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Antimicrobial and Antioxidant Potential of Silver Nanoparticles Synthesized from *Primula vulgaris*

Belgin ERDEM [1], Harun ÇİFÇİ [2], Yunus Emre ŞAHİN[3]

[1] Vocational School of Health Services Kirsehir Ahi Evran University, Kirsehir, Türkiye
[2] Department of Medical Biochemistry Faculty of Medicine, Kirsehir Ahi Evran University, Kirsehir, Türkiye
[3]Institute of Natural Sciences, Kirsehir Ahi Evran University, Kirsehir, Türkiye

✉: berdem@ahievran.edu.tr [1]0000-0001-9108-5561 [2]0000-0002-3210-5566 [3]0000-0001-8026-8119

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ABSTRACT

In this study, biosynthesis and in vitro phytochemical composition, antibacterial and antioxidant activities of silver nanoparticles were investigated by using aqueous leaf, flower and root extracts of *Primula vulgaris* (*P. vulgaris* subsp. *vulgaris*). The synthesized silver nanoparticles (AgNPs) were confirmed by color conversion and ultraviolet-visible (UV-visible) spectrophotometry. The appearance of a dark brown color and a UV absorption spectrum range at 440 nm confirmed the synthesized silver nanoparticles. The antimicrobial activity of silver nanoparticles synthesized from the leaf of *P. vulgaris*; *S. aureus* 25±1, *S. epidermidis* 20±1, *P. aeruginosa* 20±1, *A. hydrophila* 21±1, *C. albicans* 25±1, *C. tropicalis* 25±1, C. *parapsilosis* 22±1 and *C. glabrata* 20±1 mm zone diameter was determined. The most antimicrobial effect of *P. vulgaris* leaf aqueous extract; *S. aureus* 20±1, *S. epidermidis* 18±1, *A.hydrophila* 15±1, *P. aeruginosa* 12±2, *C. albicans* 18±1, *C. glabrata* 18±1, *C.tropicalis15*±2, and *C. parapsilosis* 15±2 mm zone diameter was revealed. The presence of flavonoids, terpenoids, protein, and carbohydrates was found to be higher in silver nanoparticles synthesized in the flower part of *P. vulgaris*, according to phytochemical screening. While saponins were detected in *P. vulgaris* root extracts, tannins and protein were detected in the leaf extract. The flower had the highest total phenolic extract content of the silver nanoparticle (29.08±0 mg GAE/g DW), while the leaf and root had the lowest total phenolic content of 9.06±0.5 and 8.64±3.3 mg GAE/g DW, respectively. The flower had the highest total phenolic extract content of the plant aqueous extracts (25.10±0.2 mg GAE/g DW), while the leaf and root had the lowest (8.28±0.5 and 5.20±0.0 mg GAE/g DW, respectively).The DPPH (1,1-Diphenyl-2-picrylhydrazil) assay was used to assess free radical scavenging activity. The antioxidant activity of AgNPs biosynthesized using *P. vulgaris* flower extract was 90.6 %, while *P. vulgaris* flower aqueous extracts were 86.3 %.
This can be concluded that silver nanoparticles synthesized using *P. vulgaris* flower extract are useful in the preparation of pharmacologically useful drugs.

Keywords: Antimicrobial, Antioxidant, *Primula vulgaris*, Silver nanoparticle

Primula vulgaris'ten Sentezlenen Gümüş Nanopartiküllerin Antimikrobiyal ve Antioksidan Potansiyeli

ÖZ

Bu çalışmada, *Primula vulgaris*'in (*P. vulgaris* subsp. *vulgaris*) sulu yaprak, çiçek ve kök ekstreleri kullanılarak gümüş nanopartiküllerin biyosentezi ve in vitro fitokimyasal bileşimi, antibakteriyel ve antioksidan aktiviteleri araştırılmıştır. Sentezlenen gümüş nanoparçacıklar (AgNP'ler), renk dönüşümü ve ultraviyole-görünür (UV-görünür) spektrofotometrisi ile doğrulandı. Koyu kahverengi rengin görünümü ve 440 nm'de bir UV absorpsiyon spektrum aralığı, sentezlenen gümüş nanoparçacıkları doğruladı. *P. vulgaris'*in yaprak ekstraktından sentezlenen gümüş nanopartiküldeki en fazla antimikrobiyal etki; *S. aureus* 25±1, *S. epidermidis* 20±1, *P. aeruginosa* 21±1, *A. hydrophila* 21±1, C. albicans 25±1, *C. tropicalis* 25±1, C. *parapsilosis* 22±1 ve *C.glabrata* 20±1 mm zon çapı ile belirlendi.*P. vulgaris* yaprak sulu ekstraktın en fazla antimikrobiyal etki*; S. aureus* 20±1, *S. epidermidis* 18±1, *A.hydrophila* 15±1, *P. aeruginosa* 12±2, *C. albicans* 18±1, *C. glabrata* 18±1, *C.tropicalis15*±2 ve *C. parapsilosis* 15±2 mm zon çapı ortaya konmuştur. Fitokimyasal taramaya göre P. vulgaris'in çiçek kısmında sentezlenen gümüş nanopartiküllerde flavonoidler, terpenoidler, protein ve karbonhidratların varlığı daha yüksek bulunmuştur. P. vulgaris kök ekstraktlarında saponinler tespit edilirken, yaprak ekstraktında tanen ve protein tespit edildi. *P. vulgaris*

