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Research Article

Green Synthesis of Silver Nanoparticles from *Alhagi graecorum* Leaf Extract and Evaluation of Their Cytotoxicity and Antifungal Activity

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Green synthesis of silver nanoparticles (AgNPs) using different plant parts has shown a great potential in medicinal and industrial applications. In this study, AgNPs were *in vitro* green synthesized using *A. graecorum*, and its antifungal and antitumoractivities were investigated. Scanning electron microscopy (SEM) image result indicated spherical shape of AgNPs with a size range of 22-36 nm indicated by using Image J program. The functional groups indicated by Fourier-transform infrared spectroscopy (FTIR) represented the groups involved in the reduction of silver ion into nanoparticles. *Alhagi graecorum* AgNPs inhibited MCF-7 breast cancer cell line growth in increased concentration depend manner, significant differences shown at 50, 100, and 150 μg/ml concentrations compared to the control. Strong antifungal activity against *Candida* species (*C. albicans.*, *C. glabrata*, *C. parapsilosis*, *C. tropicales*, and *C. krusei*) was observed and the inhibition zone range from 14-22 mm at a concentration of 0.01 mmol/ml and from 17-27 mm at a concentration of 0.02 mmol/ml. Based on our findings, it is concluded that synthesized silver nanoparticles from *A. graecorum* can be used as a potential antitumor and antifungal agent for various therapeutical applications.

1. Introduction

The passion for nanotechnology applications has increased recently, as nanoscale materials facilitated a major breakthrough in research and human life. Nanomaterials have mechanical, thermal, physiochemical, biological properties, and other properties that differ from the raw materials due to their large surface area to size ratio and quantum effect [1]. There are multiple methods for producing nanomaterial mechanically and chemically but they are not safe for the environment. As a result, scientists have looked for safe ways to produce nanomaterials, such as using fungi [2], bacteria, or plants [3]. Because of the limitations of chemical and physical methods that have been used for producing nanomaterials such as the production costs, researchers have developed alternative biological approaches which are clean, economical, and environmentally friendly [4]. Biological synthesis involved three stages by which stabilized NPs are synthesized, and these are including solvent used, environmental safe reducing agent, and nontoxic capping agent [5].

The presence of different phytomolecules in plants such as polyphenol, flavonoids, varies proteins, and sugars act as reducing and coating agents in the of NPs. However, a number of issues such as the synthesized methods used for

temperature and time, pH and pressure, and size and morphology significantly impact the synthesized nanoparticles using green technology and consciously their influence on cell behavior.

Metallic nanoparticles have several properties including stability, and activity and its distinguishing surface plasmon resonance make it as an effective drug carrier and diagnosis therapeutic agent. Engineered metals have been used to synthesized NPs by modified their surface shape and size; biomedicine studies have been performed in production of green-synthesized metallic NPs and its applications as anticancer, antimicrobial, anti-inflammatory, and antioxidant agents [6, 7]. The most common metallic NPs used as drug carrier are silver and gold due NPs to its unique properties, stability, thermal, and ability to reduce cell proliferative and promote cell death.

The use of plants to produce silver nanoparticles is of greater benefit than other biological methods, and the number of publications reported the use of plant extract significantly increasing each year (about 468 publications in 2020) [8]. The advantage of using plants in biosynthesis of nanoparticles is economic and does not use toxic chemicals or hazard process, hence, green synthesized of AuNPs with antioxidant and cytotoxic properties might be a potential direction for the development of nanomedicine.

