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Biosynthesis of Silver Nanoparticles Using Tagetes Plant Extracts: Antimicrobial and Anticancer Activity Assessment

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A B S T R A C T

Objective: The use of silver nanoparticles is increasing due to its unique properties. plant Tagets, a tall-based medicinal plant, is rich in bioactive compounds. The aim of this study was to investigate the anticancer and antimicrobial effects of silver nanoparticles synthesized using the aqueous extract of Jafari flower on cervical cancer (Hella), breast cancer (MCF7), and lung cancer (A549) cell lines.

Methods: The toxicity of silver nanoparticles was evaluated using the MTT assay, and the results were acceptable. The synthesized nanoparticles were confirmed using UV-Vis, SEM, and XRD analyses.

Results: The nanoparticles synthesized using the plant Tagets extract had a maximum absorption wavelength of 410 nm in the UV-Vis spectrum. The XRD and SEM patterns showed that the nanoparticles were mostly spherical in shape, with an average size in the nanometer range. The MTT results showed that the silver nanoparticles had dose- and time-dependent cytotoxic effects, significantly reducing cell viability. The IC50 values for the three cancer cell lines were 63.16, 40.19, and 63.39 µg/mL for MCF7, Hella, and A549, respectively, with the lowest IC50 value observed for cervical cancer.

Conclusion: Medicinal plants can be used in the synthesis of silver nanoparticles. Thus, silver nanoparticles have antimicrobial effects, which alter the morphology of bacterial membranes, increasing the permeability of silver nanoparticles, leading to uncontrolled penetration into cells, and ultimately causing cell death

Keywords: Silver nanoparticle, Cellular toxicity, Cancer, Antimicrobial, Plant Tagets

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Introduction

Nanotechnology is a field of applied science and technology that encompasses a wide range of sciences, such as pharmaceuticals, drug design, and biology [1]. In nanotechnology, materials with dimensions less than one micrometer, typically ranging from 8 to 800 nanometers, are used. Synthesis of nanoparticles involves different physical and chemical methods, including chemical reduction, lithography, electrochemistry, laser, and microwave irradiation. One of the disadvantages of chemical methods that play the role of reducing and stabilizing agents [1]. Recently, the biogenic synthesis of silver nanoparticles by natural and biological agents such as bacteria, fungi, and plants has attracted the attention of researchers. One of the biological methods is the green synthesis method, in which metal ions are converted into silver nanoparticles in a single-step reaction using plant compounds without the need for surfactants and other stabilizing agents [1]. In recent years, due to the increasing prevalence of cancer-related deaths and the shortcomings of chemotherapy and radiotherapy in advanced forms of cancer, the need for finding new methods for cancer control is felt, and one of these methods is the use of

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nanoparticles, especially silver nanoparticles [2]. Silver nanoparticles are one of the common nanoparticles used in nanotechnology, with various applications in biosensors, pharmaceuticals, and medicine. They can be used as antibacterial and anticancer agents, showing special physicochemical and biological properties. They are important antibacterial agents against a wide range of antibiotic-resistant bacteria. In general, the major properties of silver nanoparticles include non-toxicity, high stability, water compatibility, thermal resistance, and no creation or increase of resistance in microorganisms [3].

Plant Tagets is an ornamental plant with a widespread global distribution and is among the plants that can easily be propagated and grown in most climatic regions. Also, due to the presence of a large number of effective chemical compounds such as flavonoids and carotenoids, and antibacterial and antioxidant properties, etc., it has been the subject of research. Therefore, in recent years, various plant extracts have been used for the synthesis of silver nanoparticles. Helmy etal showed that all aqueous extracts and biosynthesized extracts from a combination of parsley, corn silk, and gum Arabic exhibited different degrees of antioxidant, anti-inflammatory, and antimicrobial activities, but the silver nanoparticles biosynthesized from parsley extract were the most effective [4]. Therefore, silver nanoparticles synthesized by a combination of three aqueous extracts can help to enhance antioxidant, anti-inflammatory, and antimicrobial activities. Additionally, Liberal et al expressed that there is good bioactivity against specific tested pathogens such as bacteria and fungi [5]. Consequently, Tagets leaves and flowers are a good source of natural bioactive compounds that have health benefits, and therefore, they should be part of a balanced and diverse diet. Considering the cost-effectiveness and lack of environmental toxicity of plant extracts for the synthesis of silver nanoparticles, as well as the anticancer and antimicrobial effects of silver nanoparticles, the aim of this study is to use tall parsley as a medicinal plant for biosynthesis of silver nanoparticles and to investigate their antimicrobial and anticancer effects using the MTT method.

