

Exploring the Potential of Blueberry Extracts as Natural Antifungal and Bioherbicidal Agents in Agriculture: Composition and Antioxidant Activity

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Abstract

This study aims to investigate the composition of essential oils, antioxidant activity, total phenolic and flavonoid content, as well as antifungal and herbicidal activities of the methanol extract of *Vaccinium myrtillus* collected from two different locations. Using GC/MS analysis, the plant essential oils from Muğla and Mersin locations were found to contain 20 and 19 components, respectively. The main components identified in the essential oils from Muğla and Mersin were α -Pinene (29.16%-15.75%), Eucalyptol (22.19%-26.46%), and Linalool (12.66%-25.27%). The methanol extracts of plant collected from two different areas were tested for antifungal activity against *Sclerotinia sclerotiorum* (SS), *Fusarium oxysporum* f sp. *melonis* (FOM), *Fusarium oxysporum* f sp. *cucumerinum* (FOC), and *Rhizoctonia solani* (RS). At a dose of 3000 ppm, the extracts inhibited mycelium growth by 78.00%, 73.31%, 46.33%, and 31.43% for SS, FOM, FOC, and RS, respectively. In allelopathy tests, the methanol extract of blueberry at a dose of 3000 ppm completely suppressed root-shoot development of *Taraxacum officinale*, while it significantly inhibited root-shoot development of *Rumex crispus* seed germination compared to the control. In addition, the plant exhibited significant antioxidant activity. These findings are significant as they provide insights for the development of new natural antifungal agents with potential applications in agriculture.

Introduction

Quality and yield losses in agricultural production areas can be attributed to fungal diseases and weeds. To combat these losses, fungicides and herbicides are frequently utilized. However, the indiscriminate use of these chemicals has resulted in issues concerning the sustainability of plant protection methods and the accumulation of toxic substances in water, air, soil, and food. Consequently, these factors have had negative effects on human health (Arslan and Karabulut, 2005; Işık et al., 2016). The adverse effects of pesticides have prompted researchers to explore alternative approaches. One such method is the utilization of natural components that are environmentally friendly and pose no harm to human health. Naturally grown plants contain compounds that possess potential biological effects, with these components being produced in greater quantities under stressful conditions and released into the environment. Secondary metabolites, in particular, have been linked to allelopathy and have been the focus of research in various fields, including differentologists, herbologists, ecologists, and physiologists (Li et al., 2020; Erez and Battal, 2022). These secondary compounds, which are also involved in allelopathy, are referred to as allelochemicals. Understanding the effects of these allelochemicals in plants aids in comprehending allelopathy and its mechanisms. Research has indicated that these allelochemicals influence crucial functions such as germination, root-shoot elongation, cell division, respiration, and photosynthesis (Singh and Thapar, 2003; Li et al., 2020). Moreover, studies have demonstrated that secondary metabolites, acting as allelochemicals, also exhibit efficacy against plant pathogenic fungi and pests. Some studies have shown the allelopathic (Santonja et al., 2019; Sudatti et al., 2020) and antifungal potential of secondary compounds on plant pathogenic fungi (Çakır et al., 2005; Bayan, 2016; Yılar et al., 2018).

Additionally, these secondary components, which are found in medicinal aromatic plants, generate bioactive molecules with antimicrobial and antioxidant properties. The oxidative process represents a significant pathway for the production of free radicals in food, drugs, and living systems (McCord, 2000; Sengül et al., 2009). Free radicals, possessing one or more unpaired electrons, are generated during normal and pathological cell metabolism. These free radicals play a role in the development of various diseases, including cancer, atherosclerosis, diabetes, and neurodegenerative disorders. However, antioxidants derived from medicinal aromatic plants can neutralize these radicals (McCord, 2000; Benhammou et al., 2008; Sengül et al., 2009). Antioxidants are commonly used as food additives to protect against oxidative degradation caused by free radicals (Wu et al., 2009; El Ouariachi and Bouyanzer, 2014). It has been reported that *Vaccinium myrtillus* fruit, due to its antioxidant content, reduces the risk of cancer and is particularly effective in preventing the development of Alzheimer's disease, which negatively affects brain function (Çelik, 2006).

