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ORIGINAL ARTICLE

Evaluation of Anticancer and Anti-bacterial Effects of Silver Nanoparticles Synthesized by *Origanum majorana*L. Extract on Cancer Cells MCF-7, HeLa and A549

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KEYWORDS

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ABSTRACT: *Origanum majorana* L. is an annual, sometimes biennial herbaceous plant with straight stems and oval opposite branches and leaves. This plant is useful in traditional medicine and is used to treat gastrointestinal diseases, rheumatism and infections. This research investigated the antimicrobial effects and toxicity of Silver nanoparticles synthesized using the extract of the medicinal plant *O. majorana* L., on 3 cancer cell lines such as A549, MCF-7, and HeLa. Silver nanoparticles Silver nanoparticles were biologically synthesized using the extract of *O. majorana*. After physical and chemical evaluation, the antimicrobial properties of the synthesized nanoparticles were evaluated in *Escherichia coli* and *Staphylococcus aureus*. Finally, the inhibitory effect of synthesized nanoparticles was assessed by the MTT assay on 3 cancer cell lines. With an average size of 15 nm, the nanoparticles synthesized by *O. majorana* extract had a significant inhibitory and lethal effect on 2 bacteria. The anti-cancer effect of the synthesized nanoparticles was on all 3 cell lines. However, with increasing the concentration of nanoparticles on the survival of cancer cells decreased, indicating a direct dose interaction on the inhibitory rate of silver nanoparticles. At a concentration of 50 g/mL, the synthesized Silver nanoparticles showed more than 50% inhibitory effect on different cell lines. Our results demonstrate that medicinal plants can be used in the successful synthesis of biological Silver nanoparticles. The synthesized AgNPs can be utilized as effective medicinal agents in the management of several cancers due to their coating made of effective secondary metabolites and the release of silver ions (Ag^+).

INTRODUCTION

In recent years, nanoparticles have found many applications as a powerful and advanced tool in the diagnosis and treatment of many dangerous diseases, including cancer [1]. Cancer is one of the leading causes of death worldwide and due to its high prevalence, research on cancer treatment is of great interest. Currently, surgery, radiation therapy, chemotherapy, immunotherapy as well as combination diets are the main treatment strategies for this disease.

Accordingly, the development of new treatment methods and environmentally friendly, it seems necessary, while the cost of treatment in these methods should be reduced [2]. Metal nanoparticles, including silver nanoparticles, have been widely used in the treatment of cancer. By inhibiting cancer cells by silencing the genes involved, nanoparticles can prevent the spread of disease and be very effective in improving it. In addition, these nanoparticles can be used as

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carriers of effective drugs [3]. Silver nanoparticles can cause apoptosis of cancer cells by producing reactive oxygen species (ROS), oxidative stress, and inhibition of vital enzymes [4]. In the last decade, significant advances have been made in the synthesis of metal nanoparticles with unique structures and properties. Certain spatial sizes and structures as well as the crystal shape of the synthesized nanoparticles are among the most important goals of nanoparticle chemistry. These properties have made these particles have potential applications in medicine, so that nanoparticles can be used as biosensors in the diagnosis and treatment of various diseases [5]. Improper use of chemical antibiotics has led to the development of various types of resistant pathogens [6]. Many pathogens have become resistant to one or more antibiotics, and many of the available antibiotics have lost their effectiveness. The emergence of antibiotic-resistant bacteria is becoming the biggest human health challenge in the world [7].

To address this issue, several studies on the synthesis and formulation of metal nanoparticles to combat various types of pathogenic bacteria have been conducted or are underway [8]. Among metal nanoparticles, silver nanoparticles have very good antimicrobial properties. These nanoparticles inhibit them by various mechanisms and affecting the cell wall and metabolic processes of bacteria [9-11].

Numerous new methods are being developed or improved to enhance the physicochemical properties of nanoparticles. In some methods, the synthesis process is modified to increase their optical, mechanical, physical, chemical, and biological properties. Biosynthesis of metal nanoparticles using biological compounds is one of the important achievements that has been very successful in producing different types of metal nanoparticles with different structure and size. Medicinal plants are among the environmentally friendly precursors that have been widely used in the production of nanoparticles [12]. The synthesis of metal nanoparticles using biological compounds in medicinal plant extracts is a reliable and environmentally friendly method. Among the synthesized metal nanoparticles, silver nanoparticles are more important due to their antimicrobial properties. Biosynthesis of silver nanoparticles using plant extracts is a fast and cost-effective method in which the metabolites in the extract cause oxidation and reduction of silver ions and finally different types of silver nanoparticles with different shapes and sizes are produced [13]. The aim of the present study was to synthesize silver nanoparticles biologically using marjoram extract and to investigate the antimicrobial effects and toxicity of synthesized silver nanoparticles on cancer cells.

