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*Original Article*

# Evaluation of anti-bacterial (*Escherichia coli* and *Staphylococcus aureus*) and Anticancer Effects of Silver Nanoparticles Synthesized by *Melissa officinalis* L. Extract on Several Cancer Cells (A549, MCF-7, and HeLa)

**Faezeh Bashiri Godarzi[1], Shabnam Shamaei[1]***

[1]Department of Chemistry, Khorramabad Branch, Islamic Azad University, Khorramabad, Iran

*Corresponding Author E-mail: shabnamshamaie@gmail.com

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**Abstract**

**Background:** Silver nanoparticles (AgNPs.) with antimicrobial and anticancer properties have been widely used in a variety of fields. This research investigated the antimicrobial effects and toxicity of AgNPs synthesized using the extract of the medicinal plant, *Melissa officinalis* L., on 3 cancer cell lines (A549, MCF-7, and HeLa).
**Methods:** AgNPs. were biologically synthesized using the extract of *M. officinalis*. After physical and chemical evaluation, the anti-bacterial properties of the synthesized nanoparticles were evaluated in *Escherichia coli* and *Staphylococcus aureus*.
**Results:** Finally, the inhibitory effect of synthesized nanoparticles was assessed by the MTT assay on 3 cancer cell lines. With an average size of 17 nm, the nanoparticles synthesized by *M. officinalis* L. extract had a significant inhibitory and lethal effect on 2 bacteria. The findings indicated that the synthesized nanoparticles had more inhibitory and bactericidal effects on *S. aureus* as a gram-positive bacterial strain. The MBC of nanoparticles synthesized by *M. officinalis* extract was 500 µg/mL for *S. aureus* and 700 µg/mL for *E. col.* At a concentration of 50 g/mL, the synthesized AgNPs showed more than 50% inhibitory effect on different cell lines. Our results demonstrated that medicinal plants can be used in the successful synthesis of biological AgNPs.
**Conclusion:** 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 ($Ag^+$).

**KEYWORDS:** Medicinal plant, Antibiotics, Synthesis, Cancer, *Melissa officinalis* L.

## 1. Introduction

Nanotechnology is known as one of the most widely used technologies worldwide, and the use of nanoparticles has been of interest in many sciences, including biology, medicine, electronics,

aerospace, agriculture, and food industry [1]. Nanoparticles represent unique chemical, biological, and physical properties, which can be attributed to their larger surface-area-to-volume ratio, high reactivity, and stability in chemical processes, as well as to their improved mechanical strength. Nanoparticles are used in many industrial and non-industrial products due to their low biohazards and high application safety [2].

Nanoparticles are currently considered a powerful and advanced chemical tool in the diagnosis and treatment of many dangerous diseases, including cancer [3]. Cancer is considered one of the main causes of mortality throughout the world; its very high prevalence has attracted a considerable number of studies for the treatment of cancer. Currently, surgery, radiation therapy, chemotherapy, immunotherapy, and combination diets are the main strategies for the treatment of this disease. Accordingly, the development of new and environmentally friendly therapeutic modalities seems to be necessary, while the cost of treatment should be lower in these methods [4].

Metal nanoparticles, including silver nanoparticles (AgNPs), have been widely utilized in the management of cancer. By the inhibition of cancer cells through silencing involved genes, these nanoparticles can prevent the spread of disease and improve it effectively. Furthermore, these nanoparticles can be used as carriers of effective drugs. Accordingly, in target tissues, the predominant accumulation of nanoparticles or drugs is often beneficial for improved therapeutic outcomes; however, it should be noted that a considerable degree of distribution to non-target tissues could lead to undesirable toxicity. Thus, to avoid any undesirable toxicity, tissue biodistribution and pharmacokinetic data are critical for the efficacious and safe biomedical applications of nanoparticles [5]. AgNPs can cause programmed death (apoptosis) of cancer cells by the production of reactive oxygen species (ROS), oxidative stress, and inhibition of the activity of vital enzymes.

The last decade has witnessed dramatic advances in the synthesis of metal nanoparticles with unique structures and properties. Certain stereotypical dimensions and structures, as well as the crystal shape of synthesized nanoparticles, are among the main goals of nanoparticle chemistry. These properties have led to potential applications of these particles in medicine so that nanoparticles are used as biosensors in the diagnosis and treatment of various diseases [6].