Materials and Methods

Preparation of the herbal extract for the stud

plant tagets were collected from Lorestan Province. 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. 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 [6].

Biosynthesis of AgNPs

To synthesize AgNPs, 5 mL of the aqueous extract of the studied medicinal plant was first transferred to 95mL of 1 mM silver nitrate (AgNO3) 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 (Figure1) [6].

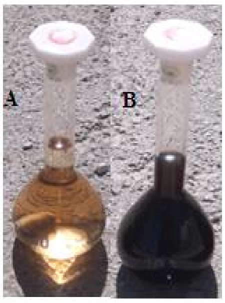

Figure1: A: Plant Extract B: AgNPs

Antibacterial Evaluation of Synthesized Silver Nanoparticles

To study the antimicrobial effects of the synthesized nanoparticles, two gram-positive and gram-negative bacteria, Staphylococcus aureus (PTCC 1112) and Escherichia coli (PTCC 1330), were used. First, the microdilution method and tetrazolium chloride were used to determine the minimum inhibitory concentration (MIC) of the synthesized silver nanoparticles. After determining

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the minimum inhibitory concentration, the minimum bactericidal concentration (MBC) was determined. For this purpose, bacterial suspensions of E. coli and S. aureus were cultured on LB agar plates in the MIC test. Then, the disk diffusion test was used to evaluate the bactericidal effect of the synthesized silver nanoparticles [6].

Evaluation of the Anticancer Effects of Synthesized Silver Nanoparticles

Cell viability was evaluated by the MTT colorimetric technique with slight modification. The anticancer activity of the synthesized silver nanoparticles was evaluated invitro against cancer cell lines after 24 h exposure and their IC50 values were determined from a graph of cell viability measured over a range of concentrations between 1 and 100 μg/mL. The IC50 was determined at a broad range of concentrations specifically 1, 10, 25, 50 & 100 μg/mL against the cell lines. For this data, a line graph was plotted between concentrations (Xaxis) versus % inhibition (Y-axis) and then an intersection drawn at 50% inhibition on Yaxis and then correlated to the concentration value on X-axis. To measure the cytotoxic effects of the synthesized silver nanoparticles on cell viability, the MTT assay was used. The cancer cell lines Hela (cervical cancer), MCF7 (breast cancer), and A549 (lung cancer) were purchased from the Pasteur Cell Bank in Tehran, Iran. In this method, a yellow-colored salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, abbreviated as MTT) was used. The active cells are converted to insoluble and purple formazan by mitochondrial dehydrogenase enzymes, which are then dissolved in DMSO and their optical absorbance is measured. Cancer cells were cultured in 96-well plates at a concentration of 10,000 cells per well for 24 hours. Then, the cells were treated with different concentrations of synthesized silver nanoparticles (1, 6.25, 12.5, 25, 50, and 100 mg/mL) for 72 hours. After this period, 20 microliters of MTT solution (5 mg/mL) were added to each well. After 4 hours, the plates were removed from the incubator and their contents were discarded. Then, 100 microliters of DMSO solution (dimethyl sulfoxide) were added to each well todissolve the formazan. The absorbance of the resulting solution was measured using a microplate reader. The results showed that the synthesized silver nanoparticles had a significant cytotoxic effect on the cancer cells, with higher concentrations resulting in more significant cell death. The MTT assay provided valuable information on the potential anticancer effects of the synthesized silver nanoparticles and can be used in future studies to evaluate their efficacy and safety. A concentration that inhibited the growth of cancer cells by 50% was considered as the inhibitory concentration (IC50) 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.[6].

Results

The addition of the plant extract to the AgNO3 solution triggered redox reactions and darkened the color of the reaction mixture. In many papers, a change in the color of an extract-AgNO3 mixture has been reported as the first sign of nanoparticle synthesis [7]. The darkened AgNO3 in the presence of the plant extract can be attributed to surface plasmon [8]. In the section on biosynthesis of silver nanoparticles using the aqueous extract of the plant Tagets, the color of the reaction medium changed from yellow to dark brown after 30 minutes, indicating the synthesis of silver nanoparticles. The reaction was allowed to proceed for 24 hours for complete conversion.

The synthesized silver nanoparticles were confirmed and characterized by the following methods; UV-Vis spectroscopy, scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT-IR).