yaprak ekstraktından sentezlenen gümüş nanopartiküllerin antimikrobiyal aktivitesinin, *P. vulgaris* yaprak sulu ekstraktının antimikrobiyal aktivitesinden daha aktif olduğu bulundu. Çiçek, gümüş nanopartikülün en yüksek toplam fenolik ekstrakt içeriğine (29.08±0 mg GAE/g DW) sahipken, yaprak ve kök, sırasıyla 9.06±0.5 ve 8.64±3.3 mg GAE/g DW ile en düşük toplam fenolik içeriğe sahipti.
Bitki sulu ekstraktları arasında en yüksek toplam fenolik ekstrakt içeriği çiçekte bulunurken (25.10±0.2 mg GAE/g DW), yaprak ve kök en düşük (sırasıyla 8.28±0.5 ve 5.20±0.0 mg GAE/g DW) bulundu. DPPH (1,1-Difenil-2-pikrilhidrazil) tahlili, serbest radikal süpürme aktivitesini değerlendirmek için kullanıldı. *P. vulgaris* çiçek özütü kullanılarak biyosentezlenen AgNP'lerin antioksidan aktivitesi %90,6 iken, *P.vulgaris* çiçek sulu özütleri %86.3'tür.
Buradan *P. vulgaris* çiçek ekstresi kullanılarak sentezlenen gümüş nanopartiküllerin farmakolojik olarak faydalı ilaçların hazırlanmasında faydalı olduğu sonucuna varılabilir.

Anahtar Kelimeler: Antimikrobiyal, Antioksidan, Gümüş nanopartikül, *Primula vulgaris*

INTRODUCTION

Bioactive nanoparticles (NPs) from plant extracts have an important place in researching new anti-cancer agents and developing more effective drugs. Non-toxic, ecological, metal based, and size of less than 100 nm NPs (such as gold, silver, palladium, manganese, zinc) using obtained plant extracts from plants by different methods can be synthesized. The recommended practical approach for generating NPs without the use of high pressure, high temperatures, or harmful chemicals is biological synthesis, often known as green synthesis [1]. Alkaloids, amino acids, flavonoids, terpenoids, and other phenolic compounds are found in plants that serve as bioactive compound reservoirs, and these compounds work as excellent reducing agents for the bioreaction of metals in NPs, which have a wide range of biological applications [2-3]. Plant or algae extract-mediated bioreaction for photosynthesis of AgNPs involves mixing the aqueous extract with silver nitrate solution [4-5]. The plant-mediated green synthesis system seems to be a method that leads to the product of stable NPs in a quicker time and provides constant construction. In this case, herbs including phytochemicals with a high therapeutic impact seem to be a principle for NP synthesis as they are released from toxic chemicals [6].

The *Primula* genus has around 400 species that belong to the *Primulaceae* family and are found throughout the northern hemisphere's temperate and cold zones. Saponins, alkaloids, tannins, terpenes, and phenolic chemicals are abundant in *Primula* species [7-8]. *Primula* species are used as popular ornamentals, traditional medicinal plants, and model organisms [9]. *Primula* is a very important medicinal plant and they are used in medicine for the treatment of cramps, spasms, paralysis, and rheumatic pains. *Primula* includes saponins, which are expectorant, and anodyne, which is the main element of aspirin, and salicylates, which have anti-inflammatory and febrifuge impacts. Flowers are anodyne, diaphoretic, diuretic, and expectorant [10]. The essential oils of *Primula vulgaris* were evaluated for antibacterial activity against nine bacterial species and shown to be effective against *M. smegmatis* [11].
P. vulgaris leaves and root extracts show antibacterial action against *E. coli* and *P. aeruginosa*, according to Majid et al. [12]. Saponins and phenolic glycosides are found in the *Primula* genus [13]. Various species of Primula have been shown to have antioxidant, antibacterial, antimycobacterial, antifungal, cytotoxic, antiviral, antiangiogenic, anti-inflammatory, and antimitotic properties, as well as cytotoxic, antiviral, antiangiogenic, anti-inflammatory, and antimitotic benefits, according to some studies [14-15]. Cellular stress occurs when the body's free radicals and antioxidants are out of balance, resulting in cellular damage. DNA is a common target of oxidative stress, and DNA damage induced by reactive oxygen species (ROS) is linked to a variety of disorders, including cancer, heart disease, and diabetes. Antioxidant molecules found in plants, such as polyphenolic chemicals, protect cells from the harmful effects of reactive oxygen species (ROS). The antioxidant effect of phenolic compounds is defined by their ability to give electrons to reactive oxygen species (ROS), chelate metal ions, and stimulate antioxidant enzymes. Various investigations have revealed that *P. vulgaris* extracts have antioxidant properties [14-16].
In the literature reviews, there is no before a published report on the antimicrobial and antioxidant activity of aqueous extracts and the biosynthesized silver nanoparticules (AgNPs) from leaf, flower and root of *P. vulgaris.*
This article presents the first report on antimicrobia and antioxidant activities of the biosynthesized AgNPs from P. vulgaris grown in southern Turkey.
In this study, we aimed to investigate the antimicrobial and antioxidant activities of biosynthesized silver nanoparticles and aqueous extract from *P. vulgaris.*