Improper use of chemical antibiotics has resulted in the development of various types of resistant pathogens [7]. Many pathogenic bacteria are now resistant to one or more antibiotics, and most of the available antibiotics have lost their effectiveness. The emergence of antibiotic-resistant bacteria continues to be a major human health challenge worldwide. To deal with this issue, the synthesis and formulation of metal nanoparticles for pathogenic bacteria have been examined in numerous studies and are under investigation [8]. Among metal nanoparticles, AgNPs have excellent antimicrobial properties and control bacteria with multiple mechanisms and effects on their cell walls and metabolic processes [9–11].

A number of novel approaches are being developed or enhanced to improve the physicochemical features of nanoparticles. In some methods, the synthesis process is modified in a way to increase their physical, mechanical, optical, chemical, and biological characteristics. Biosynthesis of metal nanoparticles using biological

compounds is one of the important achievements that has have been successfully used in the production of various metal nanoparticles with different structures and sizes. Medicinal plants are among the environmentally friendly precursors that have been frequently used in the production of nanoparticles [12].

The synthesis of metal nanoparticles using biological compounds found in medicinal plant extracts is considered a reliable and environmentally friendly method. Among the synthesized metal nanoparticles, AgNPs have received more attention due to their antimicrobial properties. Biosynthesis of AgNPs using plant extracts is a fast and cost-effective method, in which the metabolites present in the extract cause the oxidation and reduction of silver ions, and, finally, different types of AgNPs are produced with different shapes and sizes.

*M. officinalis* is one of the most widely used medicinal plants in traditional Iranian medicine. *M. officinalis* is a perennial herbaceous plant in the mint family and native to south-central Europe, the Mediterranean Basin, Iran, and Central Asia. *M. officinalis* is a perennial herbaceous plant in the mint family Lamiaceae. *M. officinalis* contains eugenol, tannins, and terpenes, 10-α-cadinol, 3-octanol, 3-octanone, α-cubebene, α-humulene, β-bourbonene, caffeic acid, caryophyllene, caryophyllene oxide, catechin, chlorogenic acid, cis-3-hexenol, cis-ocimene, citral A, citral B, copaene, δ-cadinene, eugenyl acetate, γ-cadinene, geranial, germacrene D, isogeranial, linalool, luteolin-7-glucoside, methylheptenone, neral and nerol.

The synthesis of AgNPs using herbs has been investigated in many studies. *M. officinalis* is one of the most widely used medicinal plants in Iran. Therefore, in this study, we tried to investigate the antimicrobial and toxicity effects of AgNPs synthesized by aqueous extract of *M. officinalis* on cancer cells.

## 2. Materials and methods

### 2.1. Characterization

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. The 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).

### 2.2. Preparation of the aqueous extract of M. officinalis

Aerial organs of *M. officinalis* L. were collected from the mountains of Lorestan Province (Makhmal kouh) 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 60 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 [13].

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.

### 2.3. Biosynthesis of AgNPs

To synthesize AgNPs, 5 mL of the aqueous extract of the studied medicinal plant was first transferred 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, AgNPs with a certain concentration were produced to prepare the aqueous extract [13].

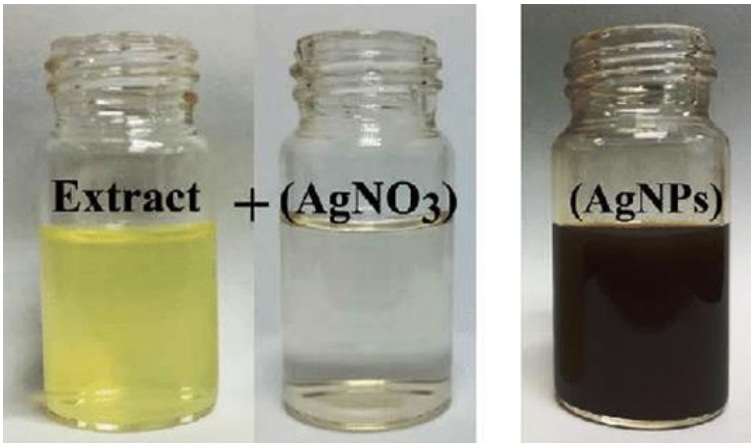


**Figure1.** A: plant extract B: Ag NO3 solution, C: Ag NPs

### 2.4. 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 AgNPs was first evaluated by spectrophotometry at wavelengths of 300–700 nm. The wavelength at which AgNPs showed the highest absorbance was determined based on the absorbance levels. The nature, structure, and size of the sample of the synthesized AgNPs were evaluated through scanning electron microscopy (SEM) and XRD spectroscopy [13].

