Original Research Article

Evaluation of silver nanoparticles and alcoholic extract of *Amaranthus retroflexus* on pathogenic bacteria

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ABSTRACT

In recent years, application of medicinal plants increase in the treatment of infections rather than antimicrobial drugs. The aim of this study was to compare the effects of silver nanoparticles and alcoholic extract of *Amaranthus retroflexus* on some pathogenic bacteria. The alcoholic extract of this plant was prepared and the effect of concentrations of 50 to 400 mg/ml of this extract and concentrations of 10 to 80 mg/ml of silver nanoparticles by well diffusion method on *S. aureus, B. cereus, E coli* and *P aeruginosa* were evaluated. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined on bacteria by dilution method. The results showed that the inhibition effect of *Amaranthus retroflexus* on gram-positive bacteria is more than gram negative bacteria while the inhibitory effect of silver nanoparticles on gram-negative bacteria is more than gram-positive bacteria. The results of this study showed that the alcoholic extract of *Amaranthus retroflexus* had an antibacterial properties that can be considered as an adjunct to further studies for *in vivo* conditions and replacement with common chemical agents in the treatment of infections.

***Keywords*:** Medicinal Plants, silver nanoparticles, *Amaranthus retroflexus*

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INTRODUCTION

Undoubtedly, medicinal plants are the oldest human approach to the treatment of diseases, and throughout the development of all civilizations there is a close connection between man and plant. However, many plant species remained unknown before the discovery of new and valuable vegetable resources. In this way, plants are considered as a source of potential chemical substances. These potentially useful chemicals could be used not only as a medicine but as an unrivaled model as a starting point for making pharmaceutical analogues, as well as an interesting tool for understanding and understanding better biological phenomena [1-7]. One of the most important therapeutic challenges is coping with infectious diseases due to its high prevalence. After recognizing penicillin and extending its use in treatment, new antibiotics were introduced every day for the treatment of infections. The result was the spread of clinical use of natural antibiotics and in the treatment of clinical infections. The excessive use of these antimicrobials resulted in increased drug resistance to different antibiotics in most of the bacteria [8]. This is one of the reasons for the growing use of plants as low-risk, affordable and inexpensive natural substances for synthetic antibiotics in the treatment of bacterial infections. Also, these herbal drugs are more likely to be consumed by people [9-11]. These reasons are due to the increase of extensive studies in the world and the introduction of antibacterial effects of different plants in recent years [12]. *Amaranthus retroflexus* is a red rosette of broad-leaved weed and one year old Amaranthaceae family that has a special place among the common weeds in Iranian and world farms [13]. In Iran, this weed is abundant in the provinces of Tehran, Khorasan, Fars, Kermanshah, Hamedan, Kurdistan, Qazvin, Azerbaijan, Ilam and Khuzestan. Seeds of *Amaranthus retroflexus* grow in the summer. Seed germination is one of the most critical events for the success of many weeds because it has been shown that germination is the first step for a weed to compete for ecology conditions [14]. Another way to tackle germs without increasing drug resistance is to use nanotechnology and nanoparticle production technology. Nanotechnology researchers identified a wide range of nanoparticle applications that may play a huge role in medicine, disease prevention and treatment. One of these nanoparticles whose antimicrobial effects are proven is silver nanoparticle which is prepared in a variety of ways [15]. One of the most promising branches in the field of nanotechnology is the

application of nanotechnology. In this technology, silver ions are placed in a colloid solution in suspension silver affects metabolism, respiration, and reproduction of microorganisms [16]. In various studies, the antimicrobial properties of these nanoparticles and their useful application in the field of biotechnology are investigated. Silver nanoparticles, without increasing drug resistance, inhibit the respiratory system of bacteria. This element has specific properties in microbial decontamination and is easy to prepare and is also is inexpensive [17]. Application of these two solutions together could solve many problems, and result in better and more affordable results. The aim of this study was to investigate the antibacterial effects of plant and silver nanoparticles on some pathogenic bacteria.

MATERIALS AND METHODS

The vegetative samples from natural landscapes around Marand city in the east of Azarbaijan province, were obtained randomly at the end of March in 2016 in two stages. Then, samples collected by botanists and herbarum in the botanical laboratory of Marand branch of Islamic Azad University and confirmed by Genus and species. The specimens were cleaned after collection and transfer, and were dried in a large space suitable for sunlight. After complete drying of the specimens and separating the aerial parts (stems and leaves) from the roots of the plant, extraction was performed using soxhlet method, so that 300 g of herbal powder was poured into filter paper and soaked in a little methanol and inside soxhlet. A balloon containing 500 ml of methanol in a soxhlet was heated and then used to obtain a pure extract from a rotary evaporator. Then, using 5 % DMSO as a solvent, concentrations of 50, 100, 200, 400 mg/ml, were prepared by well diffusion test and MIC/MBC were determination (according to CLSI protocol) [18-20]. Strains of *S.aureus* (ATCC: 25923), *B.cereus* (PTCC: 1052) *E.coli* (ATCC: 25922), *P.aeruginosa* (ATCC: 27853) were prepared as a lyophilized from Pasteur Institute of Iran. In this method, firstly, the susceptible bacteria were equivalent to the half-MacFarland standard and spread through a sterile swab from each bacterial sample on the surface of the muller hinton agar culture medium. Immediately on the above medium, wells with a diameter of 5 mm and a distance of 2 cm were created and a certain amount of dilutions of the extract, which was first mentioned, was inoculated into the well. As a positive control, streptomycin antibiotics (10 micrograms) was used as a negative control of DMSO. At the end of the work, all media were placed in an incubator at 37 °C for 24 h, and then using