UV–Vis spectroscopy is generally recognized as an important technique to examine the metal nanoparticles in aqueous suspensions [9].

The UV-Vis spectrum of the synthesized silver nanoparticles using the aqueous extract of the plant was examined. The wavelength at which silver nanoparticles showed the highest absorbance was determined based on the absorbance levels. After mixing silver salt in a solution of the extract, a sharp absorption peak at 410 nm was observed after 24 hours at a concentration of 10 mM and pH of 5.5(Figure 2).

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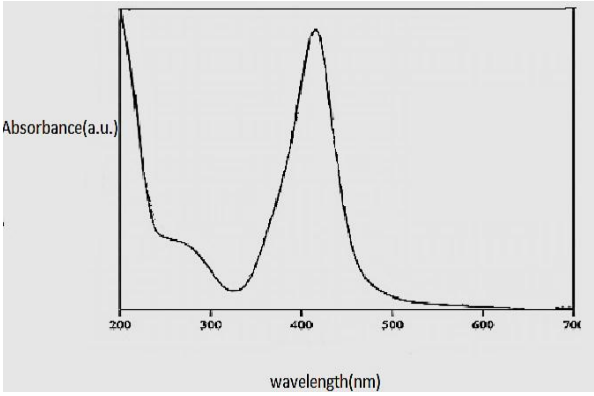

Figure 2: The UV-Vis spectrum of the synthesized silver nanoparticles

The crystalline nature of silver nanoparticles was confirmed by the analysis of XRD studies.To confirm the synthesis of silver nanoparticles using the aqueous extract of the plant tagets, the crystal structure of the nanoparticles was studied using X-ray diffraction (XRD). The presence of diffraction peaks at angles of 73.43°, 22.55°, 44.59°, 40.80°, 69.82°, 99.86°, and 52.89° in the plant confirms the cubic structure of the synthesized silver nanoparticles.

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 3).

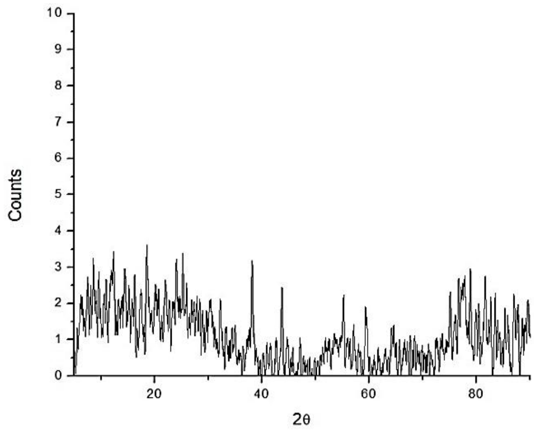

Figure 3: The X-ray diffraction pattern of the synthesized silver nanoparticles

EDX spectrometers confirmed the presence of elemental silver signal of the silver nanoparticles. The vertical axis displays the number of x-rays counts whilst the horizontal axis displays energy in KeV. Silver nanoparticles generally show typical absorption peak approximately at 3 KeV due to surface plasmon resonance and these correspond with peaks in the spectrum, thus giving confidence that silver has been correctly identified.

The EDX analysis validated the elemental composition of the synthesized AgNPs. The EDX spectrum of the synthesized silver nanoparticles using the plant tagets is shown in Figure 4, indicating the presence of silver. Other elements are also observed in the spectrum, indicating the presence of organic compounds remaining from the plant extract on the surface of the nanoparticles.

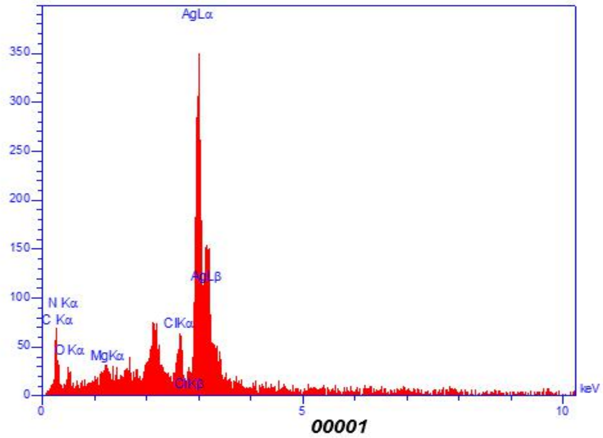

Figure 4: EDX spectrum for elemental analysis of silver nanoparticles synthesized

Scanning electron microscopy (SEM) was performed to observe the topology and size of the NPs.SEM was used to obtain the topological, compositional, and morphological information revealing the surface morphology of the nanoparticles. The images taken by scanning electron microscopy show the morphology of the silver nanoparticles synthesized using the which plant, indicating that the size of the produced nanoparticles is variable and in the range of 10 to 26 nanometers in the plant tagets. (Figure 5).