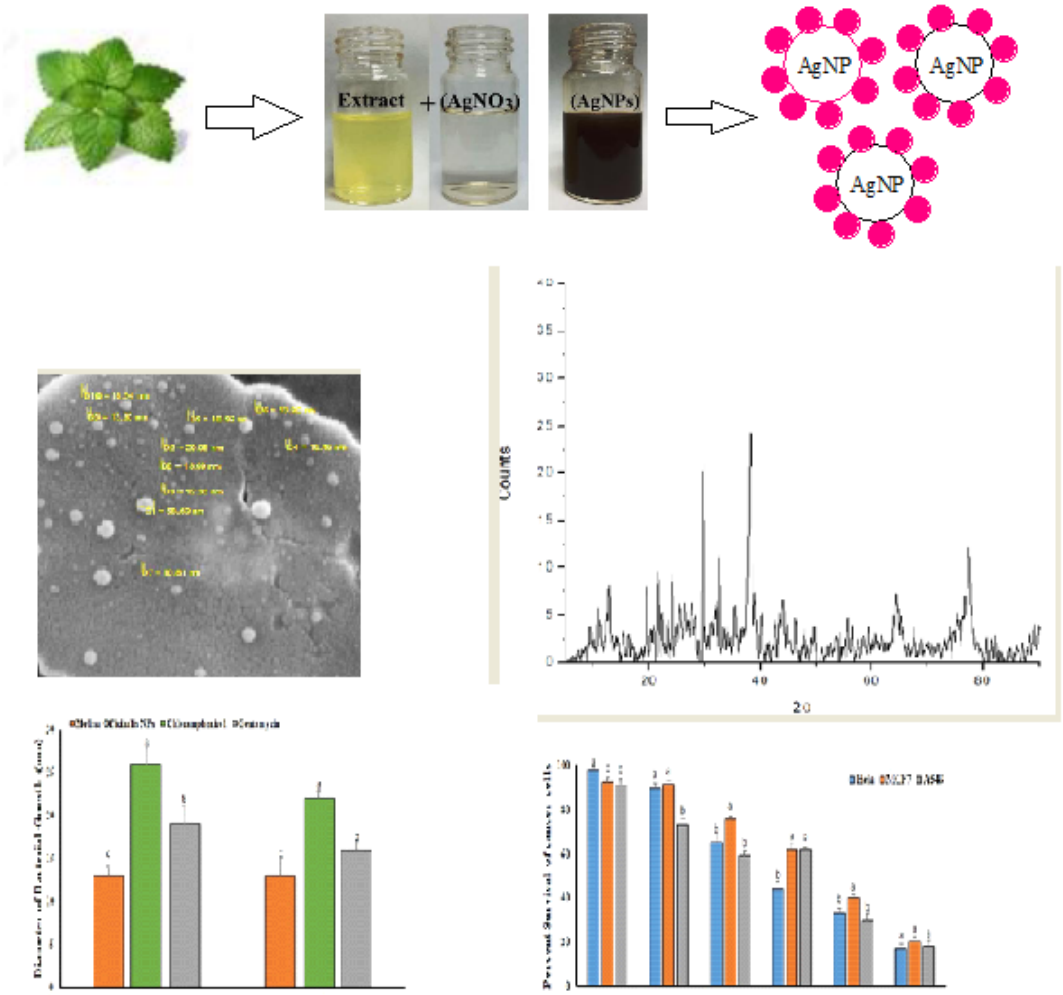


**Figure 2.** Synthesized nanoparticles

### 2.5. 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 [13].

### 2.6. Evaluation of the anticancer impact of the synthesized AgNPs

The anticancer impact of the synthesized AgNPs was evaluated by the MTT assay [13]. 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; the cell concentration was about $5 \times 10^4$ 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), 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). Each well was supplied with 20 µL 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 [13].

