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Research Article

DETERMINATION OF ANTIMICROBIAL ACTIVITIES OF ENDEMIC *Veronica cinerea* Less AND *Helleborus vesicarius* Aucher ex Boiss

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ABSTRACT. Endemic plants containing biologically active substances play an essential role in microbiological studies. Investigating the antimicrobial potential of these endemic plants will lead to new perspectives. This potential encouraged us to investigate the antimicrobial effects of natural plants collected from Kahramanmaraş, province of Turkey, on six bacterial strains. Two plant species, *Veronica cinerea Less* and *Helleborus vesicarius* Aucher ex Boiss, were the focus of this study. Analyses showed that *Helleborus vesicarius* has a high antioxidant potential, and similar antimicrobial effects were observed in *Veronica cinerea*, an important finding for potential applications. These data highlight the importance of Turkey's natural plant diversity and the potential of plant resources in medicine and nanotechnology. However, more research needs to be done, and safe use needs to be ensured for these plants. Investigating the applicability of natural compounds and environmentally friendly methods in medicine and material sciences will contribute to future progress.

Keywords: *Veronica cinerea, Helleborus vesicarius, Antimicrobial effects, Eco-friendly*

INTRODUCTION

From 5000 BC to the present, plants have been widely used for nutritional and therapeutic purposes. Plants produce hundreds of chemical compounds to defend mechanisms against insects, fungi, diseases, and herbivorous mammals [1]. Additionally, plants have been used as food, spices, and medicines for thousands of years, and herbal drugs have evolved to include the isolation of active compounds [2]. Today, approximately 25-48% of the world's medicines are derived from plants [3].

Turkey's geological structure, geographical location, and climate diversity allow it to harbor a rich plant diversity. Turkey has about 11,400 plant species, and about 3,700 are endemic [4]. One plant species examined in this study is *Helleborus vesicarius*, which belongs to *Helleborus*.

Helleborus is a genus of poisonous plants belonging to the Ranunculaceae family. In Turkey, species such as *Helleborus orientalis* and *Helleborus vesicarius* are endemic to Turkey and known as the "poppy flower". Helleborus species have been used to treat diseases such as arthritis, oedema, and ulcers and contain active compounds with anti-inflammatory, antioxidant, and antimicrobial effects. Some studies have also reported that *Helleborus* species have anticancer effects [5].

With the extraction of plant extracts, the emergence of natural compounds and the investigation of their use in different fields have come to the fore. For example, phenolic compounds found in plants have antioxidant activity and are used in traditional medicine to treat conditions such as respiratory diseases, influenza, and wound healing. In addition, some veronica species are reported to have potential anticancer activity [6].

Helleborus species contain active compounds with anti-inflammatory, antioxidant, and antimicrobial effects. There are also studies on its anti-diabetic and antitumoral properties [7]. However, despite these potential benefits, *Helleborus* species are considered poisonous, but poisonings are often associated with incorrect dosage [7].

'Green nanotechnology' is a synthesis method involving the reduction of metal salts through plants. It is a cheaper, environmentally friendly, and valuable technique compared to other nanotechnological methods. This field is used in many areas, such as cancer treatment, prevention, and treatment of microbial-induced diseases [8]. Therefore, it involves using plant extracts and herbal compounds since this method enables the reduction of metal salts through phytochemicals derived from plants [9, 10, 11]

Green nanotechnology has emerged from studying perfectly designed nano-sized materials and living things already existing in nature [12]. However, it can also be used in the production of silver nanoparticles with the use of phytochemical compounds obtained from plants. Silver nanoparticles are known for their antimicrobial effects and are used in medicine to treat infections.

Therefore, green nanotechnology allows the development of new materials with antimicrobial properties by synthesizing and using silver nanoparticles with natural compounds provided by plants [13].

With the discovery of natural compounds provided by plants and the use of environmentally friendly methods such as green nanotechnology, the potential for developing plant-derived drugs and materials is inspiring. Turkey's rich plant diversity and endemic taxa offer an excellent opportunity for potential research and discoveries in this field.

MATERIALS AND METHODS

In this study, *Veronica cinerea* and *Helleborus vesicarius* plant species growing naturally in Turkey were collected from Kahramanmaraş province of Turkey. The above-ground parts of the plants were washed thoroughly in tap water and dried in a cool and moisture-free environment.

