

Synthesis and Characterization of *Veronica beccabunga* Green Synthesized Silver Nanoparticles for The Antioxidant and Antimicrobial Activity

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Abstract: The use of nano-agents in the healthcare field is an important alternative in reducing the increasing pathogen resistance. One of the main purposes of the nano mechanism is that the secondary metabolites produced naturally in plants become more effective through metals. Silver (Ag) nanoparticles were obtained by green synthesis using *Veronica beccabunga* plant extract and AgNO₃ in our study. Scanning electron microscopy was used for SEM, SEM / EDX images of synthesized silver nanoparticles. The interaction of *V. beccabunga* with Ag was explained by X-ray diffraction (XRD) analysis. The characterization process was performed using an ultraviolet-visible (UV-vis) spectrophotometer and Fourier converted infrared spectroscopy (FT-IR). It was determined that silver nanostructures have an important antioxidant potential as a result of 2,2-difenil-1-pikrilhidrazil (DPPH) analysis. The antimicrobial activity of synthesized metal nanoparticles was investigated, against some pathogens causing disease in humans, by the disk diffusion method. Ag NPs /Vb has been found to have antibacterial effects against *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25952, *Pseudomonas aeruginosa* ATCC 27853 microorganisms. In our current study, Ag NPs / Vb appears to provide a remarkable effect for its use in medicine, pharmacology.

Keywords: *Veronica beccabunga*, antimicrobial, antioxidant, nanoparticle, characterization

1. Introduction

Metallic nanoparticles produced in different sizes and shapes can be used in many application areas. Nanoparticles come to the fore in applications such as medicine, electronics industry, agriculture, wastewater applications, antitumor and anticancer studies. The importance of metallic nanoparticles synthesized by biological applications for their antimicrobial and cytotoxic effects is increasing day by day (Patil and Kim, 2017). In particular, the antimicrobial effects of nanoparticles such as silver, copper, zinc, and gold have been reported (Mittal et al., 2013).

Antibiotics used today affect the cell wall, protein structures, lipid structures, and DNA structure in bacteria (Tenover, 2006; Wang et al., 2017). Nanoparticles (NPs), on the other hand, come into direct contact with the bacterial cell wall

without penetrating the cell. Thus, although bacteria develop resistance against antibiotics, they cannot develop resistance against NPs.

Plants are known to have the potential to over-accumulate metallic ions and reduce them biologically. Because of these properties, plants have been considered to be a more environmentally friendly instrument for the bio-synthesis and detoxification of metallic nanoparticles (Mittal et al., 2014). Plant extracts and metal ions are subjected to bioreduction to form nanoparticles with the green synthesis method (Shah et al., 2015). Plant metabolites play an important role in the reduction and stabilization of metal ions to nanoparticles. Many plant species contain bioactive alkaloids, phenolic acids, polyphenols, proteins, sugars, and terpenoids (Koçak et al., 2020). Plants provide important advantages due to their low cost and not harming the environment. For this reason,

plants are seen as the best choice for biological nanoparticle synthesis (Singh et al., 2016).

The *Veronica beccabunga* herb is a perennial plant belonging to the Asteraceae family. It is a shrubby plant that usually grows on riverside, fields, slopes, and steppes (Yaldız ve ark., 2010). *V. beccabunga* can grow in Europe, Asia, North Africa, and North America. In Turkey, it can be grown in the Black Sea, the Mediterranean, and in certain places in Anatolia (Yaşar et al., 2017). *V. beccabunga* is a bitter and aromatic herb that has been used as a medicine since ancient times. Nowadays *V. beccabunga* is used in the treatment of many diseases. It has been stated that the *V. beccabunga* has a good antifungal effect and a low degree of antibacterial effect. It has traditionally been used as an antiseptic, balsamic and antidepressant, against cholera and digestive disorders, as well as in the treatment of diuretic conditions, leukemia, and sclerosis. The *V. beccabunga* is known to be effective as a mild sedative for muscle relaxation and the treatment of anxiety (Dewick, 2001; Djilas et al., 2002).

The aim of this study was to investigate the effect of *V. beccabunga* collected from rural areas of Van-Turkey on human health. In this context, some biological and biochemical parameters were investigated.