## 3. Results

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 [19]. The darkened $AgNO_3$ in the presence of the plant extract can be attributed to surface plasmon [2]. Based on the spectrophotometric results, the synthesized nanoparticles showed the highest absorbance at 453 nm (Figure 3).

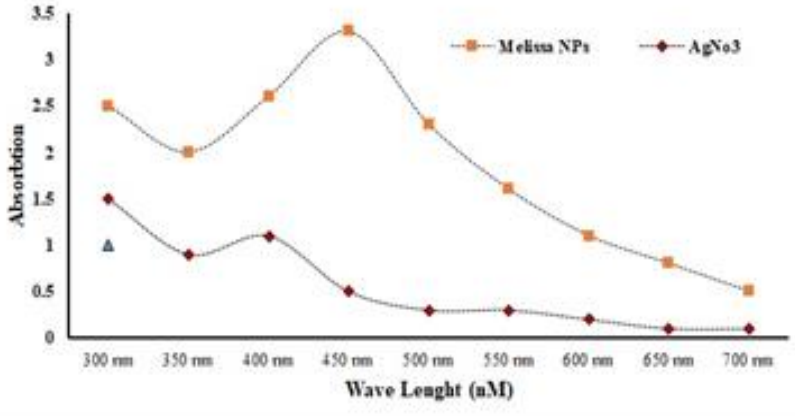


**Figure 3.** The UV-Vis absorption peak of Ag NPs

SEM was used to obtain the topological, compositional, and morphological information revealing the surface morphology of the nanoparticles. The average size of 100 nm was indicated by the SEM study (Figure 4). 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 5). The results of SEM revealed that AgNPs synthesized by the extract of *M. officinalis* were between 11 and 59 nm (17 nm on average) and spherical (Figure 4).

