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Green synthesis of silver nanoparticles using aqueous extract of dill leaves and evaluation of its antibacterial activity

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ABSTRACT

Interest in the biosynthesis of nanoparticles has increased in recent years by researchers. Nanoparticles have numerous applications in various fields. Synthesis of nanoparticles by green methods is environmentally safe and should be widely investigated, because different plants have a high ability to form these nanoparticles. In this study, the aqueous extract of dill (Anethum graveolens) leaves was used for the biosynthesis of silver nanoparticles. The antimicrobial activity of silver nanoparticles against microorganisms Erwinia amylovora, Pseudomonas syringae, Xanthomonas campestris, Salmonella typhi, Staphylococcus epidermidis and Listeria monocytogenes was investigated according to the disk diffusion agar, well diffusion agar, and minimum inhibitory concentration and bactericidal concentration methods. Increasing the concentration of silver nanoparticles from 20 to 110 mg/ml increased the diameter of the zone of inhibition from 7.75 mm to 11.17 mm in the disk diffusion agar and from 8.05 mm to 11.85 mm in the well diffusion agar method. X. campestris and P. syringae were identified as the most sensitive strains, and L. monocytogenes and S. epidermidis were identified as the most resistant strains to silver nanoparticles. The results of this study showed that dill leaf extract is capable of synthesizing silver nanoparticles and the produced nanoparticles showed a suitable antimicrobial effect on pathogenic strains in vitro.

1- Introduction

Due to their smaller particle size, different shapes, and increased surface area, nanoparticles exhibit very different properties compared to their parent materials and are recognized as interesting candidates for various applications, especially in biomedical sciences [1]. Attempts have been made to synthesize these nanoparticles using physical, chemical, and biological methods. Physical methods apply mechanical stress, thermal energy, electrical energy, and high-energy radiation to cause abrasion, melting, evaporation, or condensation of materials to produce nanoparticles. Chemical methods are the most suitable methods for the synthesis of metal and metal oxide nanoparticles, in which metal ions are reduced by chemical reducing agents and capping agents are used to stabilize the nanoparticles. Biological methods mainly use plant extracts (leaves, fruits, roots, etc.), microorganisms (bacteria, fungi, algae, etc.) and biomolecules as templates, as reducing and stabilizing agents. The green synthesis method is environmentally friendly, economical, convenient and an efficient process [2-4]. The use of plant extracts in reducing and stabilizing agents has aroused research interest in the synthesis of nanoparticles, especially in the field of food safety. The plant extract-based method is simple, cost-effective, environmentally friendly and has less or no possibility of contamination. Also, plant-mediated biosynthesis is better compared to other biological methods (based on microorganisms) because it is safe, rapid, unique, single-step and can be produced on a large scale. Various parts of plants such as stems, roots, leaves, flowers, fruits, seeds, bark or whole plants are capable of synthesizing nanoparticles in a green manner [5].

Among various metal nanoparticles, silver nanoparticles are gaining increasing momentum worldwide and since 2009, the number of publications on silver nanoparticles synthesized from plant extracts has been gradually increasing. Silver in nanoform has relatively higher antimicrobial activity compared to its macroscopic counterpart. Furthermore, silver nanoparticles have been shown to have better antioxidant and anticancer properties and have the potential to be developed as novel therapeutic agents [1, 2, 6].

Dill is an annual herb of the *Apiaceae* family, genus *Anethum* and species *A. graveolens*. This plant is rich in polyphenols, flavonoids, antioxidants, essential minerals and vital vitamins such as folic acid, riboflavin, niacin, vitamin A, beta-carotene and vitamin C [7]. Dill essential oil and extract have been reported to exhibit a broad spectrum of antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Shigella flexneri* [7-9]. Given that there are very few studies on the role of dill extract in the synthesis of nanoparticles in the scientific literature and also the positive biological properties of dill extract, in the present study, the role of dill leaf aqueous extract as a reducing agent for the synthesis of silver nanoparticles with potential biomedical applications was investigated, focusing on its antimicrobial activity against pathogenic microorganisms.