2. Materials and Methods

2.1. Preparation of plant extract

V. beccabunga plant was collected in rural areas of Van-Turkey. The collected *V. beccabunga* was washed with distilled water and kept 7 days at room temperature to dry. The dried plants were powdered thoroughly with an electric blender. The powdered plant of 50 g was added into 250 mL of distilled water and heated in a mixer (Thermomac) at 80 °C for 15 minutes. The resulting extract was filtered through Whatman 1 filter paper and kept at 4 °C for further processes.

2.2. Green synthesis of silver nanoparticles

For the synthesis of Ag NPs, 1 mM 500 ml AgNO₃ aqueous solution was prepared. *V. beccabunga* leaf extract of 100 ml was allowed to react in a 1000 ml flask at room temperature under constant conditions. The formation of silver nanoparticles was determined by the change of colorless solution to dark brown within 30-40 minutes. The nanoparticles obtained by green synthesis were separated by centrifugation (NF-1200R) at 10.000 RPM for 5 minutes. This process was repeated three times to remove free silver ions. Obtained substances were left to dry in the oven and stored at + 4 °C for further processing.

2.3. Characterization of Ag NPs / Vb

The formation of silver nanoparticles was performed using a UV device (Shimadzu UV-2450), which measures the ultraviolet-visible (UV-Vis) absorption spectrum in the range of 200-800 nm. The Ag NPs / Vb complex revealed the presence of phytochemicals with Fourier Transform-Infrared (FT-IR) named (Perkin Elmer-Spectrum) in the range of 500-4000 cm⁻¹. Measurements for X-ray diffraction (XRD) to illuminate the crystal structure of nanoparticles were made on a device (Panalytical Empyrean, Turkey), which contains an X-ray generator with copper (Cu) K α radiation (40 kV, λ = 1.5406 Å). By scanning the sample surface with a scanning electron microscope or scanning electron microscope (SEM), the topography and composition on the surface were taken. At the same time, the elemental analysis was performed with EDX (Zeiss Smart EDX).

2.4. Antioxidant activity 2,2-difenil-1-pikrilhidrazil (DPPH) of Ag NPs / Vb

The DPPH quenching activity of Ag NPs / Vb, the extract of the study subject, was calculated according to the Blois method (Blois, 1958). Butil hydroxyanisole (BHA) and Butil hidroxytoluene (BHT) were used as positive controls in this method. The experiment was performed using methanol solutions of 0,1 μ g ml⁻¹ DPPH. DPPH and extracts in the same ratio were prepared in 7 different concentrations of 5 μ g ml⁻¹, 10 μ g ml⁻¹, 15 μ g ml⁻¹, 20 μ g ml⁻¹, 25 μ g ml⁻¹, 50 μ g ml⁻¹, and 100 μ g ml⁻¹. Ag NPs / Vb of 3 ml extract and positive control were taken and DPPH solution was added to them. The mixtures formed in the tubes were incubated for 30 minutes at room temperature in the dark. At the end of this period, absorbance values were read at 517 nm. This graph is obtained using Equation 1.

$$\% \text{ Inhibition (I)} = [(A \text{ control} - A \text{ sample}) / A \text{ control}] \times 100 \quad (1)$$

As a result of these processes, a graph of Ag NPs / Vb concentration versus increasing DPPH ethanol concentration was obtained. This graph was obtained using the above equation.

2.5. Antimicrobial activity

Six pathogens were used to examine the antimicrobial activity of silver nanoparticles obtained from the *V. beccabunga*. The microorganisms used in the study are given in (Table 1). Standard strains were obtained from the Van Yüzüncü Yıl University of Eastern Anatolia in Turkey the Faculty of Science, Department of Biology. Neomycin (10 μ g) antibiotic was used as the control group. The antimicrobial activity of Ag

NPs / Vb was determined by the disk diffusion method (Ibraheem et al., 2016). Tryptic soy broth was used for the activation of microorganisms and Nutrient Agar media were used for the Disk diffusion method.