This study aimed to determine the essential oil composition, total phenolic and flavonoid content of *Vaccinium myrtillus* leaves—an important medicinal plant. Furthermore, the study investigated the plant's antifungal, allelopathic, and antioxidant activities.

Materials and methods

Isolation of plant materials and essential oils Blue berries were collected from the Mersin and Muğla provinces during the 2020–2021 vegetation period. The plant specimens were identified by Melih YILAR. The collected plant materials were dried and ground in a shaded environment. The ground plant samples were stored until the extraction process. Plant essential oils were obtained using the hydrodistillation method with a Neo-Clevenger apparatus. For the extraction of volatile compounds, 100 g of plant materials were weighed, and then 300 mL of deionized water was added and distilled for 2 hours. The essential oils were separated, dried with anhydrous sodium sulfate, and stored in dark bottles at + 4°C until analysis (Akşit et al., 2022).

GC/MS analysis For the GC/MS analysis, a 1.33% w/v solution of the sample in acetone was prepared and 1 µL was injected with a split mode (60:1). Gas chromatographic (GC) analyses were performed using a Thermo Scientific Trace 1310 GC/MS system, equipped with a DB-5MS capillary column (30 m x 0.25 mm ID and 0.25 µm) according to our previous report (Alkan et al., 2021). Helium (1.2 mL/min) was used as a carrier gas. The injection site temperature was set at 280°C. The column oven temperature was programmed as follows: the initial column oven temperature was 60°C, hold for 3 min, ramp to 200°C at a rate of 3°C/min and held for 0 min and, ramp to 240°C at a rate of 5°C/min and hold for 5 min. The total run time was 60 min. The mass spectrometer conditions were as follows: transfer line and ion source temperature were 280°C and the ionization energy was at 70 eV. The percentages of the essential oil components were calculated using peak areas without any correction factors. The retention indexes were calculated for all the components using the Van den Dool and Kratz equation based on homolog n-alkane series (C8-C20) retention times. The components were identified by comparing their mass spectral

fragmentation patterns with NIST and Wiley mass spectral database and confirmed by comparison with RI's with previous reports on DB-5 column(Akşit et al., 2022).

Preparation of Plant Extracts One hundred grams of each ground plant material was weighed and placed into a 1-liter Erlenmeyer flask. An organic solvent, methanol, was added to cover the plant parts. The mixture was then mixed at 120 rpm using an orbital shaker at room temperature for 72 hours. Subsequently, the extracts were passed through filter paper, and the organic solvent was removed by evaporation at 40°C using a rotary evaporator. The remaining dry extracts were dissolved in 50% aqueous acetone to obtain different concentrations of 1000, 2000, and 3000 ppm for use in the study (Kadioğlu and 2004).

Preparation of Antioxidant Extracts For antioxidant tests, 200 mg of the ground plant sample was taken for hexane, ethyl acetate, and methanol extracts. For the hexane extract, 10 mL of hexane/chloroform (5/1) was added; for the ethyl acetate extract, 10 mL of ethyl acetate/chloroform (5/1) was added, and for the methanol extract, 10 mL of methanol/chloroform (5/1) mixture was added to the sample. The samples were vortexed, and extraction was performed by placing them in an ultrasonic bath at 30°C for 30 minutes. Afterward, the extraction solutions were removed using a rotary evaporator, and stock solutions were prepared at a concentration of 1 mg/mL. The stock solution was stored at + 4°C for use in antioxidant activity tests and total phenolic and flavonoid analyses.

Free Radical Scavenging Activity (DPPH) Test Free radical scavenging activity was determined with some modifications to the Liyana-Pathirana method (Liyana-Pathirana and Shahidi, 2005). Stock solutions of different amounts of plant extract were placed into test tubes, and their volumes were adjusted to 3 mL with ethyl alcohol. Then, 1 mL of DPPH solution (0.26 mM) was added to each test tube, and the mixture was vortexed. After incubating for 30 minutes in the dark, the absorbances were measured at 517 nm. The DPPH radical scavenging activity was calculated as IC₅₀.