2-Materials and Methods

2.1. Extract preparation

Dill leaves were obtained from Ahvaz and were verified by our colleague in the Phytomedicine Department of Khuzestan University of Agricultural Sciences and Natural Resources. For aqueous extraction, 20 g of dried dill leaves were mixed with 200 mL of water and boiled for 10 min. After the mixture was cooled, it was filtered and the obtained extract was concentrated to a final volume of 100 mL [10].

2.2. Preparation of silver nanoparticles

A conventional one-step method was used to produce silver nanoparticles. For this purpose, 2.5 mL of dill extract was added to 50 mL of 1 mM $AgNO_3$ aqueous solution (Merck, Germany) and the resulting solution was incubated at 33°C. The color change of the solution from colorless to yellow indicated the formation of silver nanoparticles. The incubation was continued for 60 min until all the reagents were consumed in the reaction and more stable nanoparticles were formed [7].

2.3. Antimicrobial activity

The antimicrobial activity of silver nanoparticles against the microorganisms *Erwinia amylovora*, *Pseudomonas syringae*, *Xanthomonas campestris*, *Salmonella typhi*, *Staphylococcus epidermidis* and *Listeria monocytogenes* was investigated according to the disk diffusion agar, well diffusion agar, and minimum inhibitory concentration and bactericidal concentration methods presented by Sosani Gharibvand et al. with the necessary modifications [11]. All microbial strains were cultured in Mueller Hinton Agar medium (Merck, Germany) and incubated for 24 hours at 37 °C. Subsequently, a microbial suspension with a turbidity of 0.5 McFarland (equivalent to 1.5×10^8 CFU/ml) was prepared from the bacteria.

3.2.1. Disk diffusion agar

In this method, 100 µl of microbial suspension was cultured on the surface of Mueller Hinton agar culture medium. Subsequently, paper disks were impregnated with silver nanoparticles at concentrations of 20, 50, 80 and 110 mg/ml and then placed at specific intervals on the agar surface. In the next step, the inoculated plates were incubated at 37°C for 24 hours. Finally, the diameter of the growth inhibitory zone around the disks was measured in millimeters.

3.2.2. Well diffusion agar

In this test, wells with a depth of 6 mm were created on the surface of Mueller Hinton agar culture medium. A microbial suspension equivalent to 0.5 McFarland was evenly spread on the agar surface. Next, 50 µL of synthesized nanoparticles at concentrations of 20, 50, 80, and 110 mg/mL were added to the wells and the Petri dishes were incubated for 24 hours at 37°C. After that, the diameter of the growth inhibitory zone around the wells was measured in millimeters using a ruler.

3.2.3. Minimum inhibitory concentration

To determine the minimum inhibitory concentration (MIC), a sterile 96-well plate and broth microdilution method were used. Initially, serial dilutions of silver nanoparticles were prepared (2, 4, 8, 16, 32, 64, 128, 256, and 512 mg/mL) and then 100 µL of each concentration was

added to the wells of the microplate, followed by 20 µL of 0.5 McFarland microbial suspension to each well. The microplate was incubated at 37°C for 24 hours. After incubation, 10 µL of 5 mg/mL triphenyltetrazolium chloride solution was added to each well. The first concentration that did not show bacterial growth (as indicated by no change in red or purple color) was recorded as the MIC of the nanoparticle.

3.2.4. Minimum bactericidal concentration

The minimum bactericidal concentration (MBC) was determined based on the results of the previous experiment and from wells without microbial growth. For this purpose, 100 µl of the nanoparticle dilution was cultured in Mueller Hinton agar medium. Then the Petri dishes were placed at 37 ° C for 24 hours. The first concentration of nanoparticles that inhibited 99.9% of bacterial growth was considered as the MBC.