Table 1. Microorganisms used in the study

Bacteria
<i>Bacillus cereus</i> ATCC 10876
<i>Bacillus subtilis</i> ATCC 6633
<i>Escherichia coli</i> ATCC 25952
<i>Pseudomonas aeruginosa</i> ATCC 27853
<i>Staphylococcus aureus</i> ATCC 29213
Fungus
<i>Candida albicans</i> ATCC 90028

3. Results and Discussion

3.1. Characterization of silver nanoparticles

SEM / SEM-EDX, FT-IR, XRD, and UV-Vis techniques respectively were used for the structural and morphological characterization of Ag nanoparticles prepared with green synthesis using *V. beccabunga* plant. SEM images of the Ag NPs / Vb sample taken at different scales and the EDX spectrum obtained from one of these images (Figure 1). It can be seen from SEM images of different scales that Ag nanoparticles are homogeneously distributed. In the EDX spectrum, it is seen that there are Ag, carbon (C), and oxygen (O) elements that form the structure of the Ag NPs / Vb sample.

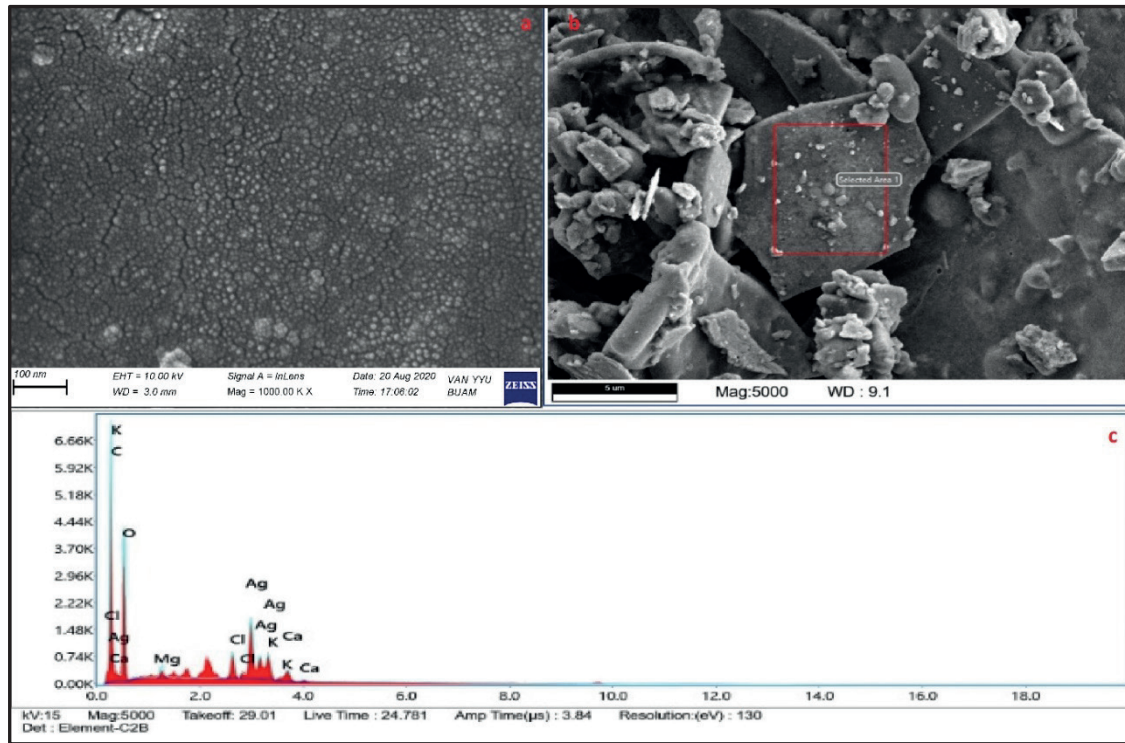


Figure 1. (a-b) SEM images and (c) corresponding EDX spectrum of Ag NPs / Vb

In addition, it has been observed that there are different metals thought to be in the structure of the plant.