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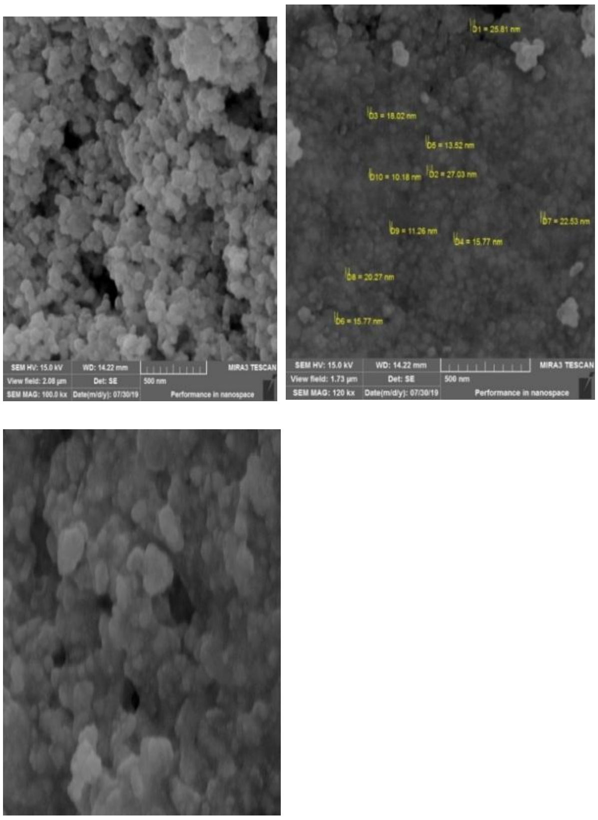

Figure 5: Scanning electron microscope images of silver nanoparticles synthesized

The antimicrobial effect of the synthesized nanoparticles was evaluated after confirming the synthesis of nanoparticles and examining their physical properties. The minimum inhibitory concentration (MIC) is the lowest concentration of a chemical that prevents visible growth of bacteria under specific conditions [10]. The results of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests to determine the antimicrobial effects of the synthesized nanoparticles on Gram-negative and Gram-positive bacteria such as Escherichia coli and Staphylococcus aureus showed that the nanoparticles synthesized with plant tagets aqueous extract inhibited both Gram-negative and Gram-positive bacteria. Regarding the effect of nanoparticles on different bacteria, the results indicated that the synthesized nanoparticles had a greater inhibitory and bactericidal effect on Gram-positive Escherichia coli. The minimum bactericidal concentration for Escherichia coli was 800 micrograms per milliliter, and for Staphylococcus aureus, it was 700 micrograms per milliliter for the nanoparticles synthesized with plant tagets extract (Figure 6).

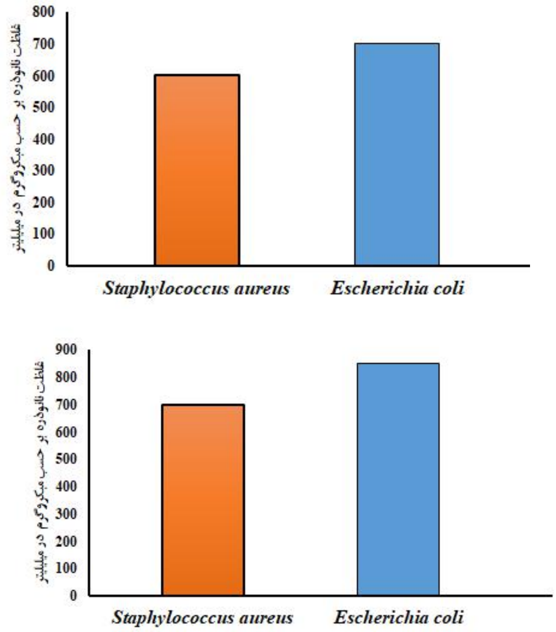

Figure 6: Results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests of silver nanoparticles on Escherichia coli and Staphylococcus aureus bacteria.

