Original Research

Appraisal of the antimicrobial and cytotoxic potentials of nanoparticles biosynthesized from the extracts of *Pelargonium quercetorum* Agnew

Berrak Dumlupınar[1], Gökçe Şeker Karatoprak[2], Mehmet Fırat[3], Esra Küpeli Akkol[4,*]

[1]*Faculty of Health Sciences, Nutrition and Dietetics, Istanbul Gelisim University, 34310 Istanbul, Turkey,* [2]*Department of Pharmacognosy, Faculty of Pharmacy, Erciyes University, 38280 Kayseri, Turkey,* [3]*Deparment of Education, Faculty of Biology, Yüzüncüyıl University, 65090 Van, Turkey,* [4]*Department of Pharmacognosy, Gazi University, 06560 Ankara, Turkey*

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1. Abstract

Aim: The aim of this study is the synthesis of nanosilver particles (AgNPs) from *Pelargonium quercetorum* Agnew. (Geraniaceae) and evaluation of the antimicrobial and the cytotoxic potential of AgNPs. **Methods**: The synthesized AgNPs were evaluated for antimicrobial and anticancer efficacy using the minimum inhibition concentration method and MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide) assay. **Results**: The AgNPs inhibited approximately 90% the growth of gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* and yeast *Candida albicans* pathogens at a concentration of 500 μg/mL. The synthesized AgNPs showed excellent toxicity in MCF-7 cells, and specifically, pq70 AgNP inhibited the growth of MCF-7 cells by 52% at a concentration of 3.125 μg/mL. **Conclusion**: It was determined that the AgNPs, which had been synthesized from extracts that contained a high phenolic composition, were smaller in size, and showed high anticancer and antimicrobial properties.

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2. Introduction

Since the beginning of the 21st century, nanosilver particles (AgNPs) have been used in almost every field, widely used in physical, biological, and pharmaceutical applications [1]. Due to the broad spectrum and high efficiency of AgNPs, their antimicrobial anticancer activities have attracted attention in the field of biomedicine. Besides antimicrobial and anticancer properties, other medical applications of AgNPs include bone healing and wound repair, enhancing the immunogenicity of vaccines, dental applications, antidiabetic agent and biosensing [2]. However, it has been reported in the literature that these nanoparticles may have negative effects on people and the environment [3–7]. Physical and chemical, and biological synthesis methods for AgNPs have been described in the literature [8, 9]. Physical synthesis can obtain AgNPs with uniform size distribution and high purity. Chemical synthesis is the most commonly used method to obtain AgNPs due to the size and shape control [2]. The use of hazardous chemicals that may cause potential environmental and biological risks in most of the physical and chemical methods used in the synthesis of AgNPs is the main factor in not selecting these methods [5]. In addition, chemical synthesis methods can absorb certain toxic substances on the surface and prevent their medical use [10]. Biosynthesis of metal nanoparticles is a simpler and less harmful method than a chemical method. Since cheaper and more efficient use of biosynthesized AgNPs than chemical AgNps is a subject that needs to be investigated, appropriate environmental and economic methods should be used for synthesizing these nanoparticles. The search for such a method has led to the need for biomimetic production of AgNPs. Therefore, biological methods using microorganisms and plants are an alternative method applicable for AgNP synthesis.

It has been determined by studies that AgNPs can arrest the cell cycle at certain phases, inhibit cell proliferation and lead cancer cells to apoptosis by inducing oxidative stress. Reactive oxygen species damage cellular enzymes (cellular respiratory chain), leading to disruption of the cellular membrane and DNA damage, leading to cell lysis and death. Cellular uptake of smaller nanoparticles may be easier compared to larger particles. Thus, the higher cytotoxicity of smaller particles compared to larger ones may be related to the amount of reactive oxygen species (ROS) produced in the relatively larger surface area of the small nanoparticles. Alternatively, smaller nanoparticles may exhibit or release more silver ions on its surface than larger nanoparticles. The molecular mechanism of AgNPs to show antimicrobial and anticancer properties by penetrating into the cell can be explained in this way.