Cation Radical Scavenging Activity (ABTS) This analysis was conducted following the method described by Re et al. (Re et al., 1999). A mixture of 2 mM ABTS (2,2'-Azino-bis 3-ethylbenzothiazoline-6-sulfonic acid) and 2.45 mM sodium persulfate (Na₂S₂O₈) solutions, prepared in a 1:2 ratio with 0.1 M pH 7.4 phosphate buffer, was incubated in the dark for 6 hours. Stock solutions of different amounts of plant extract were placed in test tubes, and their volumes were adjusted to 3 mL with 0.1 M phosphate buffer (pH 7.4). Then, 1 mL of the ABTS solution was added to each test tube, and the mixture was vortexed. After incubating at room conditions for 30 minutes, the absorbance was measured at 734 nm. The ABTS cation radical scavenging activity was calculated as the IC₅₀ value.

Ferric Reducing Antioxidant Power (FRAP) Activity The FRAP analysis was carried out by modifying the method described by (Oyaizu, 1986). A volume of 0.25 mL of plant extracts was adjusted to 1.25 mL with 0.2 M phosphate buffer (pH 6.6). Then, 1.25 mL of potassium ferric cyanide [K₃Fe(CN)₆] solution (1%) was added. The mixture was kept at 50°C for 20 minutes. Next, 1.25 mL of TCA (10%) and 0.25 mL of FeCl₃ (0.1%) solutions were added to the cooled mixture at room temperature. The mixture was vortexed, and the absorbance was measured at 700 nm. The results were calculated as Trolox equivalent (TE).

Cupric Reducing Antioxidant Capacity (CUPRAC) A volume of 0.1 mL of the obtained sample solutions was taken, and the volume was adjusted to 1 mL with methanol. Then, 1 mL of CuCl₂ (0.01 M), neocuproine (7.5×10⁻³ M), and ammonium acetate solutions were added, and the mixture was vortexed. After incubating at room temperature for 30 minutes, the absorbance value was measured at 450 nm. The results were evaluated as Trolox equivalent (TE) (Apak et al., 2004; Elmastas et al., 2018).

Determination of Total Phenolic Compound Content The determination of total phenolic compounds was performed using the Folin-Ciocalteu reagent method (Singleton et al., 1999). A volume of 0.2 mL of the prepared stock solutions of plant extracts was taken and adjusted to 4.6 mL with distilled water. Then, 0.3 mL of Na₂CO₃ solution (2%) and 0.1 mL of Folin-Ciocalteu reagent were added, and the mixture was vortexed. After incubating for 2 hours at room temperature, the absorbance was measured at 760 nm using a spectrometer. The results were calculated as gallic acid equivalent (GAE).

Determination of Total Flavonoid Content A volume of 0.2 mL of the stock solutions of plant extracts was taken, and the volume was adjusted to 4.8 mL with methanol. Then, 0.1 mL of Al(NO₃) (10%) and 0.1 mL of NH₄CH₃COO solutions (1 M) were added. After vortexing, the mixture was incubated at room temperature for 40 minutes, and the absorbance was measured at 415 nm. The results were calculated as quercetin equivalent (QE) (Chang et al., 2002).

Biological activity

Obtaining Fungal Cultures Plant pathogenic fungi were obtained from stock cultures at Ahi Evran University, Faculty of Agriculture, Department of Plant Protection, Phytoclinic laboratories. For the experiments, young fungal cultures derived from these stock cultures were grown for 7 days at a temperature of 25 ± 2°C in 90 mm Petri dishes containing 20 ml of potato dextrose agar (PDA).

In vitro Antifungal Activity The prepared potato dextrose agars (PDA) were autoclaved and cooled down to 40°C. Different plant methanol extracts were obtained and mixed with the sterile melted PDA to achieve final concentrations of 1000, 2000, and 4000 ppm. The PDA mixture was transferred to 60 mm diameter Petri dishes (10 mm). Mycelium discs measuring 5 mm in diameter, taken from previously obtained 7-day-old fungal cultures, were transferred to the Petri dishes. The fungal cultures were incubated for 7 days at 25 ± 2°C after inoculation. Fungal growth was recorded at the end of each day for a total of 7 days. The percent growth of mycelium was calculated according to Pandey et al. (1982) by comparing the inhibition in growth with the growth in the control. Thiram 80% (Thiram), a standard fungicide, was used as a positive control. The experiment was conducted with 3 replications and 2 repetitions.