MATERIALS AND METHODS

Characterization

Samples of *Origanum majorana* were collected from the mountains of Khorramabad city, Lorestan Province (Makhmalkouh) in west of Iran and used after botanical approval.

The UV-Vis spectral analysis was performed using a Phystec miniature UVS-2500 spectrophotometer; the UV-Vis absorption spectrophotometer has a resolution of 1 nm within the 190–1100 nm range. The FTIR spectra were recorded on an Avatar Thermo Spectrophotometer System. To analyze the surface morphology and particle size, a scanning electron microscope by SEM Tescan Mira3 was utilized. Analysis also included the X-ray diffraction (XRD) (Philips PW 1730); the analysis within the angle range of 20º–80º confirmed that the synthesized copper nanoparticles are of amorphous nature; they were then dried in vacuum for medium-term at 25°C. The XRD studies were performed utilizing a Bruker D8 advanced X-ray diffractometer via Cu Kα (I=1.54 A) [14].

Preparation of the aqueous extract of Origanum majorana

Samples of *Origanum majorana* were collected from the mountains of Lorestan Province and used after botanical approval. Using distilled water, the plant samples were washed; then, they were dried in an oven at 45°C for 24 h and ground into small pieces by a mill. The dried sample (10 g) was added to 90 mL of sterile distilled water and placed in a bain-marie at 60°C for 120 min. The mixture was then incubated at room temperature (25°C) for 24 h. The prepared extracts were filtered twice by Whatman filter paper (No. 24), transferred to 50 mL Falcon tubes, and then centrifuged at 5000 rpm for 15 min [14].

The supernatant was passed through a syringe filter (0.22 µm). To prepare an aqueous extract with a known concentration, the filtered extracts were first transferred to sterile Petri dishes and then incubated in an oven at 45°C for 24 h. The obtained precipitate was removed from the Petri dish surface using a sterile surgical blade. The dry extract was weighed and dissolved in a known amount of sterile deionized distilled water to prepare the aqueous extract with a certain concentration [14].

Biosynthesis of AgNPs

To silver nanoparticles, 5 mL of the aqueous extract of the studied medicinal plant was first transferred separately to 95 mL of 1 mM silver nitrate ($AgNO_3$) solution and shaken at room temperature (25°C) for 24 h. The solution containing the synthesized nanoparticles was then transferred to sterile 50 mL Falcon tubes and centrifuged at 5000 rpm for 15 min. The formed precipitate was washed using sterile deionized distilled water. Then, Silver nanoparticles with a certain concentration were produced by the mentioned method to prepare the aqueous extract (Figure1) [14].

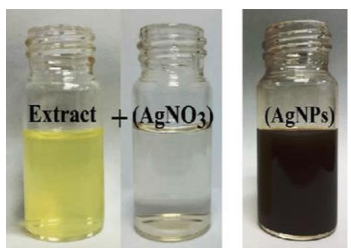

Figure 1. A: plant extract B: $AgNO_3$ solution, C: Silver Nano Particle.

Evaluation of the synthesized nanoparticles

Changes in the color of the extract and $AgNO_3$ solution were considered as the first sign in the biosynthesis of AgNPs. The solution containing the synthesized Silver nanoparticles was first evaluated by spectrophotometry at wavelengths of 300–700 nm. The wavelength at which Silver nanoparticles showed the highest absorbance was determined based on the absorbance levels. The nature, structure, and size of the sample of the synthesized Silver nanoparticles were evaluated through scanning electron microscopy (SEM) and XRD spectroscopy [14].