3.3. Statistical analysis

The experiments were performed in triplicate and the results were analyzed using Minitab software (version 16) with Tukey's test ($p < 0.05$).

3- Results and Discussion

Silver nanoparticles are effective antimicrobial compounds. According to the results of the disk diffusion agar method presented in Figure 1, the type of bacteria and the concentration of silver nanoparticles showed a significant effect on the diameter of the zone of inhibition ($p < 0.05$). Increasing the nanoparticle concentration from 20 to 110 mg/mL increased the diameter of the zone of inhibition from 7.75 mm to 11.17 mm. In addition, *P. syringae* with a diameter of 10.15 mm and *L. monocytogenes* with a diameter of 8.75 mm were the most sensitive and resistant strains to silver nanoparticles, respectively. The interaction effect results showed that the largest (12.20 mm) and smallest (7.20 mm) diameters of the zone of inhibition were related to *P. syringae* and *L. monocytogenes*, respectively ($p < 0.05$).

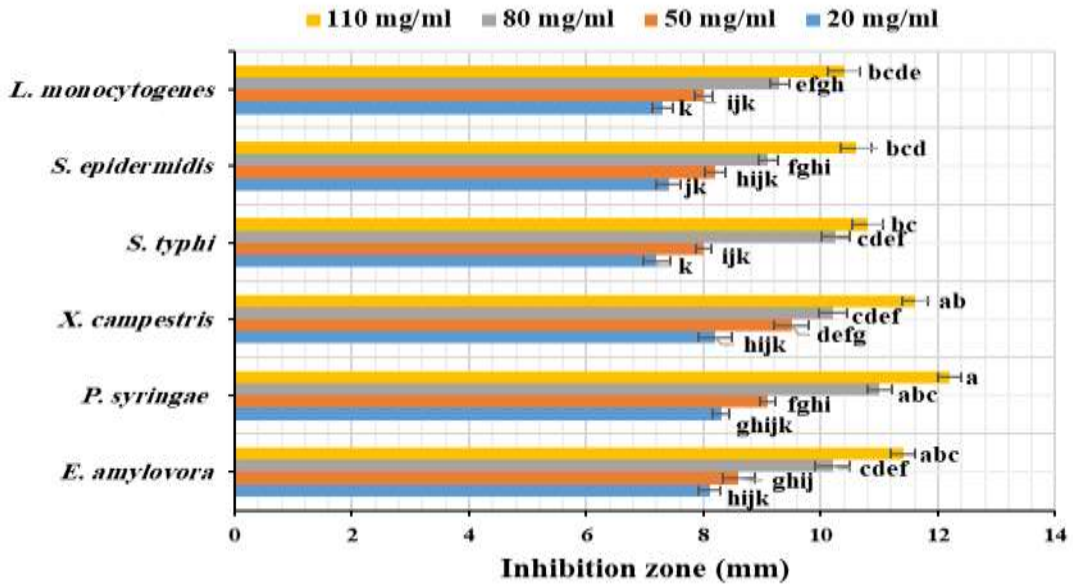

Figure 1. The antibacterial activity of silver nanoparticles based on disc diffusion agar method. Treatments labeled with different letters show significant differences at $p < 0.05$.

Similar results were observed in the well diffusion agar method (Figure 2). The concentration of nanoparticles had a significant effect on the average diameter of the inhibition zone; the diameter of the inhibition zone increased significantly from 8.05 mm to 11.85 mm in the presence of concentrations of 20 and 110 mg/mL, respectively. The type of bacteria also had a significant effect on the diameter of the inhibition zone, with *X. campestris* (10.87 mm) and *P. syringae* (10.65 mm) being the most sensitive and *L. monocytogenes* (9.17 mm) and *S. epidermidis* (9.26 mm) being the most resistant strains to silver nanoparticles. It should be noted that the average diameter of the inhibition zone in the well diffusion agar was significantly larger than that in the disk diffusion agar assay. This may be due to the direct contact of nanoparticles with microbial strains in the well diffusion agar method, whereas in the disk diffusion agar method, the antimicrobial agent must diffuse from the disk into the culture medium to exhibit its antimicrobial effect [12-15].

