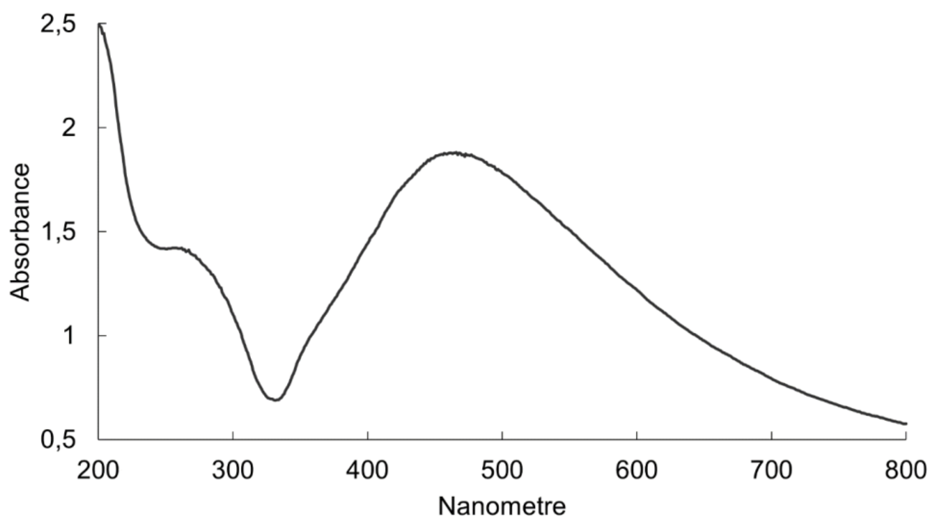

Figure 1. UV-Vis spectra of synthesized AgNPs.

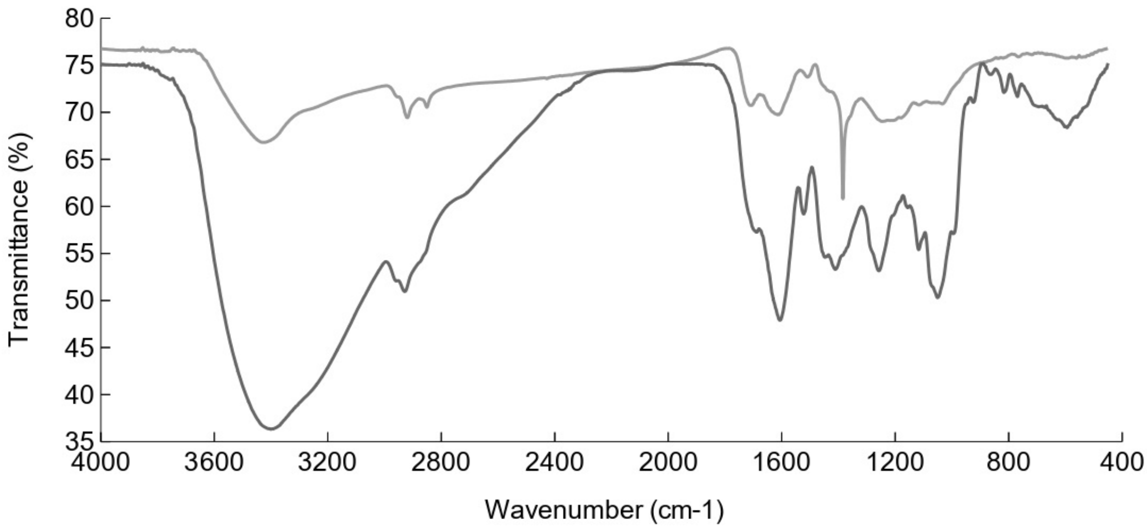

Figure 2. FT-IR spectrum of *O. glandulosum* extract (dark grey), and AgNPs (light grey).