The family Geraniaceae is represented in Turkey by 4 genera, comprising *Biebersteina*, *Geranium*, *Erodium*, and *Pelargonium*, and a total of 62 species. Two species of *Pelargonium*, the most important genus of this family, have been recorded in the vegetation of Turkey [11–13]. *Pelargonium quercetorum* is one of these species and is generally found in Northern Iraq, although it also grows in Hakkari Province in Turkey. This plant, which thrives at high altitudes and humidity, is popular in the spring in the region and is known as Tolk or Tolik. *P. quercetorum*. It has been given importance by the local people and also has a commercial value in the region. In addition to using this plant for medicinal purposes, the local people also use it for food purposes [14]. The traditional use of *P. quercetorum* as an antiparasitic is known in the Kurdistan region of Iran [15]. In Turkey, it is used to treat throat disorders and skin wounds, and its seeds and leaves are used to blast boils. It is also used for chronic headaches, neck pains, and migraines [14]. Studies have shown that *P. quercetorum* has a high content of total phenols and flavonoids, and gallic acid and apocynin have been identified in the root extracts of the plant [16]. Although there are limited studies on this traditionally used plant, it has been shown to cause apoptosis in lung and breast cancer cell lines. In a previous study, it was proved by us that it exhibits antioxidant activity [17–19].

It has been shown that silver nanoparticles synthesized by *Pelargonium sidoides* and *Pelargonium. endlicherianum* from the Geraniaceae family have strong antimicrobial activity [20, 21]. Besides, the strong effects of *P. quercetorum* and different *Pelargonium* species on cancer cell lines led us to evaluate the effects of nanoparticles synthesized by *P. quercetorum* [17, 18, 22]. For this reason, the biosynthesis of AgNPs was reported for the first time herein using different extracts that were obtained directly from the *P. quercetorum* plant. The main objectives of this study were (1) the biosynthesis of AgNPs directly using extracts of *P. quercetorum*; (2) characterization of these AgNPs using zeta potential, scanning electron microscopy (SEM), and UV-vis to its assess quality, morphology, and dimensions; and (3) determination of the antimicrobial and anticancer activity of AgNPs.

3. Material and method

3.1 Plant material, chemicals, and reagents

P. quercetorum was collected in May 2014 in Hakkari Province, Turkey, and a voucher specimen was deposited in the herbarium of Hacettepe University (HUB 30648). Silver nitrate ($AgNO_{3)}$, ethanol, and methanol were obtained commercially from Sigma-Aldrich (St. Louis, MO, USA). Nutrient agar (NA) (Cas no: 105450), nutrient broth (NB) (Cas no: 105443), Sabouraud dextrose agar (SDA), and Sabouraud dextrose broth (SDB), for appropriate microorganism culturing, were obtained from Merck (Darmstadt, Germany). Dulbecco's modified eagle medium (DMEM, Sigma-Aldrich) was used for the cell culture.

3.2 Preparation of the extracts

First, the standardized extract of *P. quercetorum* (EPs 7630) was prepared with 11% ethanol, and then the dried root parts of the plant were roughly powdered and extracted 3 times for 8 h at 37 °C in a shaking water bath with the 11% ethanol (pq11) and 70% methanol (pq70). The obtained extracts were combined and concentrated in a rotavapor (37–38 °C) under vacuum. The aerial part of the vegetable material was extracted in the same way as the 70% methanol (pqh) and then concentrated in the rotavapor. All of the extracts were lyophilized and stored at –20 °C until analysis.

3.3 Instrumentation and characterization

Formation of the AgNPs was characterized using SEM, UV-Vis spectrometry, dynamic light scattering (DLS) and Zeta potential. SEM images were obtained using a ZEISS EVO LS10 SEM (Oberkochen, Germany) with a working voltage of 25 kV. The effective diameter and surface charge of AgNPs were measured using Zetasizer (Malvern Panalytical Ltd., Malvern, UK).

3.4 Formation of AgNP

For this, 1 mg/mL concentrations of pqhAgNP, pq11AgNP, and Pq70AgNP were prepared as stock solutions. The AgNPs were synthesized from the *P. quercetorum* extracts, according to the method of a previous study [20].