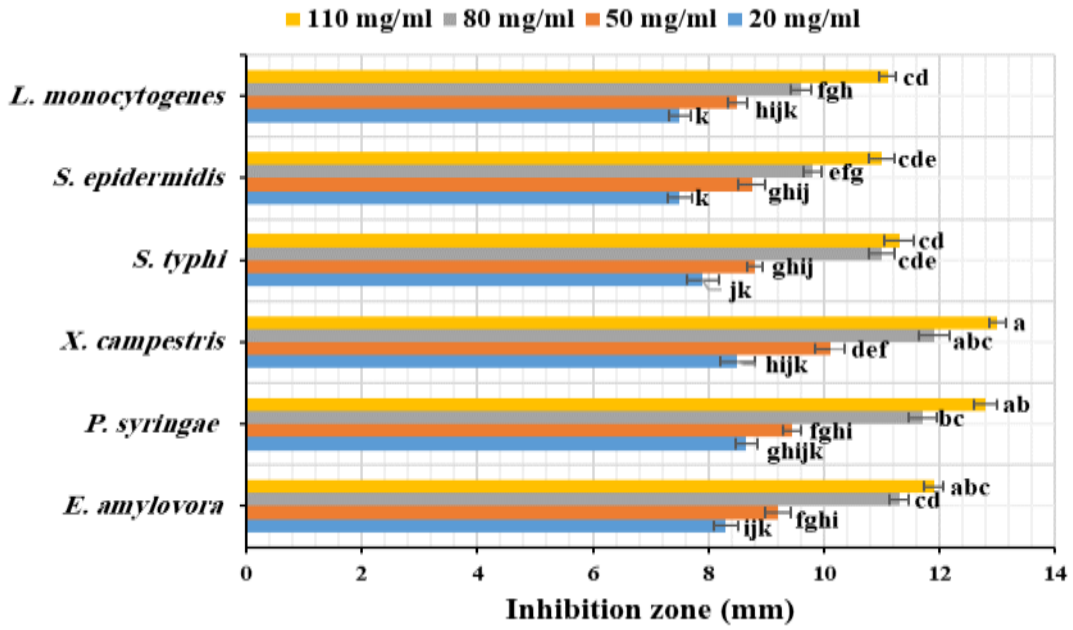

Figure 2. The antibacterial activity of silver nanoparticles based on well diffusion agar method. Treatments labeled with different letters show significant differences at $p < 0.05$.

The results of the minimum inhibitory concentration (MIC) test are shown in Figure 3. According to the results, the lowest MIC (4 mg/mL) was observed for *P. syringae* and the highest (32 mg/mL) was observed for *S. typhi*, *S. epidermidis*, and *L. monocytogenes*.