Alhagi plant species, known as camelthorns or manna tree, is belonging to the family Fabaceae. The herbs of the *Alhagi* plant are widespread in central, northern, and southern Africa, as well as in the Middle East, Europe, and northwest China and North America [9]. The plant is found in abundance dry lands, which is associated with less rainfall and areas of salinity and high alkalinity [10]. It is in the form of green, noxious bushes 1-2 meters high with simple leaves and thorny branches with a deep root system extending for about 15 meters, and these intakes grow randomly [11]. In folk medicine, dried *A. graecorum* plant is used to treat rheumatic pain and is used as a laxative and for treating bilharzias worms [12]. In addition to its usage as treatment for urinary tract infections, liver disorders, and many gastrointestinal disorders, many parts of the plant have also been used to treat bleeding cases [13, 14]. Chinnathambi and Alahmadi [15] reported the synthesis of ZnONPs using *A. maurorum* leaf extract, and the spherical particles were in the size of 27.92 nm with effective antioxidant and antiosteosarcoma. Similarly, Malik and Sehrawat [16] used *A. maurorum* leaf aqueous extract for the synthesis of AgNPs, and the size was in the range of 16-30 nm and the functional groups of hydroxyl, amine, and carboxyl. Also, gold NPs were synthesis from *A. maurorum* flower, and the average rang of size particles was of 12-24 nm and with high stability and antimicrobial activity which suggested an efficient and consistent synthesis method [17] .

One of the most important public health problem for therapists is antimicrobial resistance, so using nanotechnology to find better drug delivery has triggered researcher. It has been reported that mycogenic AgNPs synthesized from *Penicillium chrysogenum* showed an effective antibacterial and biofilm inhibitory activity against pathogenic *A. baumannii* [18)]. The current study was designed to achieve the synthesis of AgNPs from aqueous extract of *A. graecorum* and determined the characteristic properties of newly synthesized NPs and assessment of its antifungal activity.

2. Materials and Methods

2.1. Chemicals. Cell culture medium (RPMI-1640) and associated reagents were obtained from Invitrogen (Life Technologies Ltd, Paisley, UK), and sabouraud dextrose agar fungal media and silver nitrate ($AgNO_3$) were purchased from (Sigma Aldrich, Banglore, India). Isolates of pathogenic yeast (*Candida species*) were obtained from a number of patients who visited a number of hospitals in the city of Baghdad. The yeast was diagnosed by standard biochemical methods in the Department of Biology—College of Education Ibn Al-Haitham for Pure Sciences—University of Baghdad. All other reagents and chemicals, unless otherwise stated, were obtained from Fisher Scientific Ltd (Loughborough, UK).

2.2. Plant Sample Collection. Fresh samples of *Alhagi graecorum* plant were collected from the Abu Ghraib area (located west of the Capital, Baghdad). The leaves were cleaned with tap water, then rinsed with distilled water and left to dry at room temperature. After drying, electrical grinder was used to grind the leaves into powder form and saved in sealed plastic bags until use.

2.3. Preparation of Plant Extract. One gram of prepared powder was suspended in 10 ml of ionic distilled water and boiled for 15 min, then filtered through filter papers Whatman (no. 1), and stored in glass tube at 4°C [19].

2.4. Preparation of Nanoparticle Solution. AgNPs were synthesized by reaction mixture containing 5 mL of prepared plant extract and 25 mL of aqueous solution of (0.01 mmol/ml and 0.02 mmol/ml) $AgNO_3$. The reaction was carried out by placing the reaction mixture in a glass flask in a shaking water bath at 35°C for 15 minutes [19].

2.5. Characterization of Green Synthesized AgNPs

2.5.1. UV/Vis Spectrophotometer. The color change that occurred in the medium containing the plant extract and the silver nitrate solution ultraviolet spectroscopy (sp-3000 plus optima) was used to verify and record optical absorption spectra of biosynthetic silver nanoparticles within a range of 300-800 nm [20].

2.5.2. Fourier Transform Infrared (FTIR). The biosynthesized silver nanoparticle solution was mixed with potassium bromide at a ratio of 1 : 100 and was examined using an infrared spectrometer within the range of 400-4000 cm^{-1} to study the molecular vibrations of the sample particles.

2.5.3. Scanning Electron Microscope (SEM). The morphology, size, and shape of synthesized silver nanoparticles were examined with a scanning electron microscope, which was characterized by a magnification power between x1000000_ x6 through this examination [21] and Image J program used to indicate size particles.