MATERIALS AND METHODS

Analytical grade reagents were utilized throughout. The Milli-Qwater purification system was used to obtain ultrapure water for all of the aqueous solutions (Millipore Corporation, MA, USA). Silver nitrate salt ($AgNO_3$) and DPHH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma-Aldrich- Germany. The fresh plant parts (leaves, roots, and flower) of *P. vulgaris* were collected from Salıpazarı (Samsun), Turkey (Latitude 41° 4' 50¨ N, longitude 36° 49' 36¨ E) in May 2019.

Preparation of the *Primula vulgaris* Extracts

For the preparation of *Primula vulgaris* extract, the leaves, roots, and flowers of the plant sample (identification was determined by Dr. Ekrem Aktoklu, a plant systematic expert at Kırşehir Ahi Evran University) were washed with water on the same day, they were dried in a 50°C oven for 24 hours before being pulverized using a mechanical muller. 10.0 gram of powdered all the extracts (leaves, roots, and flowers) were boiled for 20 minutes at 100 ºC in 100 mL of distilled water. It was then filtered through Whatman filter paper and centrifuged twice at 10,000 rpm to obtain all the extracts. The obtained extracts were stored in sterile tubes at 4 °C until use.

Synthesis of silver nanoparticle

Silver nitrate was used as a precursor in the synthesis of silver nanoparticles. Ten milliliters of aqueous extracts were mixed with 90 ml of 10^{-3} mol/L $AgNO_3$ solutions and kept in dark for the synthesis of silver nanoparticles at room temperature for 24 h .After 24 h, the solution was centrifuged at 6000×g and the pellet was collected, rewashed with distilled water and stored for 24 hours without light (25 °C).

After, it was observed that the color of the samples turned brown. The color change proves the formation/reduction of silver nanoparticles (Fig. 1a, 1b, and 1c). The reduced silver nanoparticles solutions were transported to falcon tubes and centrifuged at 4,500 rpm for 1 hour. After centrifugation, the liquid part of the falcon tubes was left, and the wet solid sample accumulated at the bottom of the tube was given to the Eppendorf tubes.

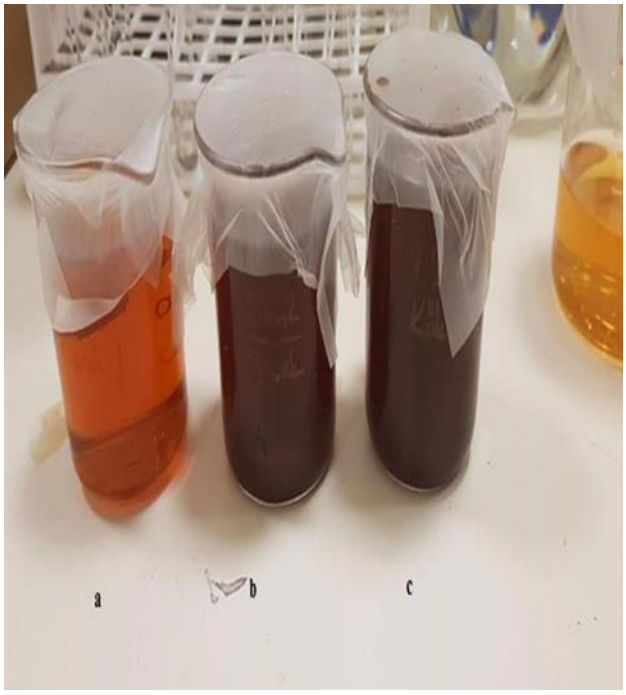

Figure 1. Color Change in Solution After Incubation 24 Hours a) AgNPs from leaf, b) AgNPs from flower, c) AgNPs from root

Analysis of the UV-VIS Spectrum

A preliminary test for the formation of silver NPs was carried out using a UV–Visible spectroscopy technique. UV–Visible spectrophotometer (Shimadzu, Japan) was used to measure a volume of 1.0 mL of the reduced silver nanoparticles solutions at wavelengths between 200 and 700 nm.