The percent mycelium growth was calculated using the following formula: $I = 100 \times (dc - dt) / dc$ Where: I = Percent mycelium growth dc = Mycelium growth in the control dt = Mycelium development in the treated samples

Allelopathy Study The seeds of the test plants (*Triticum aestivum* L., *Rumex crispus* L., and *Taraxacum officinale* F.H. Wigg), 25 each, were evenly distributed on two layers of blotting paper placed in 9 cm diameter Petri dishes. The prepared methanol extracts were moistened by adding 5 ml to the Petri dishes using distilled water at doses of 1000, 2000, and 3000 ppm, along with a control. The Petri dishes were incubated for 4 weeks at an average temperature of 25°C under a 12-hour light and 12-hour darkness cycle. At the end of the incubation period, the germination rate, as well as the root and shoot lengths of the test plant seeds, were determined. The experiment was conducted with 4 replications and repeated 2 times (Yılar et al., 2014).

Data Evaluation The obtained data were subjected to variance analysis using the SPSS 15 statistical package program, and the differences between the means were determined using the Duncan test.

Results and discussion

The essential oils composition of *Vaccinium myrtillus* populations According to GC/MS analyzes of *V. myrtillus* Muğla population, 97.87 of the oil was represented, while in Mersin population 97.25 of the oil was represented. A total of 20 and 19 components have been identified in these represented oils. In Muğla and Mersin populations, α -Pinene (29.16%), Eucalyptol (22.19%), Linalool (12.66%); Eucalyptol(26.46%), Linalool(25.27%), α -Pinene(15.75%) were determined as the main components (Table 1).

Table 1
Chemical compositions of *Vaccinium myrtillus* populations essential oils.

Pic No	RT	RI	RI lit	Companents name	Muğla	Mersin
1	5.32	932	931	α -Phellandrene	0,15	tr
2	5.53	935	933	α -Pinene	29,16	15,75
3	6.68	976	974	Sabinene	0,30	0,19
4	8.16	1022	1018	Prehnitol	0,15	0,23
5	8.31	1031	1031	Limonene	7,86	17,70
6	8.43	1039	1042	Eucalyptol	22,19	26,46
7	10.42	1063	1059	α -Terpinolene	tr	0,13
8	10.90	1098	1095	Linalool	12,66	25,27
9	13.98	1182	1182	<i>trans</i> -Sabinene hydrate	0,23	0,37
10	14.53	1195	1195	α -Terpineol	5,44	tr
11	14.77	1199	1200	Myrtenol	3,52	0,96
12	16.69	1218	1220	<i>trans</i> -Carveol	tr	0,16
13	16.09	1228	1231	Neryl acetate	0,15	0,35
14	17.19	1248	1251	Nerol	tr	2,95
15	17.26	1257	1258	Linalyl acetate	7,43	2,71
16	19.07	1263	1268	Z-Citral	0,14	0,77
17	21.11	1348	1250	A-Terpinyl acetate	2,27	0,34
18	22.55	1381	1379	Geranyl acetate	4,55	0,95
19	23.39	1401	1400	Eugenol methyl ether	0,88	1,57
20	23.91	1415	1418	<i>trans</i> - β -Caryophyllene	0,13	tr
21	25.28	1445	1448	α -Humulene	0,37	tr
22	26,57	1458	1461	Aromadendrene	tr	0,13
23	30.29	1505	1508	α -Farnesene	0,12	0,26
24	31.28	1583	1591	Caryophyllene oxide	0,19	tr
Monoterpene hydrocarbons					37,61	34,00
Oxygenated monoterpene hydrocarbons					59,46	62,86
Sesquiterpene hydrocarbones					0,61	0,39

Pic No	RT	RI	RI lit	Companents name	Muğla	Mersin
				Oxygenated sesquiterpene hydrocarbons	0,19	< 0,05
					97,87	97,25

RRI: Relative retention indices and RT: Retention time

In previous studies, the essential oil of *V. myrtillus* represented 96.4% of the total oil in GC/MS analyzes and a total of 19 compounds were identified. In this study, it was reported that 1,8-cineole (38.6%), α -pinene (21%), linalool (19.5%) and α -terpineol (5.8%) were determined as the main components (Elkiran and Avşar, 2020). The 22 components in *V. myrtillus* essential oil, including 1,8-cineole (41.07%), β -Linalool (12.72%), α -Pinene (12.17%) and Myrtenol (6.48%) they have defined (Bayar et al, 2018).