3.5 Preparation of bacterial strains

Bacterial cultures comprising gram-negative: *Escherichia coli* American Type Culture Collection (ATCC) 25922, gram-positive: *Staphylococcus aureus* ATCC 25923, and yeast: *Candida albicans* ATCC 14053 were used for the microdilution method. The bacteria were grown in NA, while the yeast was grown in DSA. The bacterial suspensions equivalent to the density of 0.5 McFarland (for bacteria 108 CFU/mL, for yeast 106 CFU/mL) were prepared by comparing the density standard, using a PhoenixSpec Nephelometer (Becton Dickinson, NJ, USA), of the fresh subcultures in broth.

3.6 Minimum inhibitory concentration (MIC)

Antibacterial activity of the AgNPs was tested using the microdilution technique of Clinical and Laboratory Standards Institute [23]. The MIC values were determined for the antimicrobial activity method of Altinsoy [24].

3.7 Cell culture

MCF-7 ATCC HTB 22 cells (human breast adenocarcinoma cell line) were obtained from the ATCC. For the MCF-7 cells, DMEM, inactivated fetal bovine serum, antibiotic mixture, and $_{L}$-glutamine were used.

3.8 Cell viability assay

After the MCF-7 cells were developed under suitable conditions, they were seeded at a ratio of 10,000 cells per well into a 96-well microplate. The grown cells were incubated with various concentrations of AgNPs (3.125–100 μg/mL) for 24 h. The cytotoxic properties of AgNPs were investigated using the using MTT method [25]. Finally, the absorbance was read using an ELISA Synergy HT microplate reader (BioTek, Winooski, VT, USA) at 570 nm. The experiments were repeated in triplicate and results were given as the mean $\pm$ SD.

3.9 Statistical analysis

The experimental data were shown as the mean $\pm$ SD. Statistical analysis was performed using ANOVA. Statistical significance was accepted as $p < 0.05$ using the Tukey pairwise comparison test.

4. Results and discussion

4.1 Synthesis and characterization of the AgNPs

The SEM images of AgNPs formed using pq11, pqh, and pq70 are shown in Fig. 1A–C. While the pq11, pqh, and pq70 mediated AgNPs were all spherical, they exhibited different sizes and size distributions, comprising ~47 nm for pq11AgNP, ~96 nm for pqhAgNP, and ~35 nm for pq70AgNP, respectively. The UV-vis spectrum of pq11AgNP, pqhAgNP, and pq70AgNP showed absorption peaks at ~434, ~460, and ~417 nm, respectively, which are all characteristic peaks of AgNPs (Fig. 1D). DLS was used to measure the effective diameter and size distribution of pq11AgNP, pqhAgNP, and pq70AgNP, as shown in Fig. 1E–G, respectively. The maximum density was found to be 43–68 nm for pq11AgNP, 78–190 nm for pqhAgNP, and 28–50 nm for pq70AgNP, respectively. The sizes of pq11AgNP (112 nm), pqhAgNP (122 nm), and pq70AgNP (61 nm) were effective diameters measured using DLS. As in the AgNPs synthesized from microorganisms in a previous study, the large dimensions can be attributed to the presence of plant phytochemicals adhering to the surface of the AgNPs and to the partial accumulation of the AgNPs. Karatoprak *et al.* [19] reported that while the total amount of flavonoids in 70% methanolic *P. quercetorum* extract was (pq70) 64.95 $\pm$ 2.93 mgCA/g, 11% ethanol extract (pq11) was found as 46.63 $\pm$ 1.93 mgCA/g, and herba extract (pqh) was found as 30.89 $\pm$ 3.58 mgCA/g [20]. It was assumed that during the pq70 formation, the presence of total phenolic, flavonoids, and flavonol contents may have had an effect, since these molecules tend to easily react with metal ions to form complexes, they can participate in the formation process of AgNp to form small-sized nanoparticles.