Microorganisms

The microorganisms were obtained from the Kırşehir Ahi Evran University microbiology laboratory. 16 bacterial strains and 4 yeast strains as *S. aureus* ATCC 29213, *S. epidermidis* ATCC 12228, *L. monocytogenes* ATCC 35152, *B. cereus* 709 Roma, *B. subtilis* ATCC 6633, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 13883, *C. jejuni* ATCC 33560, *E. aerogenes* ATCC 51342, *S. dysenteriae* ATCC 11835, *S. typhimurium* ATCC 14028, *V. angilarum* ATCC 43312, *P. aerogenosa* ATCC 27853, *A. hydrophila* ATCC 7966, *P. mirabilis* ATCC 29906, *P. vulgaris* ATCC 29905, *C. albicans* ATCC 90028, *C. parapsilosis* M006, *C. tropicalis* M007 and *C. glabrata* ATCC 90030 were used.

Assays for Antimicrobial Activity

The antimicrobial activity of aqueous extracts and biosynthesized AgNPs obtained from each of the flowers, leaves, and roots was tested against selected bacterial and yeast strains. All microbial strains are kept in Nutrient Broth at 4 °C. The antimicrobial efficacy of the plant extracts was evaluated against the given strains. Tyriptic Soy Broth (TSB) and Tyriptic Soy Agar (TSA) were used as media for the growth of microorganisms.

The antimicrobial activity of extracs and AgNPs was analyzed by using agar well diffusion method against bacterial and yeasts. Prepared TSA and sterilized at 121 °C. The medium was transferred into each sterilized Petri plate. Then 0.1 mL of diluted microbial cultures were spread over TSA. The 6-mm diameter well was drilled with a sterile drill for TSA. The plates were incubated for 24 hours at 37°C. The diameter of the resulting inhibitory zone was measured after incubation, and average results were reported [17].

Qualitative Phytochemical Analysis

To observe the common phytoconstituents, a qualitative phytochemical examination of the *P. vulgaris* extract (leaf, flower, and root) was done using standard experimental protocols. Among the phytoconstituents, flavonoid (Shinoda test), terpenoids (Salkowsi test), saponins (Frothing test), tannins (Ferric chloride), carbohydrates (Molisch test), and Protein (Biuret test). These tests yielded either positive (+) or negative (-) results [18-19].

Antioxidant Activity

Total Phenolic Content (TPC)

The Folin-Ciocalteu method was used to define the complete quantity of phenolic in the PVE with little change (−). Each test composition was done in triplicates. The total phenolic content (TPC) was measured in milligrams per gram of sample as a Gallic acid equivalent (GAE) [20].

Total Flavonoid Content (TFC)

The restricted aluminum chloride technique was used to prepare the total levels of flavonoids in the extracs [21]. The findings were duplicated three times. In the mg/g sample, the total flavonoid content (TFC) was reported as GAE.

Scavenging of DPPH Radical

0.1 mM DPPH (2, 2-diphenyl-1-picrylhydrazyl): 0.0027 g weighed and dissolved in 70 ml methanol. Aqueous leave, flower, and root extracts and AgNPs were taken in 50 µl and transferred to test tubes. After mixing 1 ml of 0.1 mM DPPH radical into methanol with vortex, it was held at room temperature for 30 minutes in the dark, and UV-Vis spectroscopic data were taken at 517 nm (UV-VIS Shimazu, Japan). As a control, ascorbic acid was employed. Silver nanoparticles were examined at different concentrations (100, 50, 25, and 12.5 µg/mL). The experiment was repeated three times.

% inhibition = [(AC – AS)/AC] × 100 was used to quantify radical scavenging activity, where AC represents the absorption of a blank sample (t = 0 min) and AS represents the absorption of the tested extract solution [22].

Statistical Analysis

The means (±) (n = 3) plus the standard deviation of the means were used to express all experimental results. Microsoft Excel was used to conduct the statistical analysis. Significant P values were defined as those less than 0.05.

RESULTS and DISCUSSION

Biosynthesized of AgNPs

UV-Vis spectra of the reduced/synthesized silver nanoparticles using leave, flower, and root extracts of *P. vulgaris* solutions are shown in Fig.1a, 1b, and 1c. Maximum absorption wavelength for reduced AgNPs solutions from root, flower, and leaf were found as 440 nm. Formations of silver nanoparticles by UV-vis spectrophotometer is a very important technique. It has been reported in different studies that strong and large surface absorption peaks were observed in the formation of various metal NPs of 2-100 nm size [23].

Despite the fact that the plasmon band of the *P. vulgaris* extracts (leaf, flower, and root) components is broad, they are read in the spectrophotometric range. UV-Visible spectroscopy (excitation band close to 450 nm for silver) revealed the synthesis of silver nanoparticles with a 24-hour absorbance rise. According to Mie theory, only a single surface plasmon resonance (SPR) band is required in the absorption spectra of spherical nanoparticles. The number of peaks increases as anisotropy increases [24-25].
In the UV-vis spectrum, extracts containing silver nanoparticles revealed a peak of 423 nm, according to Kotakadi et al. [26].
In another study, it was stated that the extracts containing silver nanoparticles showed a peak of 404 in the UV spectrophotometer. In our study, 440 nm peak of silver nanoparticles synthesized from *P. vulgaris* showed (Figure 2).