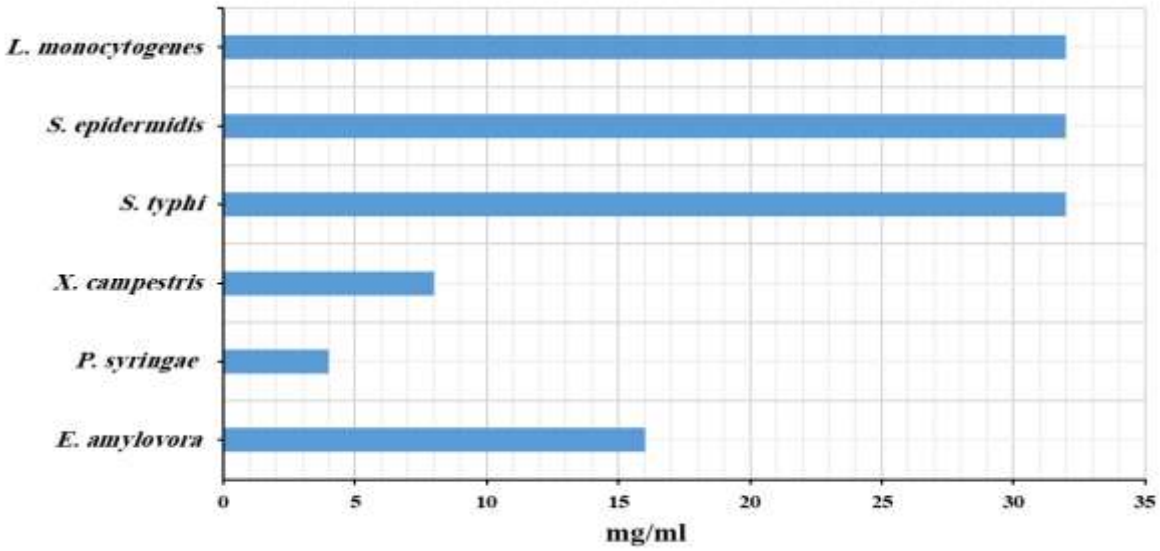

Figure 3. The antibacterial activity of silver nanoparticles based on minimum inhibitory concentration (MIC) method.

Figure 4 shows the results of the minimum bactericidal concentration (MBC) test of silver nanoparticles against pathogenic bacteria. The MBC for *E. amylovora*, *S. typhi*, and *L. monocytogenes* was 512 mg/mL, and for *S. epidermidis*, *X. campestris*, and *P. syringae* was 256 mg/mL.

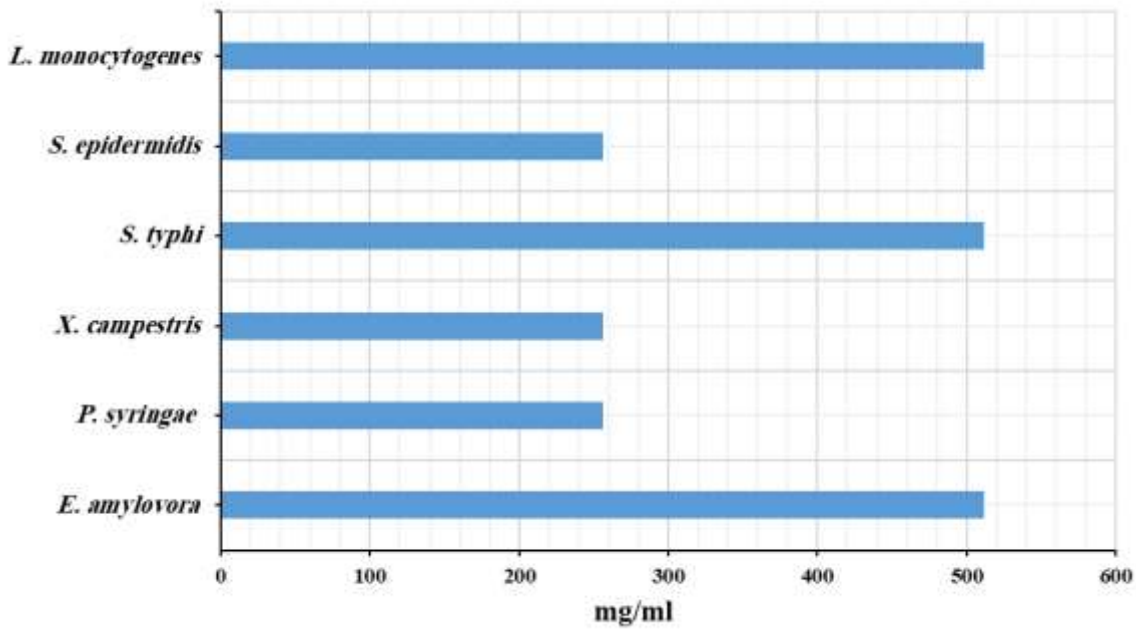

Figure 4. The antibacterial activity of silver nanoparticles based on minimum bactericidal concentration (MBC) method.