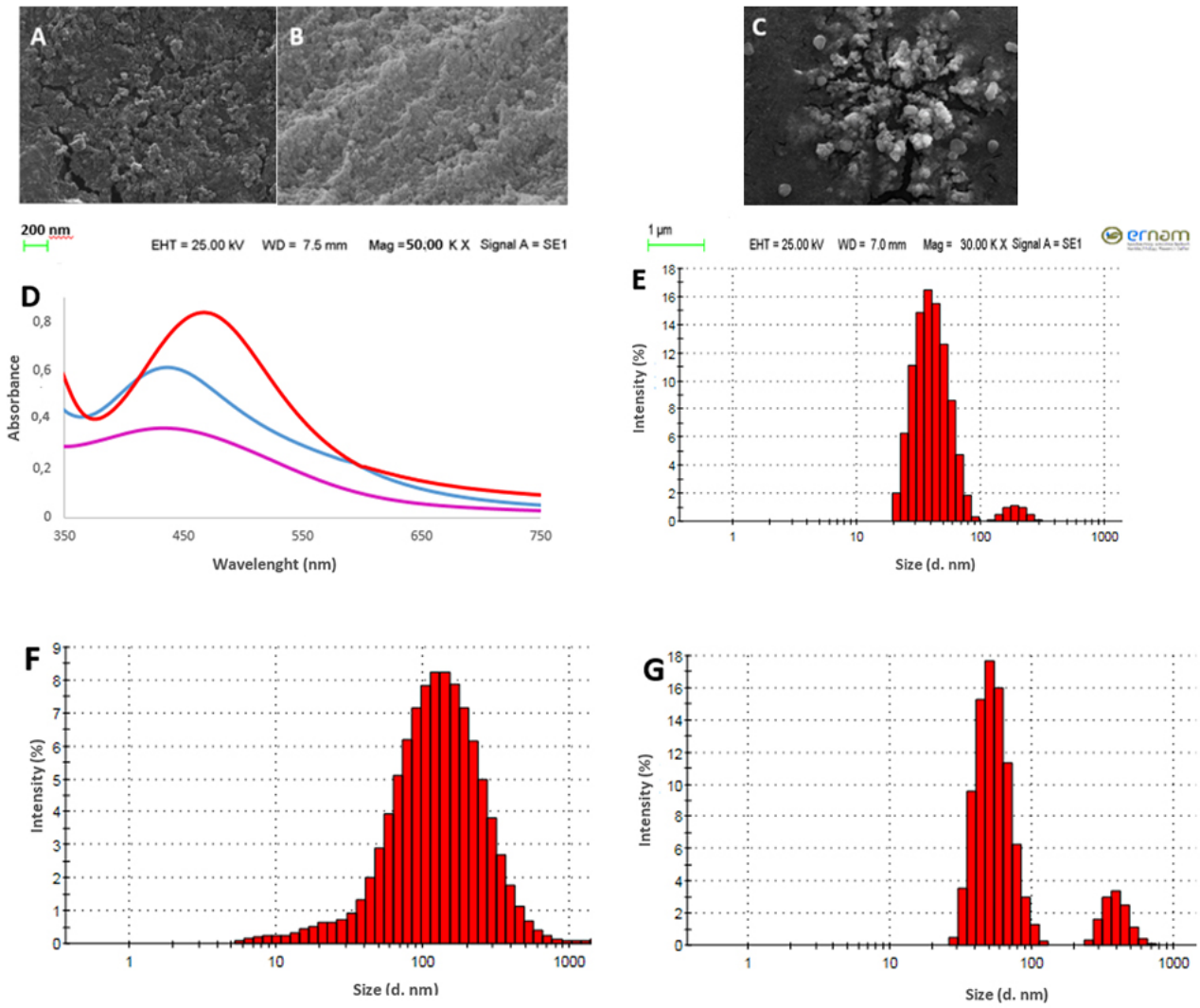

Fig. 1. Characterization of the AgNPs. SEM images (A) pqhAgNP, (B) pq11AgNP, and (C) pq70AgNP. (D) UV-vis absorption spectra of the pqhAgNP (red line), pq11AgNP (purple line), and pq70AgNP (blue line) solutions. DLS results of (E) pq11AgNP, (F) pqhAgNP, (G) pq70AgNP.