Evaluation of the antimicrobial effects of the extract and synthesized AgNPs

The antimicrobial effect of the synthesized AgNPs was evaluated using gram-positive (*Staphylococcus aureus* [PTCC 1112]) and gram-negative (*Escherichia coli* [PTCC 1330]) bacteria. First, the minimum inhibitory concentration (MIC) of the plant extract and AgNPs was determined by the microdilution method and tetrazolium chloride reagent, followed by determining the minimum bactericidal concentration (MBC). To this end, cell suspension of *S. aureus* and *E. coli* was cultured in Petri dishes containing LB culture medium in the MIC test. The bactericidal effect of the extract and the synthesized AgNPs was then evaluated by the disk diffusion test [14].

Evaluation of the anticancer impact of the synthesized Silver nanoparticles

The anticancer impact of the synthesized Silver nanoparticles was evaluated by the MTT assay. Three cancer cell lines (A549, MCF-7, and HeLa) were obtained from the cell bank at the Pasteur Institute of Iran and cultured in a suitable culture medium (RPMI) with 1% streptomycin antibiotic and 10% fetal bovine serum (FBS) at 37°C and 5% CO_2. Samples of each cancer cell with a known concentration were prepared so that the cell concentration was about 5×10^4 cells in each well of a 96-well plate. Next, using different concentrations of AgNPs (100, 50, 25, 12.5, 6.25, and 1 µg ml^{-1}), the cancer cells were treated at 37°C for 48 h. The toxicity of nanoparticles to cancer cells was assessed using the MTT reagent (5 mg mL^{-1}). Each well was supplied with 20µL of MTT solution and incubated in the dark at 37°C for 4 h. To dissolve the formazan crystals, 100 µL of dimethyl sulfoxide (DMSO) was added to individual wells. After 10 min, the absorbance level of each well was measured by a spectrophotometer at a wavelength of 570 nm. A concentration that inhibited the growth of cancer cells by 50% was considered as the inhibitory concentration (IC_{50}) of nanoparticles. The cell survival rate was defined by the absorbance ratio of the nanoparticle-treated sample to the absorbance level of the control sample. The survival rate (%) of the cancer cell samples was then obtained by multiplying the obtained value by 100 [14].

RESULTS AND DISCUSSION

The addition of the plant extract to the $AgNO_3$ solution triggered redox reactions and darkened the color of the reaction mixture. In many papers, a change in the color of an extract-$AgNO_3$ mixture has been reported as the first sign of nanoparticle synthesis. The darkened $AgNO_3$ in the presence of the plant extract can be attributed to surface plasmon [15]. Based on the spectrophotometric results, the synthesized nanoparticles showed the highest absorbance at 450nm (Figure 2).

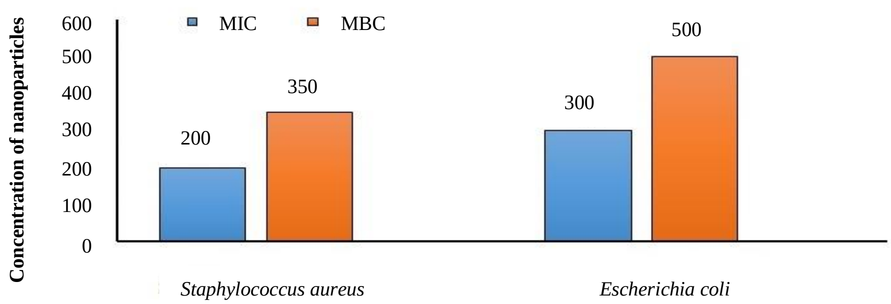

Figure 2. The UV-Vis absorption peak of: Silver NanoParticle

SEM was used to obtain the topological, compositional, and morphological information revealing the surface morphology of the nanoparticles. The average size of 19 nm was indicated by the SEM study (Figure 3). Larger sizes were found for AgNPs, possibly caused by the aggregation of smaller ones or proteins bound to the surfaces of nanoparticles. This varied shape and size is common for nanoparticles synthesized via biological systems. An Ag-rich composition was found for metal nanoparticles in (EDS) analysis (Figure 4). The results of SEM revealed that AgNPs synthesized by the extract of *Origanum majorana* were between 15 and 26 nm and spherical (Figure 3).