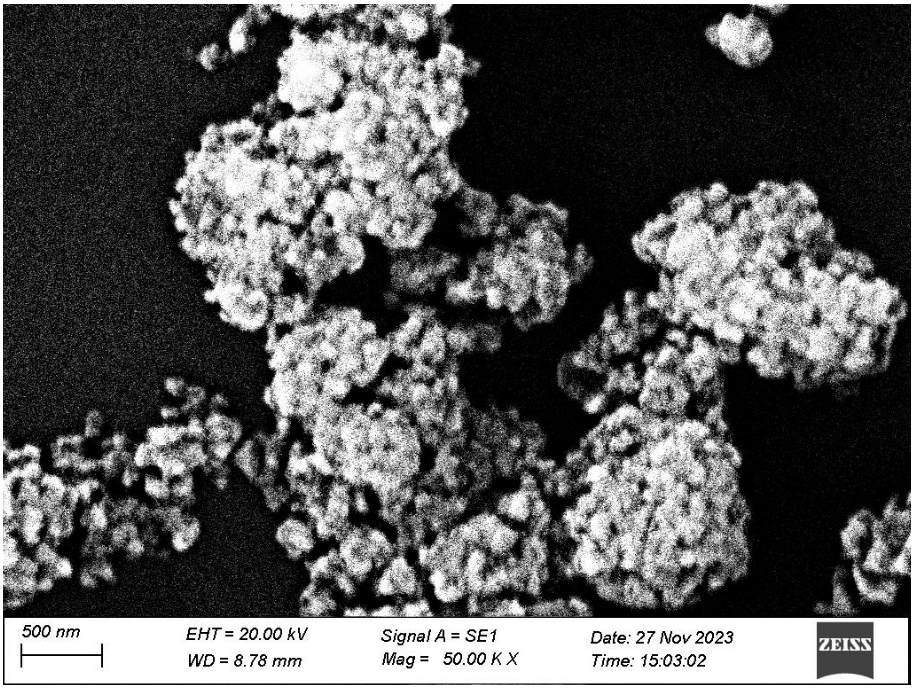

Figure 3. SEM image of the synthesized AgNPs.

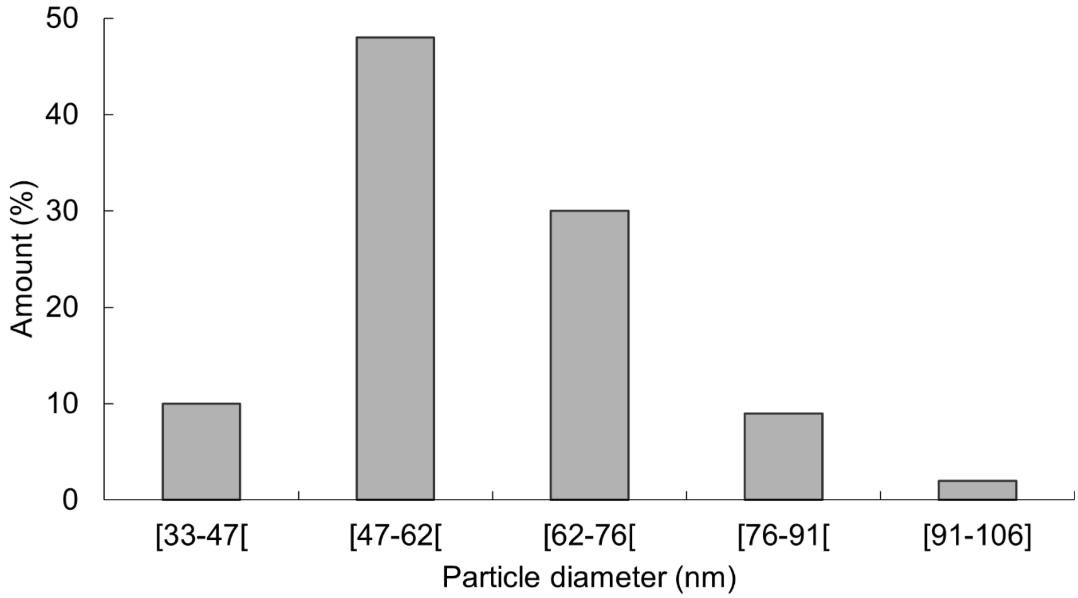

Figure 4. Histogram showing the particle diameter size distribution of AgNPs.