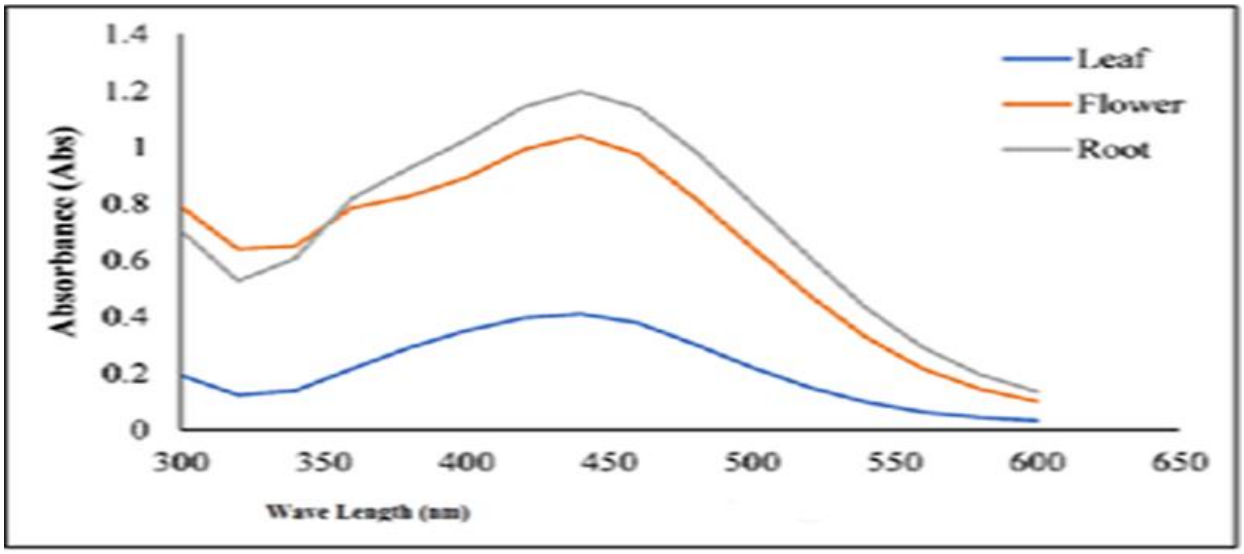

Figure 2. UV–Vis Spectra of *P. vulgaris* Leaf, Flower and Root Extract in Appearance of Ag+ Ions at 25^0C

Antimicrobial Activity of Aqueous Extracts and AgNPs

The antimicrobial activity of biosynthesized silver nanoparticles and aqueous extracts (leaf, flower, and root) was tested using the agar well diffusion method on 14 bacteria and four yeast species. Table 1 lists a few of the impacts. Antimicrobial activities of aqueous extracts and AgNPs against bacteria and yeasts were determined in millimeters. Ofloxacin (5 µg) and Fluconazole (10 µg) were used as controls. The antimicrobial effects of aqueous extracts and AgNPs were determined in triplicate for each microorganism. To various degrees, all of the aqueous extracts and AgNPs tested inhibited both Gram-positive and Gram-negative bacterial species.

Silver nanoparticles showed that they had antimicrobial activity against *E. coli* and *S. aureus* by disrupting the surface of cell walls [27].

Results revealed that *P. vulgaris* leaf extract exhibit inhibitory effects against *S. aureus* and S. *epidermidis* that showed zone of inhibition 20±1 and 18 ± 1 mm and *A. hydrophila* and *S. dysenteriae* showed zone of inhibition 15±1 mm and *B. subtilis, P. mirabilis,* and *P. vulgaris* showed zone of inhibition 13±2 and *B. cereus, E. aerogenes* and *P. aeruginosa* showed zone of inhibition 12±2 mm aqueous extract. Our results showed that leaf extract had the same effect against *C. parapsilosis* and *C. tropicalis* and the zone diameter was measured as 15 ± 2 mm. The effect of the leaf extract against *C. albicans* and *C. glabrata* 18 ± 1 inhibition zone diameter was measured.