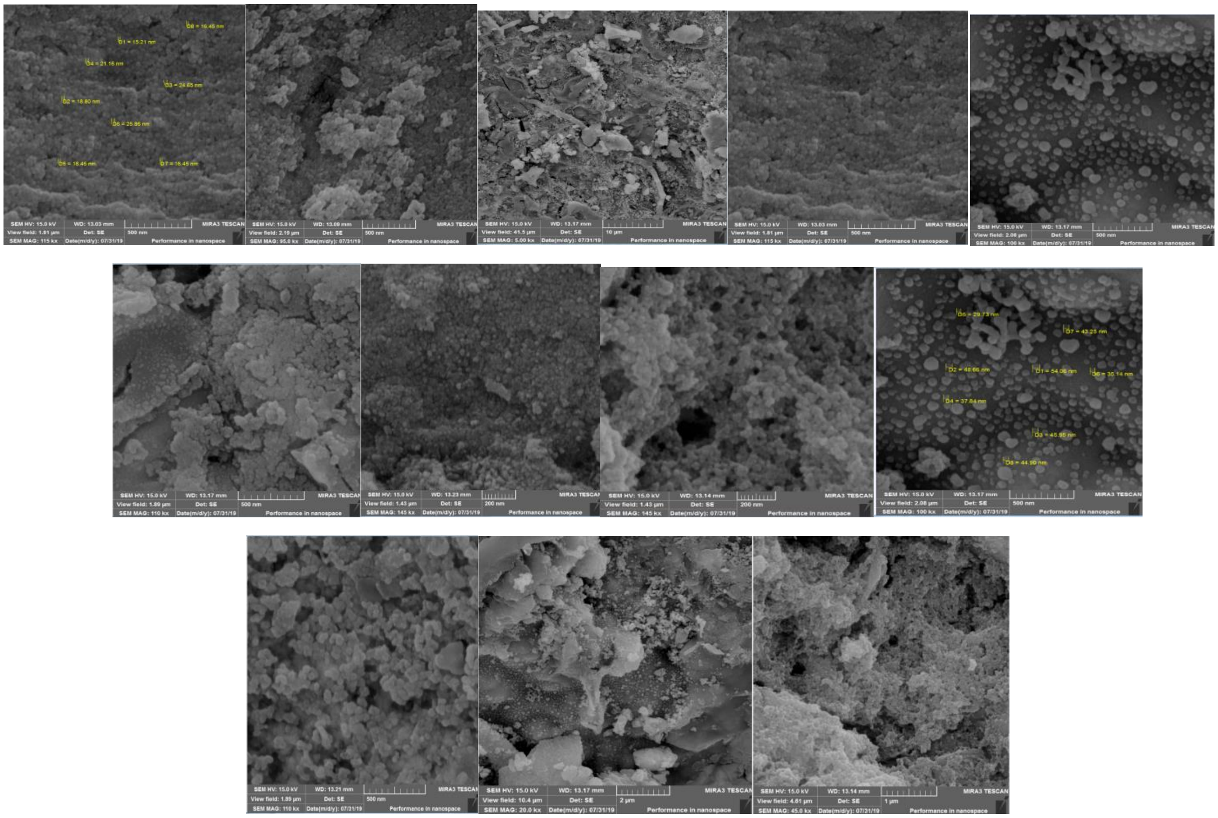

Figure 3. SEM image of Silver NanoParticle

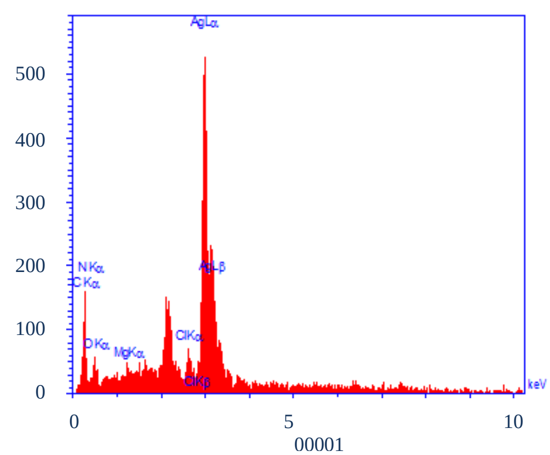

Figure 4. EDS elemental mapping of the Silver NanoParticle

Figure 5 illustrates the XRD patterns of the synthesized AgNPs; green synthesized AgNPs are very pure with no impurities. The crystallinity level was demonstrated by a sample with diffraction angles. As shown in the XRD pattern of the biosynthesized AgNPs, 4 obvious picks appeared at 38.07°, 42.26°, 64.43°and 77.35angles, which are consistent with (111), (200), (220),and (311) reflections of the face-centered cubic (fcc) phase of the AgNPs. They match the characteristics of fcc of Ag lines indexed at (220) and (200) related to the Miller indices (220) and (200), respectively (JCPDS card No: 34-1354).