The FT-IR image shows (Figure 2) the peaks formed by functional groups (phenolic, flavonoid, gallic acid, coumarin) in the structure of *V. beccabunga*, the current plant in the range of 500-2000 cm^{-1} . It is seen that these peaks decrease or disappear in Ag NPs / Vb. The peaks in between are thought to belong to many organic components,

while the sharp peak at 3000-3500 cm^{-1} belongs to the C-H organic component.

XRD technique was used to determine the crystal structure of silver nanoparticles. Considering the spectrum, different peaks are seen (Figure 3). It was determined that Ag NPs have different breaking peaks as 32.54°, 47.05°, 66.43°, and 77.02° against Ag (111), Ag (200), Ag (220), and Ag (311) planes. It is seen that the data obtained are compatible with the literature studies (Fateme et al., 2017).

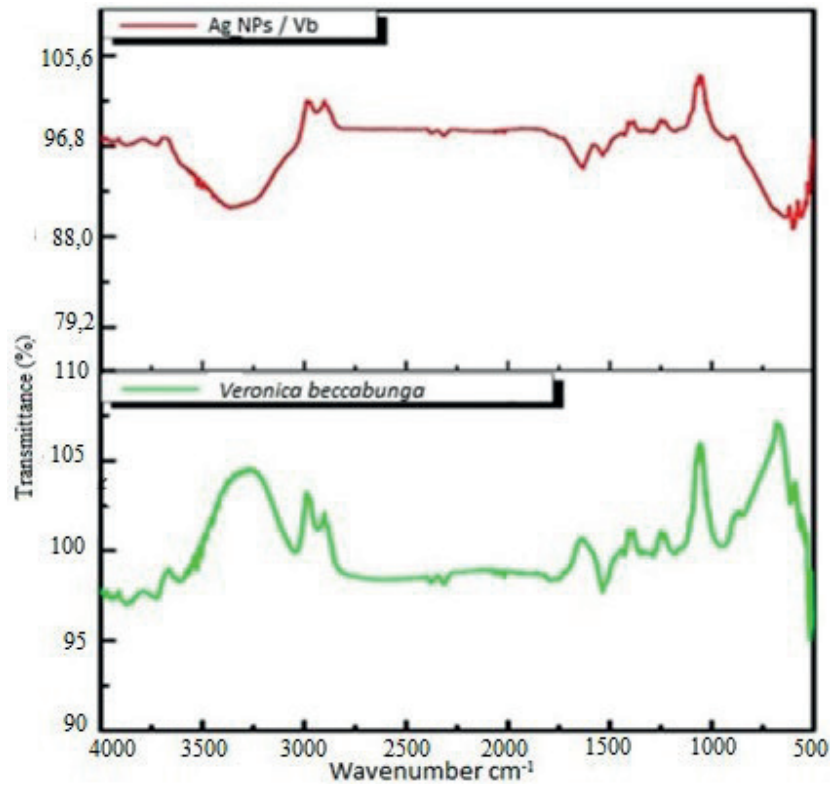


Figure 2. FT-IR spectra of Vb and Ag NPs/Vb samples

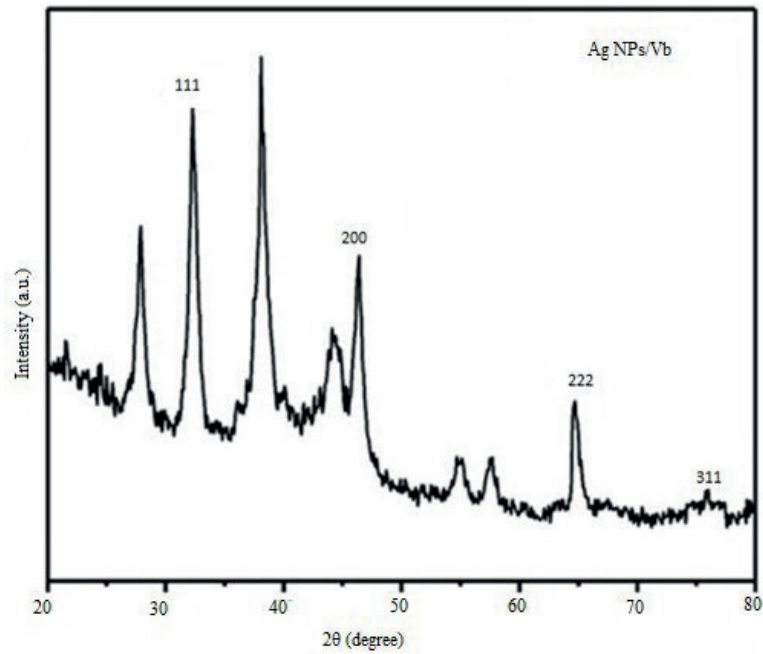


Figure 3. XRD pattern of Ag NPs/Vb

In Figure 4, it is seen that the AgNO_3 compound analyzed using UV-Vis spectroscopy gives a strong peak at 420 nm wavelength. It is understood that the given peak is in line with the literature data (Selvam et al., 2017; Nazar et al., 2018). After the Ag NPs