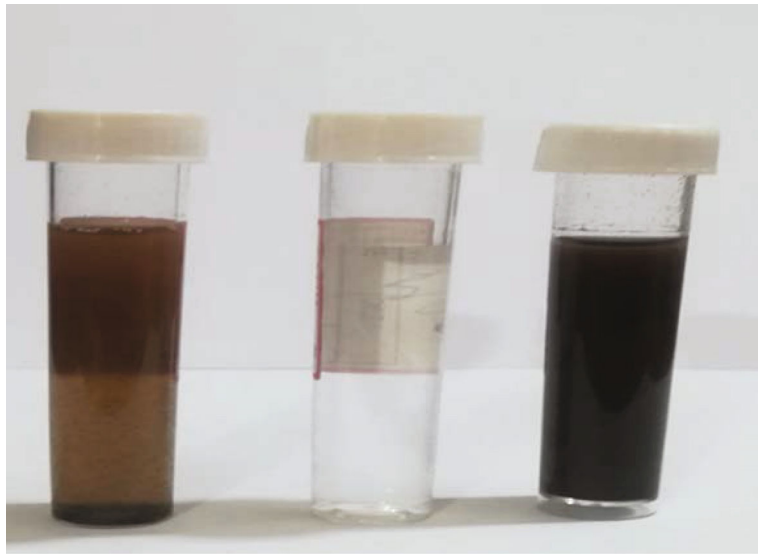

Figure 1: Synthesis of silver nanoparticles from *Alhagi graecorum* leave extract. (a) Aqueous extract of *Alhagi graecorum*. (b) Silver nitrate ($AgNO_3$, 0.01 and 0.02 mmol\ml). (c) After synthesis of AgNPs by adding extract to $AgNO_3$.

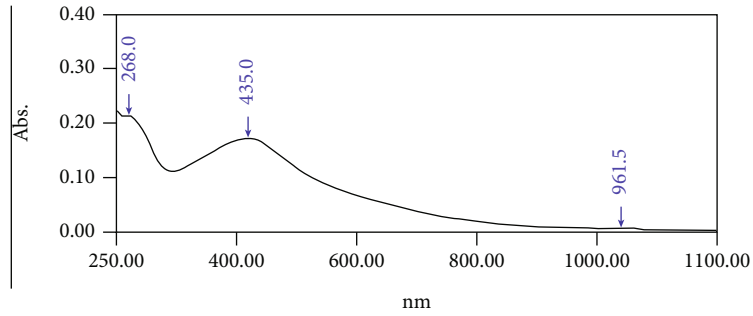

Figure 2: Different absorption spectra of synthesized AgNPs.

2.6. Test the Efficacy of Biosynthesis Silver Nanoparticles against Candida Species. The biological activity of the silver nanoparticle solution was tested against species of pathogenic yeast (*C. albicans*, *C. tropicales*, *C. parapsilosis*, *C. krusei*, and *C. glabrata*) by using well diffusion method, and SDA plate dishes were cultured with *Candida* sp. growth (100 μl) spread on the Petri dishes surfaces by using sterile cotton swab. The Petri dishes were left for a short period, and after that, holes with 5 mm diameter were made using sterile hole puncher. The hole was filled with 60 μl of aqueous extract of *A. graecorum*, fluconazole, 0.01 mmol/ml and 0.02 mmol/ml of $AgNO_3$ solution (negative control). All dishes were incubated under aerobic conditions at 37°C for 48 h. After the end of incubation, fungal growth was observed, and inhibition zone was measured in mm [22].

2.6.1. Cytotoxicity Assay. Human breast cancer cell MCF-7 was obtained from Central of Biotechnology/Al-Nahrain University and cultured under sterile conditions. Ethical approval was obtained by university of Baghdad ethics committee.

2.6.2. MTT Assay. Assay of 3-(4, 5-dimethylthiazol-z-yl)-2,5-diphenyltetrazolium (MTT) was used to determine the cytotoxicity of prepared extract and nanoparticles against MCF-7 cells. Stock solution at concentration of 10 mg/ml was prepared first, and substock solution was diluted with used culture medium. Cells were cultured at a density of 1 $\times 10^5$ cell/well in 96 well plates for 24 h, then different concentrations of *A. graecorum* leaf extract were added (50-250 μg/ml) to cultured media; cells cultured with media only served as negative control. 20 μl of MTT solution (5 mg/ml PBS) was added to each well and the plates incubated at 37°C for 3 h, then media was removed, and 200 μl of DMSO (0.5% *v*/*v*) was added to each well. The absorbance was read at 620 nm, and growth inhibition percentage was calculated according to the following equation: GI% = ((OD of control − OD of samples)/OD of control) × 100.