a caliper diameter of inhibition were measured [21-23]. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests were performed in a dilution tube. In order to determine the MIC, the alcoholic extract was diluted 0.58, 0.56, 1, 25.3, 25.6, 25, 50, 100, 200 mg / ml in muller hinton broth medium. Then, 1 ml of the prepared microbial suspension was added to each dilution. As a positive control, a tube with a mutant (bacterial culture medium without extracts) as well as a negative tube with contents (a culture medium without bacteria) were prepared. After completion of the work, all tubes were incubated at 37 °C and transmitted for 24 days. After incubation time, the tubes were examined for inoculum growth induced germination. The lowest dilution of the extract that was not observed (lack of growth) was considered as MIC. To determine the minimum concentration of extracts (MBC) of all tubes in which there was a lack of growth. muller hinton agar culture medium were cultured. The inoculated medium was incubated for 24 sec at 37 °C, the plate associated with the tube containing the lowest concentration of the extract and no bacterial growth was observed as MBC. The concentration of the extract was considered [24]. To perform antibacterial tests of silver nanoparticles, were prepared from company of Nano sany engineer. The dilution series were 10, 20, 40, 80 μg / ml. In this test, well diffusion method and MIC determination were used in muller hinton agar and muller hinton broth media. The method of preparation of culture media, bacterial strains and method of the work were similar to the test of alcoholic extract of *Amaranthus retroflexus*. The main objective of this study was to investigate the synergism (synergistic) of silver nanoparticles and alcoholic extract of *Amaranthus retroflexus* plant on pathogen bacteria. In this way, the dilution series were added together. The test procedure was the same as previous tests. The results of the experiments were analyzed using SPSS software and the required charts were drawn using excel software, one-way ANOVA and LSD test to compare. In this study, $p < 0.01$ was considered significant.

RESULTS

The effect of various concentrations of alcoholic extract of *Amaranthus retroflexus* on pathogenic bacteria revealed that this extract had a significant inhibitory effect on four bacteria and the higher concentrations of alcoholic extract, so the inhibitory effect was increased zone of inhibition became more significant. This study showed that the inhibitory effects of alcoholic extract on

gram-positive bacteria were higher than gram negative bacteria. The results of the effect of various concentrations of alcoholic extract of *Amaranthus retroflexus* on the well diffusion method are presented in table 1. MBC/MIC test showed that the herb extract was the most susceptible to *S aureus* bacteria and had the lowest susceptibility to *E coli*. The results of the MBC/MIC test of alcoholic extracts against selected bacteria by tube method are presented in table 2. The effect of concentrations of silver nanoparticles on pathogenic bacteria revealed that this extract had a significant inhibitory effect on all four bacteria tested. This study showed that inhibitory effects of silver nanoparticles on gram-negative bacteria were more than gram-positive bacteria. The results of the effect of various concentrations of silver nanoparticles by well diffusion method are given in table 3.

Table 1. Diameter of bacteria inhibition zone (mm) in different concentrations of *Amaranthus retroflexus* alcohol extract by well diffusion method

Extract concentration / Bacteria	(mg/ml) 50	(mg/ml) 100	(mg/ml) 200	(mg/ml) 400	Negative control	Positive control
Staphylococcus aureus	8.40	10.30	12.80	14.40	-	17.30
Bacillus cereus	7	9.20	11.40	13	-	16.40
Pseudomonas aeruginosa	--	--	8	10.60	-	13
Escherichia coli	--	--	7.80	9.30	-	14.30

Table 2. MBC / MIC test for bacteria at different concentrations of extract

Extract concentration / Bacteria	MIC (mg/ml)	MBC (mg/ml)
Staphylococcus aureus	12.5	25
Bacillus cereus	12.5	50
Pseudomonas aeruginosa	25	50
Escherichia coli	50	100

Table 3. The diameter of the inhibition zone of bacteria (mm) at different concentrations of silver nanoparticles by well diffusion method

Nanoparticles concentration / Bacteria	(µg/ml) 10	(µg/ml) 20	(µg/ml) 40	(µg/ml) 80	Negative control	Positive control
Staphylococcus aureus	10.20	12	14	16	-	17.26 mm
Bacillus cereus	10.50	12.30	14.70	16.30	-	17.17 mm
Pseudomonas aeruginosa	12	13.40	15.80	17.40	-	16.70 mm
Escherichia coli	11	11.80	15	16.90	-	16.53 mm

The results of this test showed that silver nanoparticles had the greatest effect on *P aeruginosa* bacteria and had the least effect on *S aureus* (table 4).