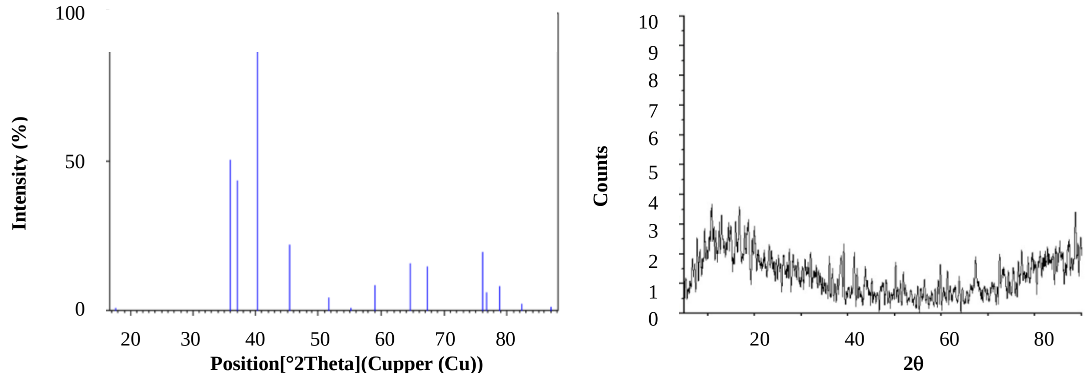

Figure 5. XRD pattern of Silver Nano Particle.

The antimicrobial effect of the synthesized nanoparticles was evaluated after confirming the synthesis of nanoparticles and examining their physical properties. According to the results of MIC and MBC for the antimicrobial effect of the synthesized nanoparticles on gram-positive (*S. aureus* [ATCC 29737]) and gram-negative (*E. coli* [ATCC 25922]) bacteria, a lower concentration of nanoparticles was synthesized by Origanum majorana extract inhibited gram-positive and gram-negative bacteria (Figure 6).

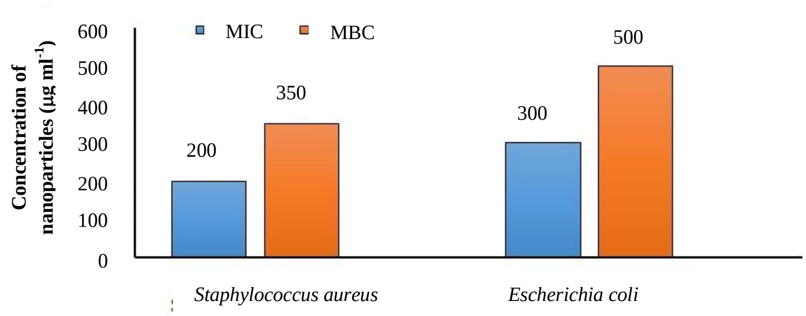

Figure 6. The results of the disk diffusion test showed Silver Nano Particle synthesized by the medicinal plant *O. majorana*

The findings indicated that the synthesized nanoparticles had more inhibitory and bactericidal effects on *Staphylococcus aureus* as a gram-positive bacterial strain.

The MBC of nanoparticles synthesized by *O. majorana* extract was 350 mm^{-1} for *S. aureus* and 500 µg mL^{-1} for *E. coli* (Figure 7).

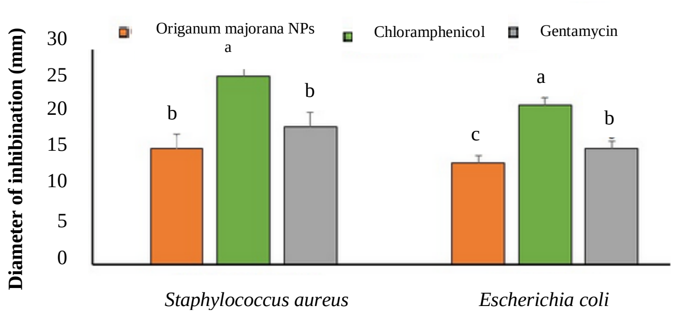

Figure 7. The results of the disk diffusion test showed AgNPs synthesized by the medicinal plant. Columns with similar letters are not statistically significant at the 5% level

The results of the disk diffusion test showed AgNPs synthesized by the medicinal plant Origanum majorana. Columns with similar letters are not statistically significant at the 5% level. The results of the disk diffusion test showed that both *E. coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 29737) were influenced by the antibacterial effect of nanoparticles synthesized by *O. majorana* extract with an average halo diameter of 15 mm.