The plant material was extracted using a Soxhlet device. Five grams of each plant sample was weighed and placed in the Soxhlet device with 50 ml of ultrapure water (solvent). The extraction process was carried out at 55°C for 8 hours. The liquid extract obtained as a result of this process was treated with a specific chemical precursor to synthesize silver nanoparticles. The precursor chemical ($AgNO_3$) was weighed, dissolved in water, and added to 100 ml. Taking 10 ml of the liquid extract, 90 ml of $AgNO_3$ and pure water solution were added, and this mixture was kept in the microwave at 360°C. The extracts that turned brown were taken into ependorf tubes and centrifuged at 6000 rpm for 6-12 minutes. The supernatant was discarded, and the pellet (precipitate) was washed with distilled water. The washing process was repeated 3-4 times. After washing, the pellet was transferred to petri dishes to remove the solvent and dried in an oven at 37°C. The portion obtained from the petri dishes at the end of the drying process was

named silver nanoparticles. The silver nanoparticle was taken into a falcon tube, dissolved in dimethyl sulfoxide (DMSO), and made ready for use.

To determine the antibacterial activity of the prepared samples, three gram-negative (*Enterobacter Aerogenes* ATCC 13048, *Klebsiella Pneumoniae*, *Salmonella typhimurium*) and three gram-positive (*Enterococcus Durons, Stophylococcus Aureus* ATCC 25923, *Bacillus Suptilus* DSMZ 1971) bacterial strains were examined [14].

The agar well diffusion method was used for the antimicrobial study. This method determined the concentration ranges of plant extracts and synthesized silver nanoparticles. Microorganisms were inoculated into the media prepared in Petri dishes. Then the wells were opened, and plant extracts and silver nanoparticles were added. After the incubation period, the inhibition zone diameters were measured.

According to the inhibition zone diameters, the Minimum Inhibitory Concentration (MIC) test was performed at the determined concentrations of plant extracts and silver nanoparticles.

Following the MIC test, the Minimum Bactericidal Concentration (MBC) test was performed. Samples were taken from the concentrations determined in the MIC test, inoculated on MHA solid medium, and incubated at 37°C for 18-24 hours. The concentration that killed approximately 99.9% of the bacteria was accepted as the MBC value[15].

RESULTS AND DISCUSSION

MIC Results

After the MIC values of the plants were measured at 600 nm with a spectrophotometer, the results were averaged and given in Table-1, taking into account the deviations together with the results in MHA cultivation.

Table 1. *MIC values of Veronica cinerea and Helleborus vesicarius plants*

	Veronica cinerea	***Helleborus vesicarius***
Enterobacter Aerogenes ATCC 13048 (-)	12,5±0,96 ml	25±1,04 ml
Klebsiella Pneumoniae (-)	12,5±1,08 ml	50±1,20 ml
Enterococcus Durons (+)	12,5±1,05 ml	25±1,15 ml
Salmonella typhimurium (-)	25±1,07 ml	25±1,18 ml
Stophylococcus Aureus ATCC 25923 (+)	12,5±1,10 ml	50±1,15 ml
Bacillus Suptilus DSMZ 1971 (+)	12,5±1,04 ml	12,5±1,02 ml

MIC between 12.5mg/ml and 25mg/ml were obtained in *Veronica cinerea* plant. The agar well diffusion antimicrobial test supported to results of the MIC test. On the other hand, the other studied *Helleborus vesicarius* plant sample was effective only on *Bacillus Suptilus DSMZ* 1971 (+) bacterial strain.

MBC Results

Table 2. *MBC values of Veronica cinerea and Helleborus vesicarius plants*

	Veronica cinerea	***Helleborus vesicarius***
Enterobacter Aerogenes ATCC 13048 (-)	25 mg/ml	50 mg/ml
Klebsiella Pneumoniae (-)	25 mg/ml	50 mg/ml
Enterococcus Durons (+)	25 mg/ml	50 mg/ml
Salmonella typhimurium (-)	50 mg/ml	50 mg/ml
Stophylococcus Aureus ATCC 25923 (+)	25 mg/ml	50 mg/ml
Bacillus Suptilus DSMZ 1971 (+)	25 mg/ml	25 mg/ml

In the *Veronica cinerea* plant, MBC results were obtained between 25mg/ml and 50mg/ml values. The agar well diffusion antimicrobial test supports each other with the MIC test. Furthermore, for *Helleborus vesicarius* plant sample, *Bacillus Suptilus* DSMZ 1971 (+) bacterial strain showed MBC at 25 mg/ml in support of the agar well diffusion antimicrobial test, while other bacterial strains did not show expected result in support of the agar well diffusion antimicrobial test.