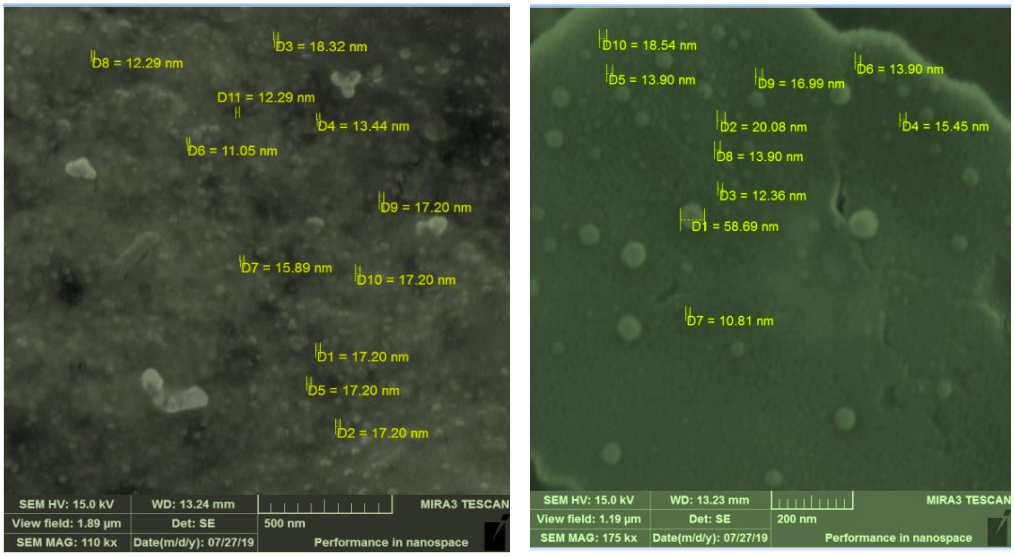


**Figure 4.** SEM image of Ag NPs taken at 500nm, and 200 nm

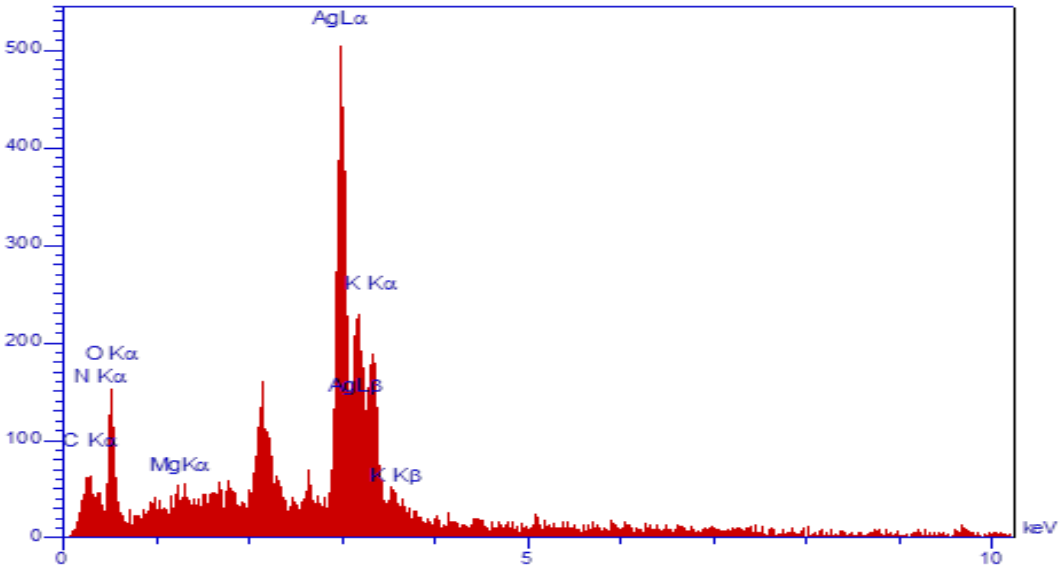


**Figure 5.** EDS elemental mapping of the Ag NPs

Figure 6 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, 5 obvious picks appeared at 38.09°, 44.1°, 64.3°, 77.5°, and 81.6° angles, which are consistent with (111), (200), (220), (311), and (222) 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). The diffraction angle found at 23.18° was caused by *M. Officinalis* leaf extract; AgNPs had an average size of 38.62 nm.

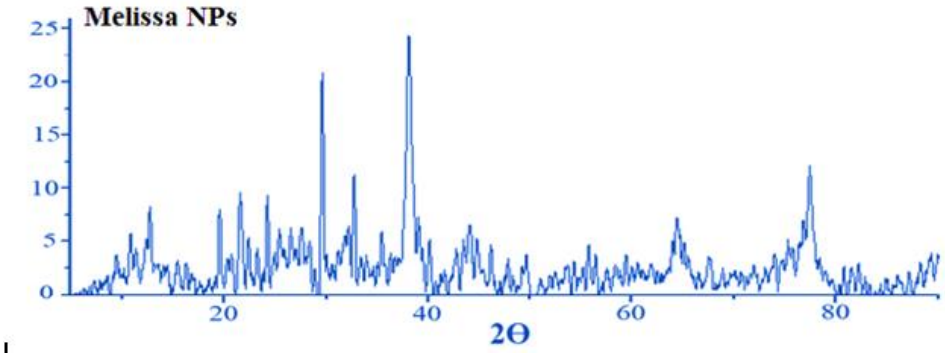


**Figure 6.** XRD pattern of Ag NPs

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 *M. officinalis* extract inhibited gram-positive and gram-negative bacteria (Figure 7).