/Vb, the existing peak appears to have dropped significantly. For this, it can be said that the peak formed has disappeared because Ag is reduced to $\text{Ag}^{+1} - \text{Ag}^0$ due to the transition between electrons in NPs / Vb.

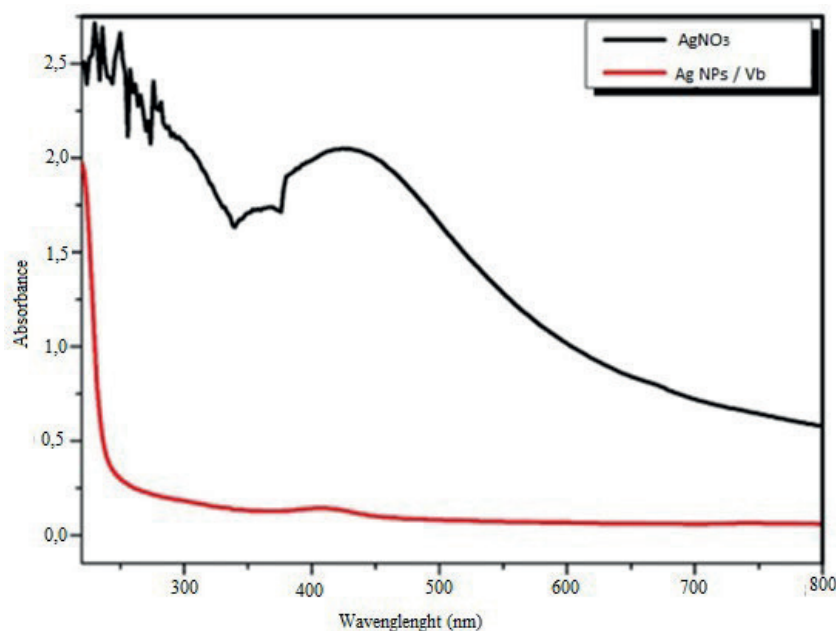


Figure 4. UV-vis spectra of Vb, AgNO₃, and Ag NPs / Vb samples

3.2. Antimicrobial activity

The antimicrobial activity of Ag NPs obtained by using *V. beccabunga* plant and AgNO₃ was investigated. Silver nanoparticles obtained from the *Musa acuminata* plant showed activity against *Pseudomonas aeruginosa* (Valsalam et al., 2019). Silver nanoparticles (AgNPs) obtained from *Ocimum sanctum* (basil) leaves formed an inhibition zone against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* strains (Aazam and Zaheer, 2016). In the study, it was

determined that the extract obtained from *V. beccabunga* created a weak inhibition zone (Table 2) against *Bacillus subtilis* ATCC 6633 and *Escherichia coli* ATCC 25952. On the other hand, Ag NPs /Vb clusters were observed to affect *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25952, and *Pseudomonas aeruginosa* ATCC 27853 (Table 2). Therefore, it was determined that silver nanoparticles have an antibacterial effect against some bacteria. In addition, it was determined that it did not show an antifungal effect against *Candida albicans* ATCC 90028.