2.7. Statistical Analysis. SPSS software version 23 was used to performed statistical analysis, all results are presented as means ± SD, and significance was considered at $P \leq 0.05$.

3. Results and Discussion

One of the characteristic of silver nanoparticles synthesis is the color change to brownish due to particle excitation of

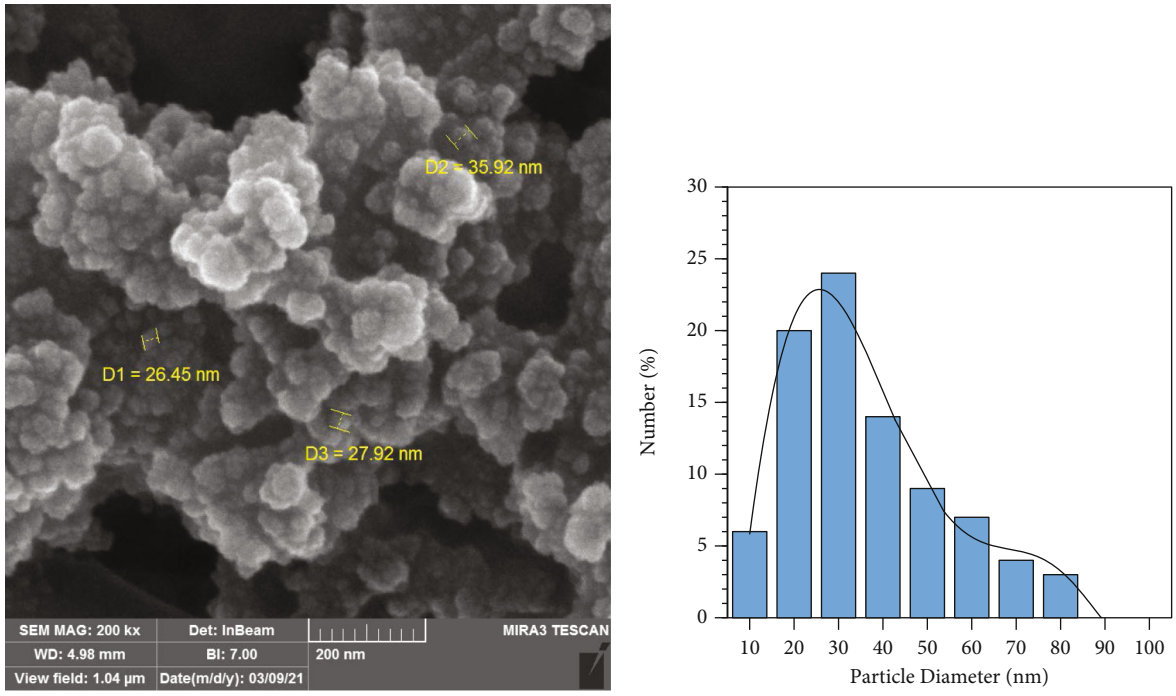

Figure 3: (a) SEM images of AgNPs synthesized at scale magnification of 200 nm. (b) Typical SEM image and corresponding size distribution of AgNPs.