Disk diffusion analysis

Antimicrobial activity of synthesized silver nanoparticles was performed using the disk diffusion method against two different pathogens including, B. cereus (Gram-positive) and E. coli (Gram-negative). Based on measuring the diameter of the cloud's lack of growth, the antimicrobial effect of Ag NPs enhanced by increasing their concentration [10].

The results of the disk diffusion test showed that the silver nanoparticles synthesized with plant tagets, with an average halo diameter of 30 millimeters, had antibacterial effects on both Escherichia coli and Staphylococcus aureus bacteria. According to the results, Staphylococcus aureus showed more sensitivity to the synthesized silver nanoparticles [11] (Figure 7).

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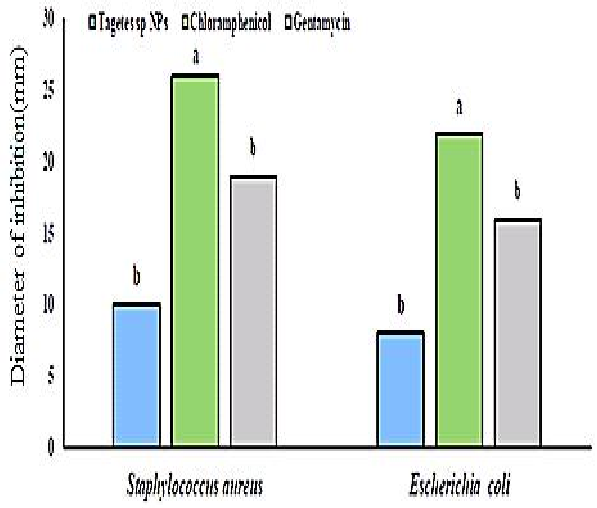

Figure 7: disk diffusion test and antibacterial effects of synthesized silver nanoparticles on Escherichia coli and Staphylococcus aureus bacteria.

Cell treatment with silver nanoparticles synthesized with plant tagets aqueous extract was evaluated for their anticancer effects on A549 lung, MCF7 breast, and Hela cervical cancer cell lines. The IC50 values for MCF7, Hela, and A549 cancer cell lines were 63.16, 40.19, and 63.39, respectively, indicating that the lowest IC50 value corresponded to the highest toxicity. Compared to the other two cancer cell lines, the IC50 value for Hela cancer cells was lower. In general, it can be concluded that the synthesized silver nanoparticles from plant tagets aqueous extract have anticancer effects, and their anticancer effects depend on changes in concentration. The viability of cancer cells decreases as the concentration of biogenic nanoparticles synthesized increases to 100 micrograms per milliliter. Among the three cancer cell lines investigated, the effect of silver nanoparticles on A549 lung cancer cells was greater, reducing their viability percentage (Figure8).

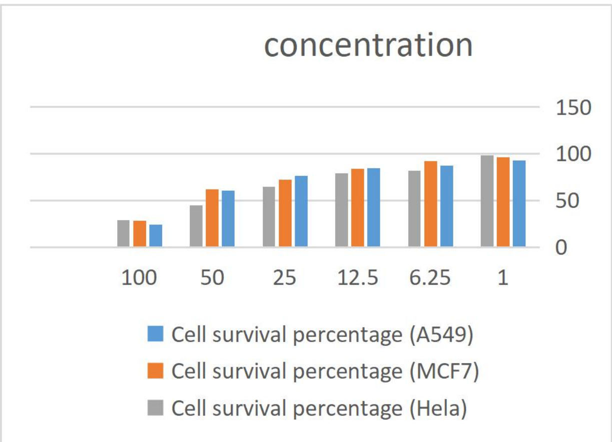

Figure8: Cell survival percentage of A549, MCF7, and Hela cell lines at different concentrations of silver nanoparticles synthesized from tall parsley plant extract.