4.2 Antimicrobial activities of the pq11AgNPs, pq70AgNPs, and pqhAgNPs

The antimicrobial effects of AgNPs against various microorganisms are widely used. Their size, morphology, surface charge, surface coating, and synthesis procedures have been reported to affect the antimicrobial performance of AgNPs [26–29]. Due to the various plant phytochemicals with antimicrobial effects, many plant extracts have widespread use as antimicrobial agents [30, 31]. In addition, essential oils rich in terpenic composition have gained importance as effective antimicrobial agents against commercial bacterial strains that cause infections [32]. In the last decade, several studies have reported the biosynthesis of Ag nanoparticles obtained from plant extracts, microorganisms, and fungi, and green synthesized AgNPs are considered as good sources of antimicrobial molecules [33]. AgNPs obtained from some microorganisms have been efficiently used against a wide range of microorganisms [34]. The antibacterial activities of the synthesized AgNPs at different concentrations (500–3.91 μg/mL) were tested against *E. coli*, *S. aureus*, and *C. albicans*. The percentage of inhibition was compared with a positive control (Meropenem and Fluconazole) and a negative control ($AgNO_3$). Antimicrobial activity results were detected using the microdilution method and the results are shown in Fig. 2 and Table 1. According to the results, the AgNPs exhibited approximately 90% inhibition against the studied gram-positive and gram-negative bacteria at a concentration of 500 μg/mL. It can be understood from Fig. 2 that AgNPs exhibited inhibition in a dose-dependent manner. The AgNPs demonstrated 70% antimicrobial activity against *C. albicans* at a concentration 500 μg/mL. It was seen that the antimicrobial effect of pq70AgNP tended to be higher than those of pqhAgNP and pq11AgNP based on the microdilution test.

Table 1. IC50 (μg/mL) values of pq11AgNP, pq70AgNP, and pqhAgNP against selected pathogens.

AgNPs	Pathogens		
	E. coli	*S. aureus*	*C. albicans*
Pq11AgNP	124.72 ± 1.95*	110.72 ± 3.90^{a}	155.47 ± 3.92^{1}
Pq70AgNP	99.25 ± 1.95**	103.04 ± 4.10^{a}	108.45 ± 1.95^{2}
PqhAgNP	140.56 ± 3.90***	117.71 ± 1.95^{b}	161.38 ± 5.93^{1}
Meropenem	<3.91****	$<3.91^{c}$	-
Fluconazole	-	-	$<3.91^{3}$

Values given as the mean $\pm$ SD are within the $\pm$95% confidence interval. Bars with the same lower case letters (*–****), (a–c), and (1–3) are not significantly ($p > 0.05$) different.
pq11, 11% ethanol root extract; pq70, 70% methanol root extract; pqh, 70% methanol herba extract.

It was determined that 125 μg/mL of concentrated pq11AgNP reduced the proliferation of *E. coli*, *S. aureus*, and *C. albicans* by 55%, 63%, and ∼43%, respectively. At the same concentration, pq70AgNP and pqhAgNP caused a decrease in viability of 68%, ∼73%, and ∼58%, and 58%, 60%, and 30% for *E. coli*, *S. aureus*, and *C. albicans*, respectively. Similarly, 31.25 and 15.62 μg/mL of pq70AgNP, respectively, showed much effective inhibition on *S. aureus* (∼53% and ∼27%), and *E. coli* (∼35% and ∼30%) when compared to *C. albicans* (∼27% and ∼20%).

When the IC50 (μg/mL) values of the pq11Ag NPs, pq70AgNPs, and pqhAgNPs on *E. coli* were examined, all of the nanoparticles were statistically different. No sample had the same significance as Meropenem. On *S. aureus*, while the pq11AgNPs and pq70AgNPs were statistically the same ($p > 0.05$), no sample could reach the activity of Meropenem. The results are summarized in Table 1.

The highest inhibition activity on *C. albicans* was found with the pq70AgNPs, while it was statistically different ($p < 0.05$) than that of the pqhAgNPs and pq11Ag NPs. Given the overall effect on all of the pathogens, it was clear that the pq70AgNPs had lower IC50 values.