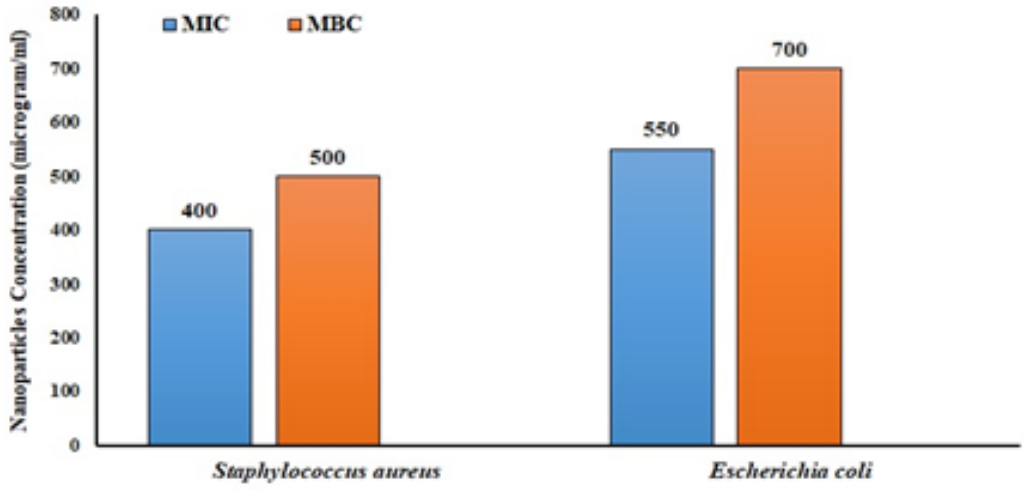


**Figure 7.** The results of the disk diffusion test showed AgNPs synthesized by the medicinal plant *M. officinalis*

The findings indicated that the synthesized nanoparticles had more inhibitory and bactericidal effects on *S. aureus* as a gram-positive bacterial strain. The MBC of nanoparticles synthesized by *M. officinalis* extract was 500 µg/mL for *S. aureus* and 700 µg/mL for *E. coli* (Figure 8).

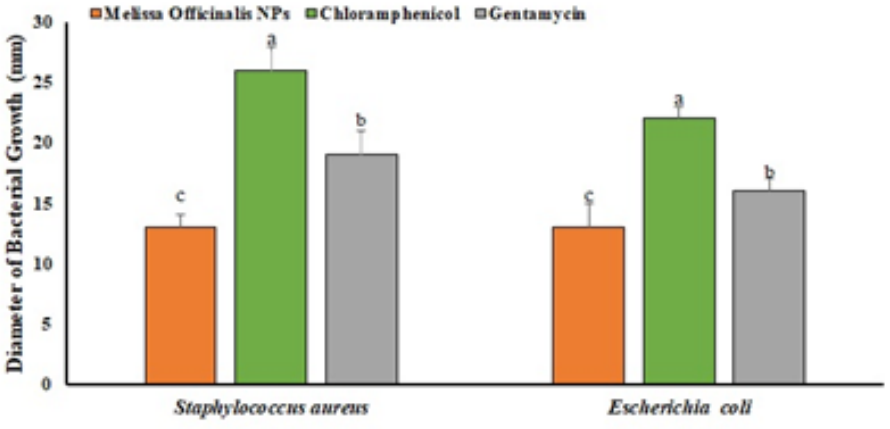


**Figure 8.** The results of the disk diffusion test showed AgNPs synthesized by the medicinal plant *M. officinalis*. Columns with similar letters are not statistically significant at the 5% level

The results of the disk diffusion test showed that both *E. coli* (ATCC 25922) and *S. aureus* (ATCC 29737) were influenced by the antibacterial effect of nanoparticles synthesized by *M. officinalis* extract with an average halo diameter of 13 mm. Based on the results of the MTT anticancer assay, the inhibitory effect of AgNPs 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 *M. officinalis* extract, which reduced the survival rate of different cell lines. The synthesized nanoparticles exerted the highest inhibitory effect at a concentration of 50 µg/mL (Figure 9).