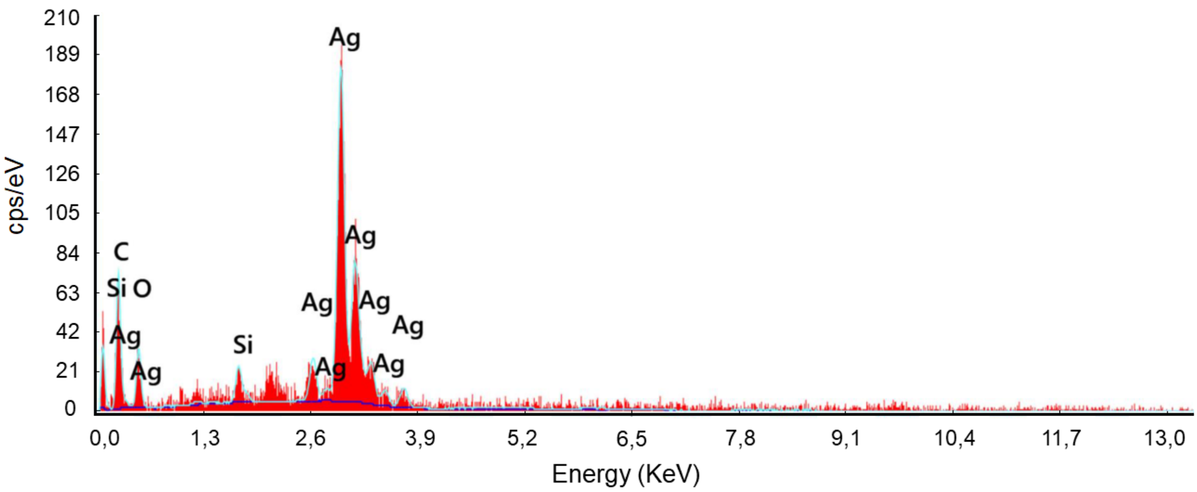

Figure 5. EDS spectrum of the biosynthesized AgNPs with labelled peaks. Abbreviations: Ag, Silver; Si, Silicon; O, Oxygen; C, Carbon; cps, counts per second; eV, electron volt.

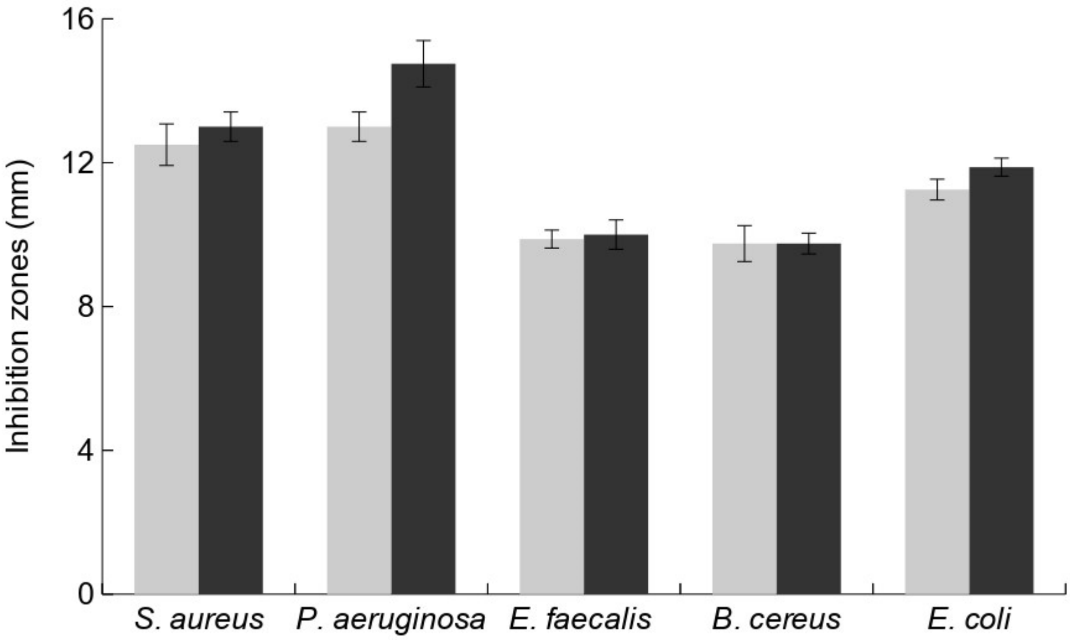

Figure 6. Antimicrobial activity of AgNPs suspensions of 1 mg/mL (light grey), and 2 mg/mL (dark grey).