Dill contains a variety of antioxidant, phenolic, and flavonoid compounds that are capable of reducing metal ions and converting them into metal nanoparticles [7, 9]. Reports indicate that when silver nanoparticles come into contact with microorganisms, they cause the release of free radicals. These free radicals can damage cell membranes and ultimately lead to cell death. In addition, silver ions are thought to bind to thiol groups of essential enzymes, inactivating them. Silver ions can also penetrate the bacterial cell wall, causing protein denaturation and resulting in cell death by rupturing the cell wall. In addition, nanoparticles can affect signal transduction in bacteria. Research has shown that phosphorylation of protein substrates affects bacterial signal transduction, and dephosphorylation occurs specifically at tyrosine residues in Gram-negative bacteria. Nanoparticles alter the phosphotyrosine profile of bacterial peptides and also increase phosphorylation of peptide substrates on tyrosine residues, which inhibits signal transduction and thus the growth of microorganisms [11, 16].

Ahmed et al. (2025) used cold plasma technology to prepare silver oxide nanoparticles using dill leaf extract as a natural reducing agent [17]. The antibacterial and antibiofilm properties of

the prepared nanoparticles were evaluated. The results obtained showed that the prepared silver oxide nanoparticles had strong antibacterial properties and showed antibacterial activity against a diverse group of pathogenic bacteria such as *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus*. The results also showed the effectiveness of the nanoparticles in preventing the formation of bacterial biofilms, as the highest inhibition was observed for the Gram-positive bacterium *Staphylococcus aureus* [17]. Alamdari-Palangi et al. (2020) synthesized spherical silver nanoparticles with an average size of 30 nm using dill leaf extract as a green, cost-effective, non-toxic and environmentally friendly source [18]. Transmission electron microscopy, particle size analysis and Fourier transform infrared spectroscopy were performed to characterize the synthesized silver nanoparticles. The antibacterial activity of the synthesized silver nanoparticles was evaluated against Gram-positive and Gram-negative bacterial pathogens. The MIC of different concentrations of silver nanoparticles was used to evaluate their antibacterial properties against the pathogens *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The results showed the favorable antibacterial properties of silver nanoparticles and suggested their use as potential antibacterial agents. In addition, the anticancer effect of green synthesized silver nanoparticles was evaluated against the MCF-7 cell line and the results showed that cell viability was dependent on the concentration of silver nanoparticles [18]. In this study, Zare Bidaki et al. (2024) used dill seed extract as a capping and reducing agent for the synthesis of silver nanoparticles [19]. The observation of surface plasmon resonance of silver nanoparticles at around 420 nm, along with a change in the color of the suspension to dark brown, confirmed the synthesis of nanoparticles. Analysis by X-ray diffraction, Fourier transform infrared spectroscopy, dynamic light scattering, zeta potential and transmission electron microscopy confirmed the production of pure, homogeneous, spherical and stable silver nanoparticles with sizes of 20-40 nm using dill extract. The produced nanoparticles showed significant antimicrobial activity against both Gram-positive (*Staphylococcus aureus*, *Streptococcus mutans* and *Enterococcus faecalis*) and Gram-negative (*Klebsiella pneumoniae* and *Escherichia coli*) bacterial strains. Antioxidant evaluations showed that silver nanoparticles at a concentration of 250 µg/mL inhibited 92% of DPPH free radicals and in the reducing power test, dill seed extract and silver nanoparticles reduced 48.7% and 71% of ferric ions to ferrous iron, respectively. Anticancer properties were investigated against a lung cancer cell line (A-549) with an IC_{50} of 242 µg/mL. Flow cytometry and lactate dehydrogenase assays showed cancer cell death [19].