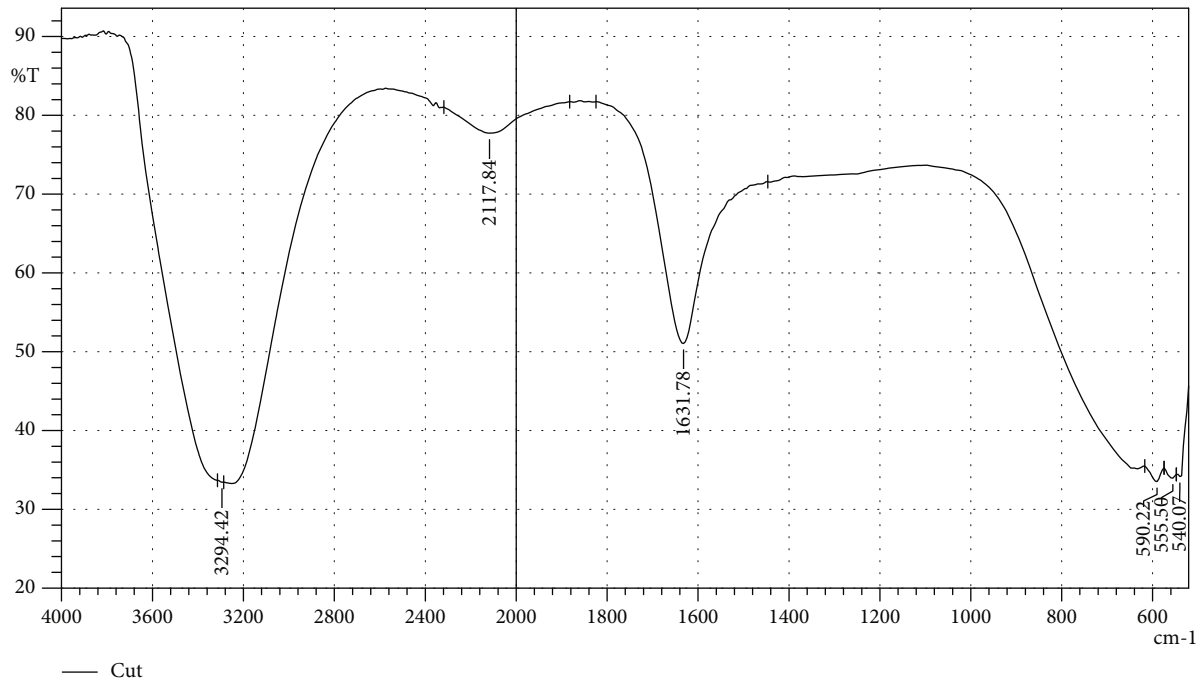

Figure 4: Fourier-transform infrared spectroscopy (FTIR) AgNPs synthesized at scale magnification of 200 nm.

surface plasmon vibrations. A gradual change in extract color from dark yellow to dark brown occurred after adding (0.01 and 0.02 mmol/ml) $AgNO_3$ as a first conformation sight of silver nanoparticle synthesis (Figure 1). These results suggested that the exciting of reducing compounds in extract solution plays a significant role in conversion of silver ion Ag^+ into Ag^0 nanoparticles. This was further confirmed by UV-vis spectrum, where the characteristic absorbance peak was at 435 nm (Figure 2). The peak might be corresponded for the longitudinal surface plasmon vibrations excited of

Table 1: The antifungal activity of silver nanoparticle synthesis using leaf extract of *Alhagi graecorum*.

Pathogenic fungi	0.01 mmol\ml	0.02 mmol\ml	$AgNO_3$	Fluconazole
	Zone inhibition mm			
C. albicans	14	16	8	3
C. glabrata	18	21	10	5
C. parapsilosis	22	27	11	5
C. tropicales	21	25	9	3
C. krusei	15	17	9	4