In a study on antifungals, it was reported that crude extract of *P. macrophylla* with benzene and ethyl acetate exhibited highest antifungal activity on *T. longifilis* and *M. canis* [28]. Crude extracts from the aerial parts of *P. longipes* were found to have antibacterial activity against Gram-positive and Gram-negative bacteria in another study [29]. *P. vulgaris* leaf extracts were reported to have inhibitory action against gram-positive bacteria (*S. pneumoniae, S. pyogenes, S. aureus,* and *S. epidermidis*) in earlier research [30],with MICs ranging from 32 to 64 µg/ml. Plants including Jatropha curcas [31], *Argimone maxicana* [32], and *Punica granatum* have been found to produce silver nanoparticles in the past [33]. Bar et al. [31] and Khandelwal et al. [32] reported on the antibacterial activity of AgNPs in a series of papers. Devanesan et al. [33] observed a zone of inhibition in *E. coli, P. aeruginosa, P. vulgaris, S. typhi, S. aureus, S. epidermidis,* and *K. pneumoniae* when the produced nanoparticles were investigated.

Our results showed that AgNPs from leaf extract had the best antimicrobial effect. In AgNPs from leaf extract, *B. cereus, B. subtilis, E. aerogenes, A. hydrophila, S. dysenteriae, S. epidermidis, S. aureus, P. aeruginosa, P. mirabilis* and *P. vulgaris* showed the widest zone 25±1-15±1mm in diameter. Other bacteria showed an inhibition zone of 8 mm or less. Yeast formed a zone diameter of 25±1- 20±1 mm from silver nanoparticles from leaf extract. Among yeasts, the highest antimicrobial activity was observed in *C. albicans* and *C. tropicalis.*

In silver nanoparticles from flower extract, *S. epidermidis, S. aureus, B. cereus, B. subtilis, E. aerogenes, P. aeruginosa* and *A. hydrophila* show an inhibition zone of 18±0-10±1 mm, while other bacteria have zones of 6 mm or less. showed. *S. epidermidis, S. aureus, B. cereus, B. subtilis, P. aeruginosa,* and *A. hydrophila* displayed inhibition zones of 10±15 mm in silver nanoparticles from root extract, while other bacteria showed inhibition zones of 5 mm or less. In the root, all yeasts formed a zone diameter of 10±0 mm. AgNPs showed more antimicrobial activity to yeasts compared to bacteria. None of the tested extracts did show inhibitory effects against *E. coli, K. pneumoniae,* and *S. typhimurium.* In antimicrobial evaluation, the zone with the diameter of < 8 mm was considered as inactive; 10±0-18±1 mm as less active, and 20±1-25±1 mm as very well active.

The release of silver cations from silver nanoparticles, which act as a reservoir for them, is responsible for silver nanoparticles antibacterial activity [34].

Silver nanoparticles from leaf extract showed good antimicrobial activity in *C. albicans, C. glabrata, C. parapsilosis* and *C. tropicalis.* Silver nanoparticles contain more antimicrobial activity to yeasts than bacteria.

Table 1. Antimicrobial Activity of Aqueous Extracts and Silver Nanoparticles

Zone Diameter (mm) of aqueous extracts and silver nanoparticles								
Zone Diameter (mm) of Aqueous Extracts				**Zone Diameter (mm) of Silver Nanoparticles**				
Microorganisms	**Leaf**	**Flower**	**Root**	**Leaf**	**Flower**	**Root**	**Ofloxacin (5µg)**	**Flukonazol (10µg)**
S. aureus ATCC 29213	20±1	13±1	12±0	25±1	15±1	15±0	20±1	0
S. epidermidis ATCC 12228	18±1	12±1	10±0	20±1	15±1	10±0	21±2	0
L.monocytogenes ATCC35152	5±1	6±1	2±1	8±1	6±1	5±1	20±1	0
B. cereus 709 Roma	12±2	10±0	10±0	15±1	10±0	10±0	30±1	0
B. subtilis ATCC 6633	13±2	12±1	10±1	15±1	15±1	10±1	15±2	0
E. coli ATCC 25922	0	0	0	0	0	0	15±1	0
K. pneumoniae ATCC 13883	0	0	0	0	0	0	20±1	0
C. jejuni ATCC 33560	6±2	6±0	2±1	8±2	6±0	5±1	20±1	0
E. aerogenes ATCC 51342	12±2	8±0	2±1	15±1	10±0	5±1	20±1	0
S. dysenteriae ATCC 11835	15±1	6±0	5±0	18±1	6±0	5±0	20±1	0
S. typhimurium ATCC 14028	0	0	0	0	0	0	20±2	0
V. angilarum ATCC 43312	6±0	3±0	2±0	8±0	5±0	5±0	15±1	0
P. aeruginosa ATCC 27853	12±2	12±0	10±0	20±1	15±0	10±0	22±1	0
A.hydrophila ATCC 7966	15±1	12±1	12±1	20±1	15±1	10±1	10±1	0
P. mirabilis ATCC 29906	13±2	3±1	3±1	15±1	5±1	5±1	10±1	0
P. vulgaris ATCC 29905	13±2	4±1	2±1	16±2	5±1	5±1	25±1	0
C. albicans ATCC 90028	18±1	15±0	8±1	25±1	18±0	10±0	0	25±1
C. parapsilosis M006	15±2	15±0	7±1	22±1	18±0	10±0	0	25±1
C. tropicalis M007	15±2	12±0	8±0	25±1	15±0	10±0	0	25±1
C.glabrata ATCC 90030	18±1	13±1	7±0	20±1	15±1	10±0	0	23±1