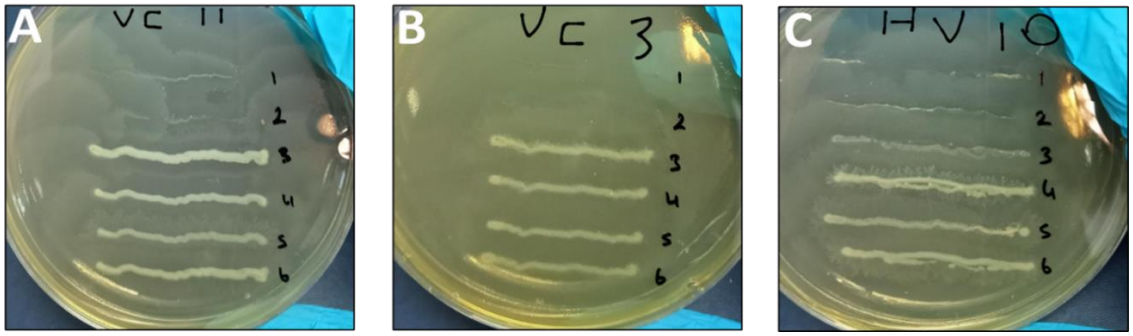


Fig. 1. *MIC and MBC analysis of Veronica cinerea and Helleborus vesicarius plants. In section 1, there is a plant concentration of 50 mg/ml, while the plant concentrations decrease by half towards section 6. Concentrations respectively; 1-50 mg/ml, 2-25 mg/ml, 3-12,5 mg/ml, 4-6,250 mg/ml, 5-3,125 mg/ml, 6-1,562 mg/ml.*

Fig. 1.A The MIC and MBC effects of *Veronica cinerea* plant on *Stophylococcus Aureus* ATCC 25923 (+) bacterial strain are shown in the photograph. Part number 3, i.e., the part where the plant concentration was 12.5 mg/ml, was determined as the MIC value, and part number 2, i.e., the part where the plant concentration was 25 mg/ml, was determined as the MBC value.

Fig. 1.B The MIC and MBC effects of *Veronica cinerea* plant on *Klebsiella Pneumoniae* (-) bacterial strain are shown in the photograph. Part number 3, i.e., the part where the plant concentration is 12.5 mg/ml, was determined as the MIC value, and part number 2, i.e., the amount where the plant concentration is 25 mg/ml, was determined as the MBC value.

Fig. 1.C The MIC and MBC effects of *Helleborus vesicarius* plant on the *Salmonella typhimurium* (-) bacterial strain are shown in the photograph. Part number 2, i.e., the part where the plant concentration is 25 mg/ml, was determined as the MIC value, and part

number 1, i.e., the part where the plant concentration is 50 mg/ml, was determined as the MBC value.

Table 3. *Agar Well Diffusion Results*

	Veronica cinerea		***Helleborus vesicarius***			
	50 mg/ml	**25 mg/ml**	**50 mg/ml**	**25 mg/ml**	**DMSO**	**IPM10**
***Enterobacter Aerogenes* ATCC 13048 (-)**	18 mm	10 mm	-	-	-	23 mm
***Klebsiella Pneumoniae* (-)**	20 mm	11 mm	-	-	-	19 mm
***Enterococcus Durons* (+)**	16 mm	7 mm	-	-	-	20 mm
***Salmonella typhimurium* (-)**	17 mm	8 mm	1 mm	-	-	22 mm
***Stophylococcus Aureus* ATCC 25923 (+)**	8 mm	2 mm	-	-	-	24 mm
***Bacillus Suptilus* DSMZ 1971 (+)**	15 mm	6 mm	2 mm	2mm	-	20 mm

(-) No zone of inhibition, (IPM10): Ipinem 10mg/ml. DMSO (10%). Well, the diameters were subtracted by 8mm.

According to the results of the antimicrobial study, *Veronica cinerea* endemic plant formed an inhibition zone diameter on all bacterial strains at 50 mg/ml and 25 mg/ml plant concentrations. In contrast, *Helleborus vesicarius* endemic plant only formed a minimal zone diameter on *Bacillus Suptilus* DSMZ 1971 (+) bacterial strain at 50 mg/ml plant concentration.