Table 2. Antimicrobial activity results

Test microorganisms	Inhibition zone (mm)		
	Extract	Ag NPs/Vb	Neomycin
Bacteria			
<i>Bacillus cereus</i> ATCC 10876			16±5.0
<i>Bacillus subtilis</i> ATCC 6633	10±1.4	14±3.2	20±4.2
<i>Escherichia coli</i> ATCC 25952	8±2.5	9±2.0	12±5.5
<i>Pseudomonas aeruginosa</i> ATCC 27853		10±4.5	
<i>Staphylococcus aureus</i> ATCC 29213			16±1.0
Fungus			
<i>Candida albicans</i> ATCC 90028			21±2.2

3.3. Antioxidant activity 2,2-difenil-1-pikrilhidrazil (DPPH)

DPPH radical quenching activity is the safest, highly valid, cheap, accurate, fast, easy, and economical method used to measure total antioxidant activity (Sharma and Bhat, 2009; Deng et al., 2011) By adding an antioxidant solution to the DPPH solution, antioxidants reduce radicals by giving them protons (Kedare and Singh, 2011). As

a result of the reduction, the color of the solution changes from purple-violet to yellow and there is a decrease in the absorbance at the measured wavelength (Haida and Hakimian, 2019). This color change is usually measured spectrophotometrically at 517 nm. In Figure 5, the antioxidant activity of Ag NPs / Vb was compared with positive control BHA and BHT. DPPH radical quenching activities at the highest concentration of 100 µg ml⁻¹ are

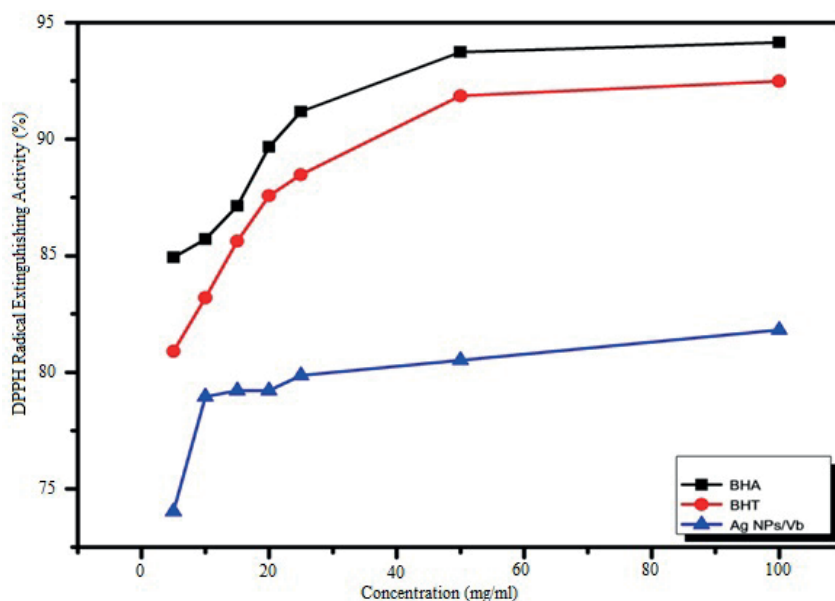


Figure 5. DPPH Radical extinguish Activity of Ag NPs / Vb

94.021% for BHA, 92.024% for BHT, and 81.245% for Ag NPs / Vb. *V. beccabunga* green synthesis study has not been found in the literature. Antioxidant studies related to the natural structure of the plant are available in the literature. In previous studies, the antioxidant activity of *V. beccabunga* plant was investigated and it was determined that it is a good antioxidant (Nikolova, 2011; Nóra et al., 2019). When compared with the studies in the literature, Ag NPs/Vb seems to increase the potential antioxidant activity.

4. Conclusions

The use of nano-sized structures at the biomedical level has made important progress in sterilization, disinfection, and pharmaceutical industries. New ones are added to promising nanotechnological studies every day. It is thought that Ag NPs / Vb synthesized in this study can form a basis for further studies and contribute to the development of pharmacological treatments and non-toxic alternatives. Analyzes show that AgNPs have good antioxidant potential. Looking at the antimicrobial properties of silver nanoclusters, it was determined that they formed an inhibition zone against some pathogen bacteria. It is predicted that Ag NPs / Vb structures can contribute to the production of new antibacterial drugs and will become an important factor in the field of health and biotechnology as a result of more comprehensive studies.

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