The minimum inhibitory concentration (MIC) values of the pqhAgNPs and pq70AgNPs were determined as 7.81 μg/mL for *S. aureus* (Table 2). Overall, the activity showed by the Pq70AgNPs was significantly higher than that of the Pq11AgNPs. In a previous study, AgNPs synthesized from *P. endlicherianum* showed a higher inhibitory effect against gram-positive *S. epidermidis* [20]. The cell membrane interactions of *S. aureus* and AgNPs predicted a very strong interaction when compared to *E. coli* and *C. albicans*. *S. aureus*, a gram-positive bacterium, has a very thick cell wall due to proteoglycan layers, which may strengthen the interactions between *S. aureus* cells and AgNPs, where the cells are effectively and rapidly deactivated. In contrast to that, gram-negative *E. coli* has thin cell walls, which may weaken the interaction between, *E. coli* cells and the AgNPs.

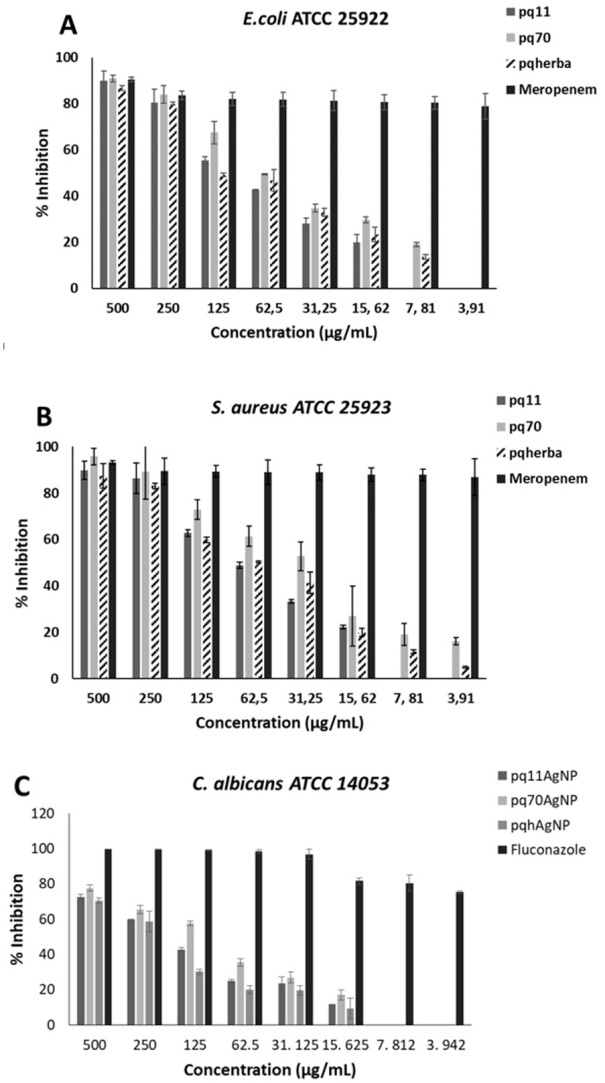

Fig. 2. Inhibitory effect of the AgNPs with respective concentrations towards (A) *E. coli*, (B) *S. aureus*, and (C) *C. albicans*. Values given as the mean $\pm$ SD are within the $\pm$95% confidence interval. pq11AgNP, 11% ethanol root extract; pq70AgNP, 70% methanol root extract; pqhAgNP, 70% methanol herba extract.

Table 2. MIC values of the pq11AgNPs, pq70Ag NPs, and pqhAgNPs (μg/mL).

AgNPs	Pathogens		
	E. coli	*S. aureus*	*C. albicans*
Pq11AgNP	15.62	15.62	31.25
Pq70AgNP	7.81	7.81	15.62
PqhAgNP	15.62	7.81	15.62
Meropenem	3.91	3.91	-
Fluconazole	-	-	3.91

Values given as the mean $\pm$ SD are within the $\pm$95% confidence interval.
pq11, 11% ethanol root extract; pq70, 70% methanol root extract; pqh, 70% methanol herba extract.