Based on the results of the MTT anticancer assay, the inhibitory effect of Silver nanoparticles at different concentrations on the studied cell lines showed a direct interaction of the dose on the inhibitory level of AgNPs. The comparison of the inhibitory effect of different levels of AgNPs revealed a good inhibitory effect of nanoparticles synthesized by *Origanum majorana* extract, which reduced the survival rate of different cell lines. The synthesized nanoparticles exerted the highest inhibitory effect at a concentration of 100 mm^{-1} (Figure 8).

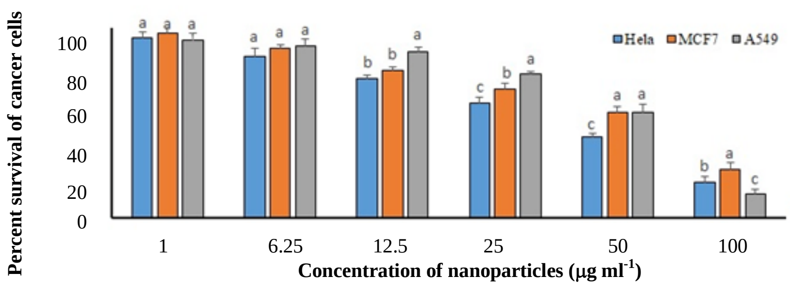

Figure 8. Survival rates of A549, MCF7, and HeLa cell lines against different concentrations of AgNPs synthesized by the medicinal plant *O. majorana* ithin 48 h. Columns with similar letters are not statistically significant at the 5% level.

An average inhibitory effect of 33% was obtained on different cells using various concentrations of *Origanum majorana* nanoparticles. The highest average inhibitory effect of Silver nanoparticles synthesized by *Origanum majorana* extract was observed on the A549 cell line. The HeLa cell line with 62% survival was ranked second in terms of sensitivity.

Biosynthesized nanoparticles are more important because of their many advantages over other methods. Medicinal plants are a rich source of biological compounds with the common name of secondary metabolites, whose medicinal function has been proven and is considered as the best option for the synthesis of silver nanoparticles [16]. In the present study, silver nanoparticles were successfully synthesized biologically. Synthesized silver nanoparticles at the wavelength of 450 nm showed the highest adsorption.

In the present study, the average diameter of silver nanoparticles synthesized by marjoram extract was 19 nm. The size and shape of nanoparticles is an important factor in their antimicrobial effect. Nanoparticles of different sizes can have different interactions with the bacterial cell wall, which ultimately greatly affects their antimicrobial properties [17].

Past studies have shown that a wide range of nanoparticles of various sizes and shapes can be obtained based on the synthesis method. In addition, the synthesis conditions will affect their physical and chemical properties. The greatest effect of cytotoxicity of nanoparticles is related to their dimensions. Spherical nanoparticles easily interact with the cell surface and begin their toxicity [18]. The results of the present study showed that silver nanoparticles synthesized by marjoram extract had a significant lethal effect on gram-positive and gram-negative bacteria. Gram-positive *S. aureus* was more sensitive than Gram-negative Escherichia coli. *Origanum majorana* is a rich source of various metabolites including polyphenols, tannins and terpenes [19]. This plant has strong antimicrobial properties and is used today as an effective natural antibiotic [20]. In recent years, due to the increasing use of chemical antibiotics, the frequency of resistant strains of pathogenic bacteria has increased. Bacterial resistance is one of the major challenges in the healthcare sector. Pathogenic bacteria use many different mechanisms to develop resistance to different types of antibiotics. Mechanisms of resistance vary between bacteria, and studies have shown that gram-positive and gram-negative bacteria differ in this respect. The first barrier against antimicrobial compounds is the bacterial cell wall. Gram-positive bacteria such as *S. aureus* have a thicker cell wall, however Gram-negative bacteria such as Escherichia coli have a higher resistance to the entry of antimatter agents than gram-positive bacteria due to its lipopolysaccharide and purine-rich outer layer. Microbes are present inside the bacterial cell [21].