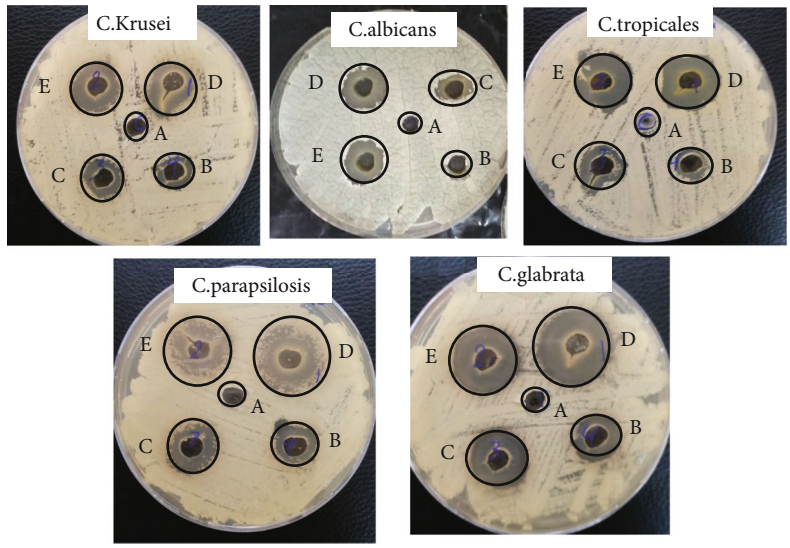

Figure 5: Antifungal activity assay for silver nanoparticles against five different species using well diffusion method. (a) Aqueous extract of *Alhagi graecorum*, (b) solution of silver nitrate (0.02 mmol/ml), (c) fluconazole, (e) synthesized silver nanoparticles at 0.01 mmol/ml, and (d) synthesized silver nanoparticles at 0.02 mmol/ml.

silver nanoparticles which also might relate for the nano-spherical shape. Strong band of AgNPs with extract might be due to the group oscillations of electrons on the surface of silver nanoparticles (plasmon resonance) [23]. Similar findings were observed by Sahu et al. [24] conjugating AgNPs to a flavonoid, which exhibited concentrated suspensions measuring approximately 10-80 nm, consistent with the findings in this study. Moreover, yellow pepper and quercetin were used for the syntheses of AgNPs with size of 5-40 nm and the stability last for four weeks as the SPR did not deviate with time and also exhibited significant antibacterial and antibiofilm activity [22].

The surface topology and size of present silver nanoparticles were analyzed using scanning electron microscopy (SEM), and the results showed spherical NPs with a size range from 22-36 nm (Figure 3). Malik and Sehrawat [16] reported the synthesis of spherical AgNPs with an average size of 14-29 nm using ethanolic extract of *A. maurorum* ethanolic extract.

FTIR spectra of *Alhagi graecorum* extract displayed a number of absorption peaks as shown in (Figure 4), which indicated a complex nature of plant material. The presence of new peaks are at 3294 cm^{-1} characteristic for the –NH and NH2 group, characteristic peaks at 2178 cm^{-1} are for C-H stretching vibrations, at 1631 cm^{-1} for amide I linkage, at 1488 cm^{-1} for CHN vibration, and between 590 and 540 cm^{-1} for hydroxyl (-OH) group. All the above analysis suggested a reduced mediator of silver ion to silver nanoparticles.

The antifungal activities of biosynthesized silver nanoparticles against *C. albicans*. *C. glabrata*, *C. parapsilosis*, *C. tropicales*, and *C. krusei* were investigated by using well diffusion method. The inhibition zone that was observed was ranging from 14-22 mm for (0.01 mmol/ml) and from 16-27 mm to the (0.02 mmol/ml). While the effect of $AgNO_3$ solution ranging from 8-11 mm and fluconazole zone inhibition was 3-5 mm, it was also noticed that there was no effect of the aqueous extract of the plant (Table 1, Figure 5).

Both silver ions and silver nanoparticles were known to have excellent antimicrobial activities and have the ability to interact with the cell membrane by destroying the cell wall structure, so they have a large surface to volume ratio and antibacterial properties [25]. McDonnell and Russell [26] showed that nanoparticles tend to interact with phosphorous or sulfur. Therefore, proteins in the cell membrane or inside the cell that contain sulfur such as DNA are among the best sites for nanoparticle binding [27]. The surface area and size of the silver nanoparticles allow them to reach and link with the cells' DNA. Because they are linked to the clicking present in the cell membrane and lead to the release of