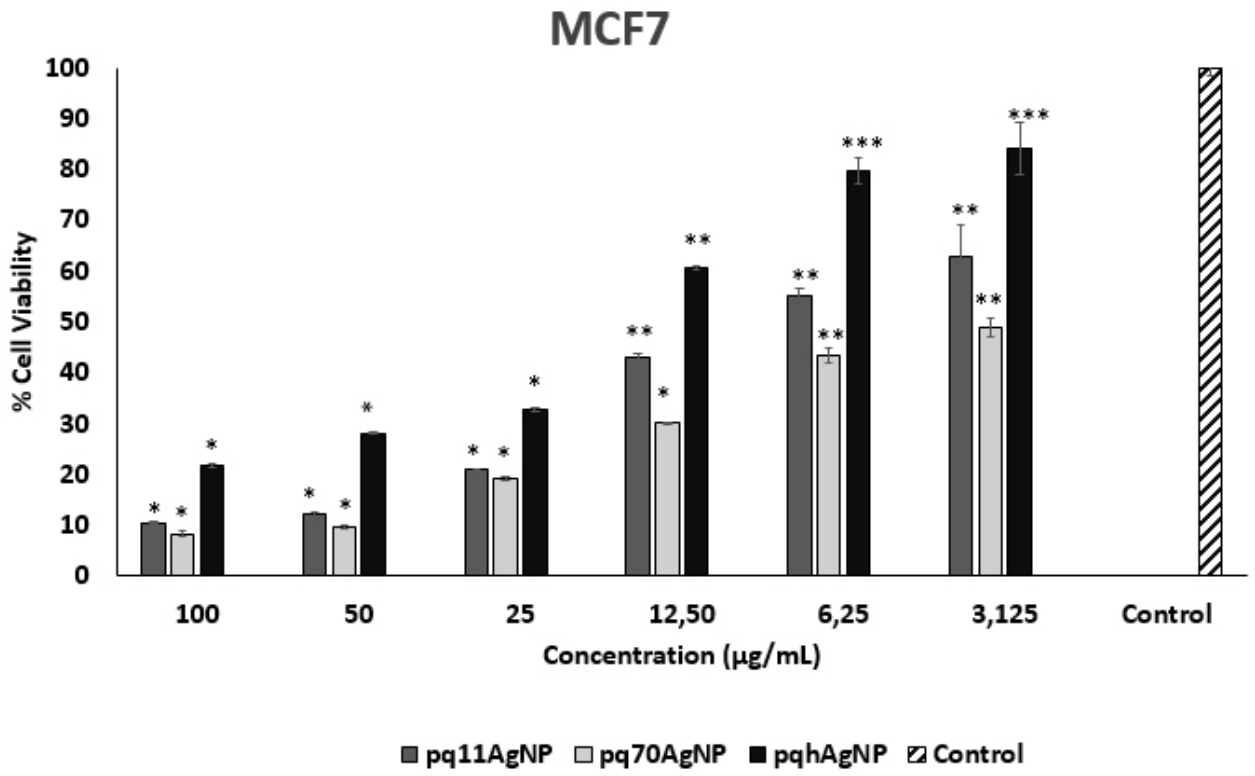

Fig. 3. Cytotoxicity of the AgNPs assessed by MTT reduction assay against the MCF-7 cell line. Data are expressed as mean ± standard deviation, n = 3. Significant differences are indicated as * $p < 0.001$, ** $p < 0.01$, *** $p < 0.05$. pq11AgNP, 11% ethanol root extract; pq70AgNP, 70% methanol root extract; pqhAgNP, 70% methanol herba extract.