Phytochemicals Analysis and Antioxidant Activity

Phytochemicals Analysis of Silver Nanoparticles

Flavonoids, saponin, and tannin were found to be active components in the plant based on the findings of phytochemical screening. Table 2 summarizes the phytochemical screening of the aqueous extract of *P. vulgaris* leaf, flower, and root, both qualitatively and quantitatively. The analysis explained the existence of several phytoconstituents in the leaf extract, including flavonoids, tannins, phenolic compounds, glucosides, and proteins. The early research showed that plant includes phenolic and flavonoids maintain powerful antioxidant abilities and from the biosynthesis of nanoparticles [35].

Tablo 2. Phytochemical screenings of Biosynthesized Silver Nanoparticles from *P. vulgaris* Leaf, flower and Root

Phytochemicals	**Test**	**Silver nanoparticles Result**		
		Leaf	Flower	Root
Flavonoids	Shinoda	++	+++	+
Terpenoids	Salkwaski	-	+	-
Saponins	Frothing	++	++	+++
Tannins	Ferric chloride	++	+	+
Carbonhydrates	Molish	++	+++	++
Proteins	Biuret	+	+	-

(+++) = strong presence, (++) = moderate presence, (+) = Less present, (-) = Absent

A yellow precipitate showed the presence of tannins in the *P. vulgaris* leaf extract. In *P. vulgaris* carbohydrates screening, a reddish-purple ring was found to be high in the flower, while it was weaker in the leaf and root. Obtaining a purple color as a result of protein screening in *P. vulgaris* extracts was found to be positive in leaf and flower extracts. Green synthesis of AgNPs was carried out using *P. vulgaris* leaf, flower, and root extract. In this way, nanoparticle synthesis with plant extract is more advantageous than known resistant antibiotics as it is cost-effective, friendly, and easy to use.

In another study, Green synthesis of gold nanoparticles and silver nanoparticles synthesized from *Pistacia*

atlantica were reported to be a very good friendly and non-toxic source [36].

According to phytochemical screening, the presence of flavonoids was determined to be weaker in the leaf and root part while a yellow coloration was observed in the flower part of *P. vulgaris.* While terpenoids were found positive in flower extracts of *P. vulgaris*, leaves and roots were also evaluated as negative. Saponins were positive in root extracts of *P. vulgaris*, while leaves and roots were also considered to be moderate availability. The presence of these phytochemicals in *P. vulgaris* is an indication that antibacterial and antioxidant activity may also occur.

In our study, silver nanoparticles were synthesized using leaf, root, and flower extracts of *P. vulgaris.* This study also can serve as the main source of *P. vulgaris* extracts phytochemicals for pharmaceutical products, so the *P. vulgaris* plant can be used for treatment in various diseases.

Total Phenolic (TPC) and Total Flavonoid (TFC) Content of Extracts and Silver Nanoparticles

The Folin-Ciocalteu reagent was used to determine the total phenolic content. Total phenolic content of the boiled water of *P. vulgaris* extracts express grams of Gallic acid equivalents (GAE).

Total phenolic compounds in sections changed, with values ranging from 8.28 0.5 mg/g for the leaf, 25.10 0.2 mg/g for the flower, and 5.20 0.0 mg/g for the fruit, expressed as gallic acid equivalents (GAE). The flower had the greatest total phenolic content of all the flowers. Orhan et al. [30] study that TPC usage of *P. vulgaris* water extract has 7.55 mg of GAE/g extract. A water extract of *P. vulgaris* had a TPC value of 89.6 g GAE/mg extract in a different investigation. Furthermore, for a concentration of 45 g/mL, the DPPH inhibition values of *P. vulgaris* water extract are 43% and 99.5 percent, respectively [15].
The total phenolic contents of AgNPs were in 9.06 ± 0.5 (leaf), 29.08 ± 0.1(flower), and 8.64 ± 3.3 (root) mg GAE/g DW range which is in agreement with values reported before [37]. The highest amount of total phenolic was observed in flowers (AgNPs), while the lowest one was observed in the root. The total phenolic content values obtained for the ethanol extracts of the primula are different from that reported for aqueous ethanol extracts of the primula, 535.4 mg GAE/ 100 g for flowers [38]. Phenolic compounds are thought to provide color, taste, aroma and beneficial effects on health. It is also believed to contribute to the quality and nutritional value. The phytochemical results may create a link between antibacterial and antioxidant activity. It can find an important place as an antimicrobial and antioxidant agent [39]. The highest amount of total flavonoids was observed in flowers (11.27 ± 0.4), while the lowest one was observed in the root (0.53 ± 2.2.2) in extract.
The content of flavonoid expressed as equivalents, varied 4.32 ±0.4 (leaf), 19.67 ± 2.2, and 1.98 ± 2.6 mg equivalent/g silver nanoparticles (Table 3). The flower showed the highest amount of flavonoid contents followed by leaf and root in AgNPs. Flavonoids show antioxidant activity and their effects on human nutrition and human health are quite high. It is well known that it handles the antioxidant effect in flavonoid plants containing hydroxyl functional groups [40].