Discussion

Using natural compounds for the production of nanoparticles is a new approach that has attracted a lot of attention, especially for the production of metallic nanoparticles. One of the methods for producing these nanoparticles is the use of plant, fungal, and bacterial extracts. The aim of this study was to produce silver nanoparticles using an aqueous extract of the Tagetes plant and to investigate their antibacterial and anticancer effects. One of the applications of silver nanoparticles is their antibacterial and anticancer effects. The results of the study showed that the synthesized silver nanoparticles had an average size of 22.89 nanometers and remarkable antibacterial and anticancer effects [12]. Studies have shown that silver nanoparticles have antibacterial effects, causing changes in the morphology of bacterial membranes [13], increasing the permeability of silver nanoparticles, and causing uncontrolled penetration of silver nanoparticles into the cell, leading to cell deathjj. Therefore, this study investigated the green synthesis method of silver nanoparticles using Tagetes aqueous extract and their antibacterial and anticancer effects. Based on the results obtained, the use of Tagetes aqueous extract for the synthesis of silver nanoparticles can be considered as a green and sustainable method for the production of nanoparticles with antibacterial and anticancer effects. Additionally, the results of the current experiments showed that the synthesized silver nanoparticles using Tagetes extract have considerable antibacterial and anticancer effects and can be used as an antibacterial and anticancer agent in various industries, especially in the pharmaceutical and medical industriess. In this study, the color change from pale yellow to dark brown, observed during the interaction of the plant extract and silver nitrate solution, indicates the synthesis of silver nanoparticles and confirms the reduction of silver ions. In bulk form, silver has an absorption peak at 410 nanometers. In this study, the presence of a peak around 400 nm, which indicates the formation of silver nanoparticles, is consistent with the findings of other researchers.

The XRD pattern of the synthesized nanoparticles showed four distinct peaks at angles of 43.73, 55.22, 59.44, 80.40, 82.69, 86.99, and 89.52 degrees, which was consistent with the results of other studies. SEM was used to investigate

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the shape and size of the synthesized nanoparticles, and the results showed that the size of the silver nanoparticles synthesized using plant aqueous extract ranged from 10 to 26 nanometers. The change in color from light green to dark brown was observed in the biosynthesis of silver nanoparticles through the

The results of this study on the antibacterial activity of silver nanoparticles synthesized using Tagetes extract were against Staphylococcus aureus (A) and Escherichia coli. These results were consistent with the study by Helmy and et al entitled Synergistic effects of silver nanoparticles biosynthesized from a combination of Tagetes, corn silk, and gum Arabic extracts, their antioxidant, anti-inflammatory, and antibacterial activities [14]. In another part of this study, the anticancer effects of silver nanoparticles synthesized using Tagetes extract on three cancer cell lines, including Hela (cervical), MCF7 (breast cancer), and A549 (lung), were investigated. The results showed that the cytotoxicity of the cells depended on the concentration of the synthesized silver nanoparticles. on the investigation of the bioactive properties and phenolic compounds of different varieties of parsley, including root, leaf, and petiole, which showed good bioactivity against tested pathogens such as bacteria and fungi [15].

The results of this study suggest that tagets plant aqueous extract can be used as a green and sustainable method for the synthesis of silver nanoparticles with antibacterial and anticancer properties [16]. The antibacterial effects of silver nanoparticles were attributed to their ability to cause changes in the morphology of bacterial membranes, leading to increased permeability and uncontrolled penetration into the cell, resulting in cell death [17]. The anticancer effects of silver nanoparticles were concentration-dependent and observed on the three cancer cell lines [18]. Overall, this study shows that the use of natural compounds such as tagets extract for the synthesis of nanoparticles is not only a sustainable method, but also has great potential for the advancement of nanotechnology applications in health and medical fields. According to the results of this study, the cytotoxic effects of silver nanoparticles synthesized using tagets plant extract on Hela, MCF7, and A549 cancer cells were dependent on their concentration, with a greater inhibitory effect observed on A549 lung cancer cells compared to the other two cancer cell lines. The viability percentage of A549 lung cancer cells also decreased with an increase in the concentration of the synthesized silver nanoparticles. These results show that silver nanoparticles synthesized with tagets extract can have anticancer properties, but more preclinical and clinical research is needed to evaluate the safety and optimal dosage and possible side effects. Medicinal plants and plant-based antioxidants [18-21] can serve as a suitable source for the synthesis of nanoparticles [19-22].

Conclusion

The plants can be also good source for synthesis of silver nanoparticles. This green chemistry approach toward the other chemical and physical synthesis of silver nanoparticles has many advantages such as, cost effective, environment friendly, easily scaled up for large scale synthesis and in this method, there is no need to use high pressure, energy, temperature and toxic chemicals. In addition, the produced AgNPs by peel extract are suitable for pharmaceutical and biological applications due to they have not any chemical contaminations.

Declaration of Competing Interest

Funding/Support

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Ethics approval

This study was performed in line with the principles of the **Declaration of Helsinki.**

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