By mixing dilutions of silver nanoparticles and alcoholic extract of *Amaranthus retroflexus*, a combined concentration was obtained in previous experiments. The results of the antibacterial test of this compound are presented in tables 5 and 6, which were performed by well diffusion methods and MIC test.

Results showed that there was a significant increase in the size of the inhibition zone bacterial, which was more pronounced in gram negative bacteria.

The results of this test show that by integrating silver nanoparticles and *Amaranthus retroflexus* extract, all bacteria affected by this combination, so that the minimum inhibitory concentration and bacterial leakage concentration were significantly reduced compared to previous tests. This compound has the greatest impact on *P aeruginosa* and has the least effect on *S aureus* bacteria.

Table 4. MBC / MIC test for bacteria in different concentrations of silver nanoparticles

Nanoparticles concentration / Bacteria	**MIC (µg/ml)**	**MBC (µg/ml)**
Staphylococcus aureus	50	100
Bacillus cereus	50	50
Pseudomonas aeruginosa	12.5	25
Escherichia coli	6.25	12.5

Table 5. The diameter of the bacteria inhibition zone (mm) in the concentrations of the silver nanoparticles and alcoholic extract of *Amaranthus retroflexus* by the well diffusion method

Extract concentration / Bacteria	50mg/ml +10µg/ml	100mg/ml +20µg/ml	200mg/ml +40µg/ml	400mg/ml +80µg/ml	**Negative control**	**Positive control**
Staphylococcus aureus	12	13.40	15.20	16.70	-	16.50
Bacillus cereus	11.60	13.30	15	16.80	-	16.40
Pseudomonas aeruginosa	13	13.20	15.60	17.80	-	16
Escherichia coli	11	12.50	14.80	17	-	16.30

Table 6. MBC / MIC test at different concentrations

Concentration / Bacteria	**MIC (µg/ml)**	**MBC (µg/ml)**
Staphylococcus aureus	6.25	25
Bacillus cereus	6.25	12.5
Pseudomonas aeruginosa	3.125	6.25
Escherichia coli	6.25	12.5

DISCUSSION

Plants played a major role in maintaining health and improving the quality of life of humans thousands of years ago. Medicinal plants have beneficial properties, including anti-bacterial, anti-parasitic, anti-fungal and anti-oxidant properties [25]. The results showed that silver nanoparticles and alcoholic extract of *Amaranthus retroflexus* both affected the pathogenic bacteria. But the combination of these two combinations created a synergistic property between them, and as a result their antibacterial activity increased dramatically. In 2012, Saeedi and his colleagues found that the ethanolic extract of *Amaranthus retroflexus* had an inhibitory effect on *S aureus* [26]. In 2015, Saritha and his colleagues determined that the alcoholic extract of *Amaranthus retroflexus* had an inhibitory effect on *K pneumoniae* and B subtilis and no activity against *S aureus* among the bacterial strains, while in case of the fungal strains the most intensive effect were exhibited against *C famata* [27]. In a 2017, Pourianfar and his colleagues found that the alcoholic extract of *Amaranthus retroflexus* and silver nanoparticles had an inhibitory effect on fungi [28]. In 2013, Ahmed and his colleagues found that the alcoholic extract of *Amaranthus viridis L* had an inhibitory effect on *S aureus*, *E coli* and some fungi [29]. Mobaiyen and his colleagues in 2017 determined that the essential oils of weeds had inhibitory effects on pathogen bacteria [4,30]. Moudgi and his colleagues in 2006 showed that the effects of nanoparticles on living organism cells depend on the diameter, size, and shape of nanoparticles [31]. Silver nanoparticles, due to their small size, the surface of the contact with the outer space had a greater effect on the membrane of the cells [32]. Khosravi-Darani and his colleagues found that the inhibitory effects of silver nanoparticles on *E coli* bacteria were more than *S aureus* bacteria [33,34]. Dost Mohamadi and his colleagues noticed that the aqueous extract of the *Malva neglecta* and silver nanoparticles had the most antimicrobial activity affecting *S aureus* and *Salmonella typhimurium* and could be useful in making new drugs [35]. Kim reported that silver nanoparticles with a diameter of 20 nm could be inhibited *S aureus* [36].

CONCLUSION

The production of new medicinal plants with the least side effects can be used as a substitute for common antibiotics against these bacteria.

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