4.3 Cytotoxicity of the AgNPs against the MCF-7 cell line

Exposure of cells to AgNP can induce changes in the form of the cells, decrease cell viability, increase lactate dehydrogenase release, and ultimately result in cell apoptosis and necrosis [35]. According to the results of this study, the AgNPs, synthesized by using a plant extract, were determined to have anticancer potential against the breast cancer cell line MCF-7. The nanoparticles, pq11AgNP, pq70AgNP, and pqhAgNP, at concentrations between 25 and 100 μg/mL were found to be statistically different from the control group ($p < 0.001$). At a concentration of 100 μg/mL, pq11AgNP and pq70AgNP showed 89.47% and 91.66% lethal activity against the MCF-7 cells, respectively. It was noticed that the cytotoxic effect of the pqhAgNPs was lower than that of the pq11AgNPs and pq70AgNPs. The pq70AgNPs at a concentration of 3.125 μg/mL inhibited 52% of the MCF-7 cell growth. Size, shape and surface charge are factors that affect the anticancer activity of AgNPs [2]. In our study, pq70AgNP with the smallest particle size showed the highest anticancer activity. The cytotoxic effect against the MCF-7 cells is shown in Fig. 3. The AgNPs, synthesized from various microorganisms, confirmed a cytotoxic effect on the MCF-7 breast cancer cell line, confirming that the nanoparticle with the smallest particle size had a higher inhibitory effect [24].

The antibacterial and cytotoxic activity of the nanoparticle depends on the shapes and sizes of the nanoparticles; this can be confirmed by examining the inhibition of bacterial growth and cancer cells by different shaped nanoparticles. Nanoparticles of various sizes and shapes were synthesized according to the plant extracts used in the plant-mediated synthesis of AgNPs. Nanoparticles synthesized from different plants were detected in size from 1 to 100 nm. Silver nanoparticles synthesized by green synthesis were obtained in various shapes such as spherical, crystal, polydispersed, circular, cubic, smooth edges, triangular, cuboidal and irregular [36]. In our previous study and this study, the nanoparticles obtained from *Pelargonium* species were found to be spherical and smaller than 100 nm in size [20]. Pal *et al.* [26], reported that triangular nanoparticles complete inhibition of bacterial growth was observed even at a total silver content of 1 μg, spherical nanoparticles, a total silver content of 12.5 μg reduced the number of colonies, bacteria grew in rod-shaped particles even at 100 silver content. Apart from the size of the silver nanoparticle, being spherical, rod or triangular causes it to show different antibacterial effects. It has been shown that the most active silver nanoparticles among them are those with a triangular shape, while those with a rod shape have the lowest activity [26].

5. Conclusions

It can be concluded that the *P. quercetorum* components function as both reducing and stabilizing agents for the synthesis of pq11, pqh, and pq70 AgNPs and induce AgNP formation with different size and size distributions. The synthesized AgNPs exhibited dramatically enhanced inhibitory properties against the pathogenic strains. In addition, these molecules had intrinsic anticancer properties and also showed promising toxicity against the MCF-7 cell line; hence, these results will shed light on studies regarding their use as potential antimicrobial and anticancer agents. Herein, the use of AgNPs synthesized in conjunction with physicochemical interactions of AgNP and plant phenolic compounds from *P. quercetorum* extracts offered a promising alternative to reduce the use of chemical agents in the treatment of infection and cancer. In future work, focus will be aimed at in vivo biological activity and toxicity studies.

6. Author contributions

Conceptualization, BD, GŞK. Design, BD, GŞK. Supervision, BD. Resources, BD, GŞK. Materials, MF. Data Collection and/or Processing, BD, GŞK. Analysis and/or Interpretation, BD, GŞK. Literature Search, BD, GŞK, and EKA. Writing, BD. Critical Reviews, BD, GŞK, MF, and EKA.

7. Ethics approval and consent to participate

Not applicable.

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10. Conflict of interest

The authors declare no conflict of interest.

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Abbreviations: pq11, 11% ethanol root extract of *P. quercetorum*; pq70, 70% methanol root extract of *P. quercetorum*; pqh, 70% methanol herba extract *of P. quercetorum*; AgNPs, Nanosilver particles; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide; SEM, Scanning electron microscopy; UV, Ultraviolet; pq11AgNP, Silver nanoparticles of 11% ethanol root extract of *P. quercetorum*; pq70AgNP, Silver nanoparticles of 70% methanol root extract of *P. quercetorum*; pqhAgNP, Silver nanoparticles of 70% methanol herba extract of *P. quercetorum*.

Keywords: *Pelargonium quercetorum*; Geraniaceae; Silver nanoparticle; Antimicrobial; Cytotoxicity

Send correspondence to: Esra Küpeli Akkol, Department of Pharmacognosy, Gazi University, 06560 Ankara, Turkey, E-mail: esrak@gazi.edu.tr