Nanotechnology is growing rapidly and today is considered an integral part in the diagnosis and treatment of diseases. In recent years, nanotechnology-based therapeutic and diagnostic approaches have demonstrated the potential of nanoparticles in the treatment of cancer. Nanoparticles have found many applications due to their unique physicochemical properties such as high surface to volume ratio and strong reactivity [22]. The biosynthesis of nanoparticles is of great interest due to its low toxicity and environmental friendliness. In the biosynthesis of nanoparticles, coating agents are used in the synthesis of nanoparticles. Nanoparticles absorb these coating agents, which are usually organic molecules, and are used to help stabilize nanoparticles. From a medical point of view, it has been shown that the biosynthesis of nanoparticles greatly increases their clinical application [23]. Based on the results of the present study, the anti-cancer effects of biosynthesized silver nanoparticles were observed on 3 different cancer cell lines. Although the lethal effects of nanoparticles on different cells increased with increasing concentration of silver nanoparticles, however, no significant difference was observed between the lethal effect and the type of cell line. Nanoparticles synthesized using marjoram extract at a concentration of 100 µg ml^{-1} were able to show more than 60% inhibitory potency on all 3 cell lines (A549, MCF7 and HeLa).

Studies on the cytotoxic effect of silver nanoparticles synthesized by pearl extract by MTT method against breast cancer cells (MCF-7) were performed. Significant cytotoxicity (3µg ml^{-1}) was observed. They stated that the anti-cancer effect of the synthesized nanoparticles was due to the spherical shape and small particle size (10 to 30 nanometers) that easily penetrated into the cancer cells. There have also been other studies showing that higher concentrations of silver nanoparticles (82 µg ml^{-1}) are needed to prevent cancer cells from multiplying [24].

Silver nanoparticles can enter the cell using endocytosis and enter the mitochondria by affecting cellular respiration to produce reactive oxygen species (ROS). In summary, silver nanoparticles can damage the DNA of cancer cells and inhibit cancer cells by inducing oxidative stress, inducing apoptosis and mitochondrial damage [25].

According to the hypotheses, the development of blood vessels helps the growth of cancer cells. The new blood vessels help the cancer cells to attack and spread throughout the body by supplying oxygen and nutrients to the cells. This phenomenon is known as metastasis [26].

The results of previous studies have shown that silver nanoparticles synthesized by medicinal plants have an important role in preventing the development and production of cancer cells by inhibiting angiogenesis [27]. Silver is naturally toxic, and converting the metal to nanoparticles may increase the risk of toxicity. However, the green synthesis method reduces the toxicity of silver nanoparticles. The toxicity of silver nanoparticles depends mainly on their coating. Plant secondary metabolites stabilize silver nanoparticles by preventing them from accumulating. Therefore, the biocompatible behavior of silver nanoparticles synthesized by medicinal plants will be suitable for the production of drugs and the use of therapeutic methods [28].

Nanoparticles provide researchers with a new perspective on detecting, protecting and treating different types of cancer cells. Due to the improvement in the speed of various diagnostic devices and treatment strategies, cancer mortality has been significantly inhibited. Unfortunately, there is no successful method for selecting and accurately binding drugs to cancer cells to prevent their toxicity and side effects. To overcome this situation, the synthesis of nanoparticles as a new technique has provided appropriate solutions to the medical [29]. Researchers are looking for drugs that are effective against diseases and use herbs to treat diseases. Studies have shown that medicinal plants due to their active ingredients and medicinal and antioxidant compounds have beneficial effects on human health and can be used for medicinal and therapeutic purposes [30-44].

CONCLUSIONS

This research work shows that the production of biopharmaceutical particles is biodegradable, with great simplicity and very low cost. Due to their small size and spherical structure, the particles are able to easily pass through the cell wall of bacterial cells and have a toxic effect on the growth and metabolism of gram-negative and gram-positive bacteria. Therefore, it contains some of the particles that contain the drug and the nutrients in it.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication and etc.) have been completely observed by author.

Authors' contribution

All authors contributed equally to the manuscript.

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