In another study, extracts of *Ocimum tenuiflorum*, *Solanum tricobatum*, *Syzygium cumini*, *Centella asiatica*, and *Citrus sinensis* were used to synthesize silver nanoparticles from silver nitrate solution [20]. The antimicrobial activity of silver bio-nanoparticles was determined by agar well assay against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*. The highest antimicrobial activity of silver nanoparticles synthesized

by *S. tricobatum* and *O. tenuiflorum* extracts was observed against *Staphylococcus aureus* (30 mm) and *Escherichia coli* (30 mm), respectively. The silver nanoparticles synthesized in this process had effective antimicrobial activity against pathogenic bacteria [20-24].

4- Conclusion

The findings of this study showed that dill leaf extract effectively synthesizes silver nanoparticles. Nanoparticles produced from dill leaves showed strong antimicrobial properties. These greenly synthesized nanoparticles are promising as antimicrobial agents to combat infectious diseases caused by a wide range of microbial strains. However, further extensive research is needed *in vitro*, in animal models, and through *in vivo* studies. In addition, exploring alternative green synthesis methods and optimizing conditions to increase the yield and stability of silver nanoparticles could lead to more effective antimicrobial agents. Further studies are needed to understand the exact mechanisms by which silver nanoparticles exert their antimicrobial effects. Also, investigating the synergistic effects of silver nanoparticles with other antimicrobial agents to increase the efficacy and resistance to challenge in pathogenic bacteria should be investigated.

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Author Contributions

All activities were carried out by the author.

Competing Interests

The author confirms that he / she has no financial conflicts of interest or competing interests in this study.

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چکیده

علاقه به بیوسنتز نانوذرات در دوره گذشته توسط محققان افزایش یافته است. نانوذرات کاربردهای متعددی در زمینه‌های مختلف دارند. سنتز نانوذرات با روش‌های سبز برای محیط زیست بی‌خطر است و باید به‌طور عمومی مورد بررسی قرار گیرد، زیرا گیاهان مختلف قابلیت بالایی برای تشکیل این نانوذرات دارند. در این پژوهش، عصاره آبی برگ گیاه شوید جهت بیوسنتز نانوذرات نقره مورد استفاده قرار گرفت. فعالیت ضدمیکروبی نانوذرات نقره در برابر میکروارگانیسم‌های *اروینیا امیلوورا*، *سودوموناس سیرینگه*، *زانتوموناس کمپستریس*، *سالمونلا تیفی*، *استافیلوکوکوس اپیدرمیدیس* و *لیستریا مونوسیتوژنز* مطابق روش‌های دیسک دیفیوژن آگار، چاهک آگار و حداقل غلظت مهارکنندگی و باکتری‌کشی مورد بررسی قرار گرفت. افزایش غلظت نانوذرات نقره از ۲۰ به ۱۱۰ میلی‌گرم در میلی‌لیتر سبب افزایش قطر هاله عدم رشد از ۷/۷۵ میلی‌متر به ۱۱/۱۷ میلی‌متر در روش دیسک دیفیوژن آگار و از ۸/۰۵ میلی‌متر به ۱۱/۸۵ میلی‌متر در روش چاهک آگار گردید. *زانتوموناس کمپستریس* و *سودوموناس سیرینگه* بعنوان حساس‌ترین و *لیستریا مونوسیتوژنز* و *استافیلوکوکوس اپیدرمیدیس* بعنوان مقاوم‌ترین سویه‌ها در برابر نانوذرات نقره شناسایی شدند. نتایج این پژوهش نشان داد که عصاره برگ گیاه شوید قادر به سنتز نانوذرات نقره می‌باشد و نانوذرات تولیدی اثر ضدمیکروبی مناسبی بر سویه‌های بیماری‌زا در شرایط برون‌تنی از خود نشان دادند.