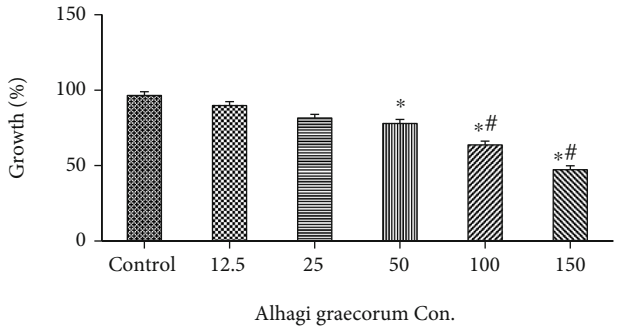

Figure 6: Cytotoxicity effect of *Alhagi graecorum* extract in MCF-7 breast cancer cell line. Data are mean ± standard error.

silver ions that work to improve the permeability of the cell membrane, nanoparticles with a high surface area allow them to have the best contact and interaction with the microbial cell [28]. Another suggested mechanism that explain the impact of AgNPs in cell wall is the thickness and composition of cell wall and the peptidogly content can along with the charged of lipopolysaccharide [22].

Cunden et al. [29] reported that AuNPs has antibacterial and antifungal activities. The antibacterial activity depends on the method, size, shape, and concentration of synthesized NPs [30]. Also, Singh et al. [31] showed that the fatal effect of microbial cells resulting from the release of nanoparticle ions as they remain in the cell's membrane, which leads to a change in its composition and thus increases the permeability of the cell membrane until the cell dies. This activity is attributed to the coating or manufacturing method on the AuNPs surface rather than the reactive contaminants left by the AuNPs core [32]. Interestedly, a comparative study between green synthesized and commercial AgNPs showed that Z. multiflora AgNPs have significantly higher antibacterial and biofilm formation inhibitory activity [18]. Additionally, it has been suggested that the silver ions released from silver nanoparticles can interact with the phosphorus part of DNA, causing DNA replication inactivation, or react with sulfur-containing proteins, leading to inhibition of enzyme function, resulting in loss of cell viability, and ultimately cell death [33].

3.1. Cytotoxicity Assay. The cytotoxicity results of *A. graecorum* extract showed dose-dependent manner (Figure 6), and significant differences ($P \leq 0.05$) were revealed at 150, 100, and 50 μg/ml concentrations as compared to the control. Also, a significant difference was observed between 100 and 150 μg/ml and 12.5, 25, and 50 μg/ml.

Several bioactive flavonoids and its glycosides have been isolated from *Alhagi* species were identified to be safe for use and treatment in human [34]. For example, kaempferol which has been reported being a multiple bioactive was displayed as an antioxidant, antitumor, anti-inflammatory, and hepatoprotective and neuroprotective activities [35]. Moreover, the epoxide of lupeol, lutein, and eugenol isolated from *A. maurorum* are found to be promising drug applicants for inducing apoptosis in human breast cancer cells [36]. Oleanane glycosides from the roots of *A. maurorum* have found to be responsible for the antiproliferative activity against MCF-7, A549, PC-3, and U937 cancer cell lines [37]. One of the suggested mechanism that possibly induced apoptosis in cancer cells by flavonoids and its glycosides was by inducing apoptosis through inhibiting cell cycle action from G1 to S or by altering the regulation of p21 and p27 which are necessary for the RB phosphorylation and E2f activation [38]. Additionally, increase free radical especially, and reactive oxygen species (ROS) are associated with cell apoptosis under oxidative stress in physiological and pathological conditions that induced lipid, protein, and DNA cellular damage. Flavonoid helps by protecting from oxidative damage, and lupeol and triterpene found to inhibit tumor responses and play a role as an effective chemopreventive agent [39]. Reports show that AgNPs treated with cells increased ROS level. The increase of ROS generation in cancer cells can further stimulate cell proliferation cause DNA mutations and promote genetic instability and the emergence of drug resistant cells [40]. In cancer cells, three mechanisms have been suggested to utilize the way by which apoptosis occur by nanoparticles, activation of death transmembrance receptor, mitochondrial damage, and injury of endoplasmic reticulum [41, 42].

4. Conclusions

The green synthesized silver nanoparticles using *A. graecorum* extract had spherical shape particles with an average size of 22-36 nm following characterization by SEM. The antimicrobial and antitumor activity results confirmed a significant antioxidant ability and highest sensitivity indicating its potential use against microbes and tumor cell line.

Data Availability

All data are presented within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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