Tablo 3. Total phenolic (mg GAE/g DW) and Total Flavonoids Contents of (mg CE/g) Aqueous Extracts and Silver Nanoparticles

	Total Phenolic (mg GAE/g DW)			**Total Flavonoid (mg CE/g)**		
	Leaf	Flower	Root	Leaf	Flower	Root
Aqueous Extracts	8.28±0.5	25.10±0.2	5.20±0.0	1.36±2.2	11.27± 0.4	0.53±2.2
Silver Nanoparticles	9.06±0.5	29.08±0.1	8.64±3.3	4.32±0.4	19.67±2.2	1.98±2.6

Antioxidant Activity of Aqueous Extracts and Silver Nanoparticles

In this study, the antioxidant activities of silver nanoparticles and aqueous extracts were evaluated using DPPH radical scavenging assay. As it is depicted in Table 4 the inhibition varied between 32.6-41.5 % for leaf, 78.4-86.3% for flower and 28.3-36.7 % for root in 12.5 and 100 (µg/mL) concentration of extracs. As it is depicted in Table 4 the inhibition varied between16.4-23.2% for roots, 16.0-17% for leaves and 14.9-24.7 %.for flowers. Table 4 shows that AgNPs from flower extracts provide the most DPPH radical scavenging activity (90.6 %) at higher concentrations (100 µg/mL). At 50 µg/mL and 100 µg/mL, silver nanoparticles from the leaf showed moderate inhibition of DPPH % 48.8 and 58.4. Also, At 50 µg/mL and 100 µg/mL, AgNPs from root extracts showed moderate inhibition of DPPH % 46.3 and % 48.4. Ascorbic acid showed the highest inhibition of DPPH at 100, 50, 25 and 12.5 µg/mL with 96.0 %, 95.1 %, 93.1 and 90.1 %. The result indicated that the % inhibition of DPPH by µg/mL was dose-dependent. The antioxidant activity obtained in the present study is different from that reported for aqueous ethanol extracts of the primula, research obtained 86.65 % [38]. Primrose water extract and ethanol are effective DPPH radical scavenging which was 99.5 and 99.4 [15]. Demir et al., [40] stated that the extract prepared with dimethyl sulfoxide from P. vulgaris flowers has strong antioxidant properties.

Table 4. Antioxidant Activities of Aqueous Extracts and Silver Nanoparticles in DPPH Assay

% DPPH inhibition by Aqueous Extracts and Silver Nanoparticles

	Aqueous Extracts			**Silver Nanoparticles**			
Concentration (µg/mL)	Leaf	Flower	Root	Leaf	Flower	Root	% DPPH inhibition by Ascorbic Acid
100	41.5	86.3	36.7	58.4	90.6	48.4	96.0
50	40.0	83.4	34.3	48.8	86.7	46.3	95.1
25	35.7	82.6	32.3	46.6	84.6	42.3	93.1
12.5	32.6	78.4	28.3	45.6	81.5	40.2	90.3

Antioxidants are of great importance in reducing oxidative stress, which plays a role in disease development by damaging biological molecules [41]. Oxidative stress plays a role in various acute and chronic pathological processes such as cellular aging, acute and chronic kidney disease, cardiovascular, cancer, neurodegenerative diseases, and biliary tract diseases [42]. The consumption of antioxidants is necessary to maintain the homeostasis balance in the human body, to prevent and treat diseases. But synthetic antioxidants have some degree of toxicity. Thus, taking natural antioxidants from food is the first choice, because natural antioxidants play an important role in the prevention and treatment of diseases [43-44].

Antimicrobial and antioxidant activities of *P. vulgaris* extracts are promising that silver nanoparticles with new antioxidant and antimicrobial activity may find a wide area of use in medicine. More research is needed, especially to get therapeutic drugs. With detailed studies on this subject, the usage areas of nanoparticles can be further expanded.

CONCLUSION

Consequently, Silver nanoparticles were synthesized by using *P. vulgaris* extracts (leaf, flower, and root) grown under in vitro conditions. Gram-positive bacteria are more vulnerable to gram-negative bacteria, according to antibacterial investigations. In short, antioxidants have an active function in inhibiting and destroying free radicals, thus helping the body's defense mechanism on disease and chronic infections.

In this study, Silver nanoparticles showed good biological activities, antimicrobial and antioxidant activity, and phytochemical properties. This may be useful for pharmacologists to discover safe and cost-effective drugs for the treatment of ailments rather than synthetic drugs.

Thus, the synthesized nanoparticles became a good alternative to developing an antimicrobial and antioxidant agent.

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