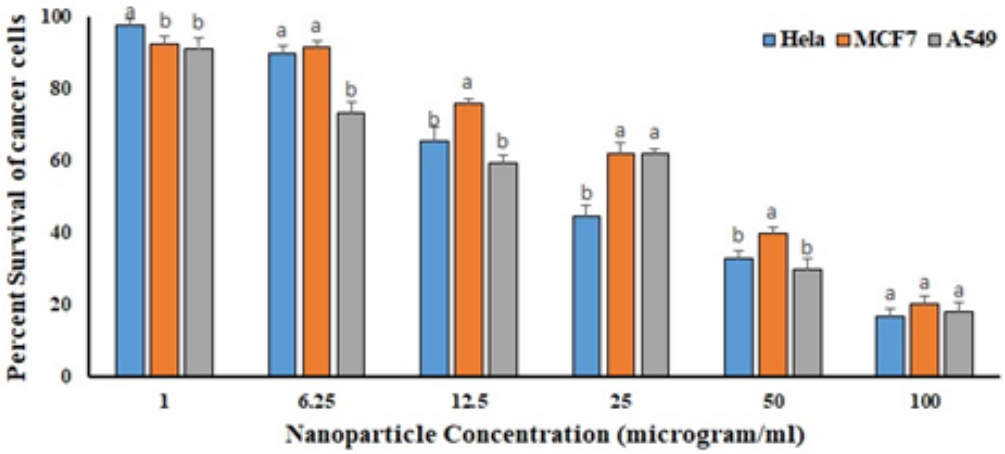


**Figure 9:** Survival rates of A549, MCF7, and HeLa cell lines against different concentrations of AgNPs synthesized by the medicinal plant M. officinalis (A) within 48 h. Columns with similar letters are not statistically significant at the 5% level.

## 4. Discussion

An average inhibitory effect of 40% was obtained on different cells using various concentrations of *M. officinalis* nanoparticles. The highest average inhibitory effect (45%) of AgNPs

synthesized by *M. officinalis* extract was observed on the A549 cell line. The HeLa cell line with 58% survival was ranked second in terms of sensitivity. According to the results, the HeLa cell line showed the lowest survival and the highest sensitivity with an average of 65% by the effect of *M. officinalis* nanoparticles (Fig. 9).

Biosynthesis of AgNPs using medicinal plants reduces production costs, and the produced nanoparticles possess special physicochemical properties that are of high value for use in a variety of medical and pharmaceutical applications. In the present study, an average diameter of 17 nm was obtained for AgNPs synthesized by the extract of *M. officinalis*. The size and shape of nanoparticles are important factors in their levels of antimicrobial effects. Nanoparticles of different sizes can have different interactions with the bacterial cell wall, being ultimately very effective in their levels of antimicrobial properties [16–17].

AgNPs synthesized at a wavelength of 450 nm showed the highest absorbance. Based on the results of previous studies, increased surface plasmon vibration and the absorbance of biological AgNPs have been reported in the range of 400–460 nm. Similarly, the present study reported the highest absorbance of nanoparticles synthesized by *M. officinalis* at a wavelength of 450 nm [18]. In a similar study, it was presented that nanoparticles synthesized by *M. officinalis* extract had a good inhibitory effect on gram-positive and gram-negative bacteria. However, *E. coli* gram-negative bacteria showed less sensitivity to the synthesized AgNPs. The maximum absorbance of biological AgNPs was estimated to be between 420 and 440 nm [19]. Since the content of secondary metabolites and their diversity are very different in medicinal plants, nanoparticles synthesized by medicinal plant extracts are highly different in terms of shape and size.

Bacterial resistance is now considered a major challenge in the healthcare sector. Pathogenic bacteria use several different mechanisms to develop resistance to different antibiotics. Mechanisms of resistance vary between bacteria, and gram-positive and gram-negative bacteria have been shown to be different in this respect. The first barrier against antimicrobial compounds is the bacterial cell wall, which is thicker in gram-positive bacteria, e.g., *S. aureus*. However, gram-negative bacteria, e.g., *E. coli*, have a higher resistance to the entry of antimicrobial agents than gram-positive bacteria due to the presence of a lipopolysaccharide, purine-rich. in outer layer [20].

Our synthesized nanoparticles showed a suitable antimicrobial effect on *S. aureus* and *E. coli*. The MBC of nanoparticles ranged between 500 and 850 µg/mL; however, *E. coli* as a gram-negative bacterial strain indicated less sensitivity. The antimicrobial effect of nanoparticles synthesized by the *M. officinalis* medicinal plant has been reported in previous studies [19–21].

Metal nanoparticles, including AgNPs, have been of interest to many researchers due to their various physicochemical properties and antibacterial effects [22]. Suppression of cancer cells using metal nanoparticles reduces the unwanted side effects of disease treatment.

The results indicated a significant difference between the inhibitory effects of the synthesized nanoparticles on different cell lines; in other words, the type of the medicinal plant was effective in the inhibitory effect of the synthesized nanoparticles. Additionally, the survival rate of cancer cells was positively correlated with the given dose of AgNPs. At a concentration of 50 µg/mL, the synthesized AgNPs led to more than 50%

inhibitory and bactericidal effects on various cancer cells. Overall, AgNPs synthesized by *M. officinalis* extract indicated a good inhibitory impact on the tested cancer cells.