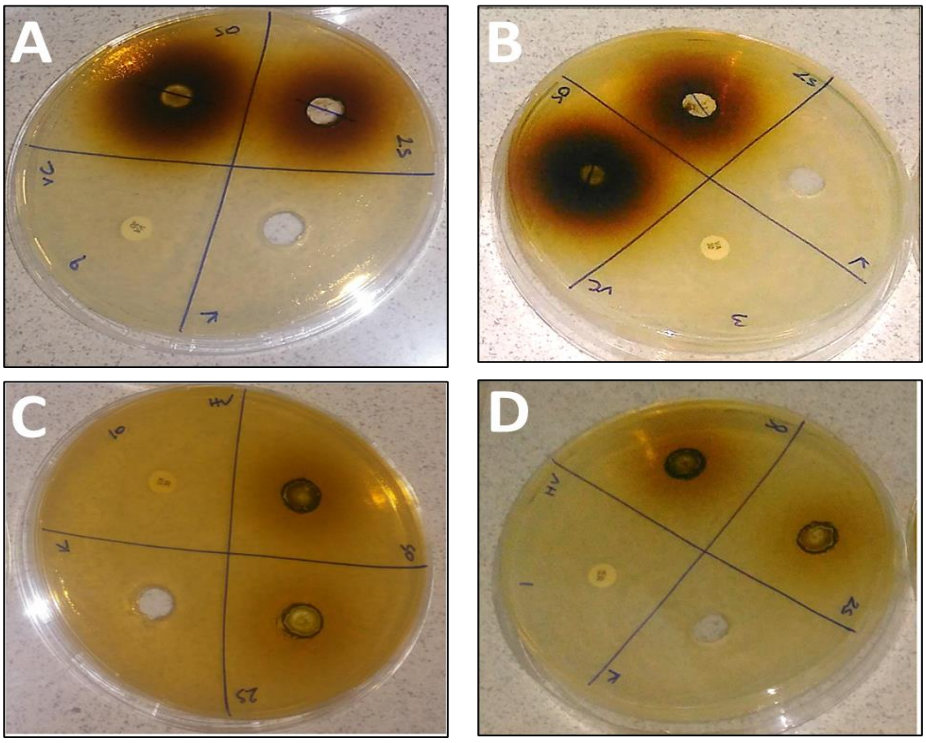


Fig. 2. *Agar well diffusion test results of Veronica cinerea and Helleborus vesicarius plants. Agar well diffusion application was performed as a control, 10mg/ml, 25mg/ml, and 50mg/ml.*

Fig. 2.A The effects of the agar well diffusion test of *Veronica cinerea* on *Enterococcus Durons* (+) bacterial strain are shown. It formed a zone diameter.

Fig. 2.B The effects of the agar well diffusion test of *Veronica cinerea* plant on *Klebsiella Pneumoniae* (-) bacterial strain are shown. It formed a zone diameter.

Fig. 2.C The effects of the agar well diffusion test of *Helleborus vesicarius* plant on *Salmonella typhimurium* (-) bacterial strain. No zone diameter was formed.

Fig. 2.D The effects of the agar well diffusion test of *Helleborus vesicarius* on *Enterobacter Aerogenes* ATCC 13048 (-) bacterial strain. No zone diameter was formed.

CONCLUSION

This study determined the antimicrobial activities of endemic *Veronica cinerea* Less and *Helleborus vesicarius* Aucher ex Boiss species. Although there is a study determining the total antioxidant and oxidant status of *Helleborus vesicarius* in the literature [16], there is no study determining the antimicrobial activity of *Veronica cinerea* Less.

Mohammed et al. showed that the plant has high antioxidant potential in their study on *Helleborus vesicarius* [16]. We present that our findings supported the antimicrobial effect of *Helleborus vesicarius* with the literature according to the results obtained.

Furthermore, we demonstrate similar antibacterial effects on *Veronica cinerea* Less species, similar to *Helleborus vesicarius*, and provide preliminary data to the literature for potential applications.

Using samples from the natural plant diversity of Turkey, this study highlights antimicrobial effects. Such studies contribute to exploring and evaluating the potential applications of natural plant compounds and eco-friendly methods, such as green nanotechnology in medicine and materials science.

As a result, it has shed light on the importance of Turkey's natural plant diversity and the potential of plant resources in medicine and nanotechnology. However, more research needs to be done on herbal medicine's safe use and possible risks. In addition, scientific studies and promoting good practices for the conservation and sustainability of existing plant diversity will form the basis for future research and medical innovations.

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