The cytotoxicity of AgNPs synthesized by plant extracts against breast cancer cells (MCF-7) was observed by the MTT assay [23]. The anticancer effect of synthesized nanoparticles was due to their spherical shape and small size, thereby easily penetrating cancer cells. Biological AgNPs could have effective toxicity against various cancer cells, and the highest lethal effect was observed in MCF-7 breast cancer cells so 65 µg/mL of AgNPs killed 50% of cancer cells, which is in line with our study [24]. The surface charge of prokaryotic and eukaryotic cells has a vital role in the toxicity of AgNPs. Eukaryotic cells are surrounded by a negatively charged phospholipid membrane. For this reason, positively charged AgNPs are expected to have a high affinity for binding to cell membranes. The membranes of cancer cells (e.g., HeLa) are richer in negative charge than that of normal cells. Therefore, the affinity to attract $Ag^+$ ions by the membranes of cancer cells is one of the factors that potentially affect the effectiveness of nanoparticles in the suppression of cancer cells [9].

The major lethal effect of AgNPs is the release of Ag+ ions as an intracellular toxicant. There is evidence that the cellular environment surrounding cancer cells is more acidic compared with normal cells. The reason is that Ag+ ions are released faster and easier in an acidic environment, and concentration increases in the environment surrounding cancer cells and will cause their eradication [21].

The size and shape of AgNPs are among the critical factors affecting their biological function. Small nanoparticles with a diameter of ≤10 nm can easily penetrate cancer cells and have a more toxic effect than larger nanoparticles [14]. In addition to the size and shape of nanoparticles, their cytotoxicity is affected by the coating type of biological nanoparticles [17]. Spherical nanoparticles (<150 nm) easily react with cell surfaces and show their toxic effect. However, the toxicity of AgNPs is more affected by the used doses [16].

As a medicinal plant, *M. officinalis* contains a variety of secondary metabolites from different groups, including alkaloids, phenols, and flavonoid compounds with proven antimicrobial and anticancer effects [25]. In the biosynthesis of metal nanoparticles, secondary metabolites are considered the most important coating factors and stability of nanoparticles. These biological compounds can be used as key factors in the synthesis of nanoparticles due to their high environmental compatibility and high consumption safety. The high antioxidant potential of secondary metabolites, including phenols and flavonoid compounds, has been demonstrated in a number of studies. Therefore, nanoparticles coated with secondary metabolites play an important role in cancer prevention in normal cells, besides having a lethal effect on cancer cells [26].

AgNPs are broadly utilized in the management of diseases and the production of drugs due to their multiple physicochemical and biological properties. Our findings revealed that medicinal plants could be successfully used in the biosynthesis of metal nanoparticles, including AgNPs, which could be utilized in the treatment and prevention of many infections and chronic diseases due to their antimicrobial properties and inhibitory effect on different cancer cell lines. As the main factor in the biosynthesis of AgNPs, secondary metabolites in medicinal plants cause the stability and coating of

nanoparticles. With beneficial biological properties, such as antioxidants, antimicrobials, and anticancer, these compounds will be very effective in the improved function of AgNPs. Despite their significant antimicrobial and anticancer properties, AgNPs are highly toxic to various human cells, including liver and brain cells. However, the biosynthesis of AgNPs reduces their toxicity. AgNPs are stabilized when coated by secondary metabolites of medicinal plants, which prevents their accumulation. The bio-coating on AgNPs makes them very suitable for medicinal applications [27]. Infectious and chronic diseases have a high prevalence and today drug resistance to infectious diseases has increased [28, 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 [30-33].

## 5. Conclusion

Our results demonstrate that medicinal plants can be used in the successful synthesis of biological AgNPs. 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 ($Ag^+$). Accordingly, further studies seem to be necessary concerning the effect of AgNPs on natural cells to use their biological benefits more safely.

**Authors' contribution:**

All authors contributed equally to the manuscript.

**Conflicts of interest:**

The authors declared no competing interests.

**Ethical considerations:**

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

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