Total phenolic, total flavonoid content of *Vaccinium myrtillus* plant Total phenolic and total flavonoid contents of different solvent extracts of *Vaccinium myrtillus* populations are given in Table 2. Total phenol content was determined by comparing with the gallic acid used as a standard, and the results were calculated as mg GAE/g extract. The total phenolic content of *V. myrtillus* plant differed according to organic solvents and location (Muğla and Mersin), and it was determined that the extract values were between 29.38 and 157.58 mg GAE/g (Table 2). Among the extracts obtained from *V. myrtillus*, the highest amount of phenolic substances was found in methanol extracts (151.44-157.58mg GAE/g extracts) obtained from plants collected from Mersin and Muğla locations, respectively, followed by ethyl acetate (76.02–77.02 mg GAE/g). extracts) and hexane (29.38–42.80 mg GAE/g extracts) (Table 2).

Table 2

Total phenolic and total flavonoid contents of *Vaccinium myrtillus* with different extraction solvents.

Solvent of extracts	Mersin		Muğla	
	Toplam fenolik içeriği (mg GAE/g extracts)	Toplam flavonoid mg QE/g extracts	Toplam fenolik içeriği (mg GAE/g extracts)	Toplam flavonoid mg QE/g extracts
Hexane	29.38 ± 0.08	14.81 ± 0.52	42.80 ± 0.20	10.52 ± 0.34
Ethyl acetate	76.02 ± 0.19	42.18 ± 0.71	77.02 ± 0.20	37.88 ± 0.52
Methanol	151.44 ± 0.37	19.34 ± 0.34	157.58 ± 0.38	23.41 ± 0.34

The table (Table 2) presents the total amounts of flavonoid substances obtained from Hexane, ethyl acetate, and methanol extracts of *Vaccinium myrtillus*. The study revealed significant differences in flavonoid content among the extracts. The ethyl acetate extract from the plant collected in Mersin exhibited the highest amount of flavonoids, with 42.18 mg QE/g extract, followed by 19.34 mg QE/g extract in methanol, and 14.81 mg QE/g extract in hexane. In Muğla, the ethyl acetate extract contained 37.88 mg QE/g extract of flavonoids, followed by 23.41 mg QE/g extract in methanol and 10.52 mg QE/g

extract in hexane. These findings indicate variations in flavonoid content among the extracts and collection locations.

In recent studies conducted on *Vaccinium myrtillus* grown in different regions, the total phenolic and flavonoid contents of the plant were determined. The *V. myrtillus* plant collected from the Eastern Black Sea Region in Turkey exhibited a total phenolic content of 1035 ± 16 mg gallic acid/100 g fw and a total flavonoid content of 150 ± 4 mg quercetin equivalent/100 g fw (Altıok et al., 2022). Furthermore, the total phenolic content of *V. myrtillus* fruits and leaves collected from various locations in the Rize Province ranged from 184.2 to 556.1 mg GAE/100 g DW and from 69.9 to 212.9 mg GAE/100 g DW, respectively (Seyis et al., 2019). In Romania, the total phenolic content of *V. myrtillus* populations collected from different locations and years varied between 144.50 and 796.30 mg GAE/100 g FW, depending on the location and year (Ciulca et al., 2021).

Antioxidant activities of *Vaccinium myrtillus*

CUBRAC Fig. 1 illustrates the total CUPRAC reducing power of hexane, ethyl acetate, and methanol extracts obtained from the *Vaccinium myrtillus* plant. The results indicate significant variations in the total CUPRAC reducing power among the different solvent extracts. Specifically, methanol extract exhibited the highest copper reducing power compared to the other solvents. However, it should be noted that the reducing power of methanol extracts was lower than that of BHT (used as a standard) and BHA (as shown in Fig. 1). Additionally, the ethyl acetate extract demonstrated the second highest reducing power after methanol, while the hexane extract exhibited the lowest reducing power among the three solvents.

ABTS Fig. 2 presents the results of the ABTS radical removal analysis. The ABTS radical scavenging activities of hexane, ethyl acetate, and methanol extracts obtained from *Vaccinium myrtillus* plants collected from different locations were compared with those of Trolox, BHA, and BHT, which were used as standards. The hexane extract exhibited the highest ABTS radical scavenging activity with IC₅₀ values ranging from 8.19 to 17.76 µg/mL. Following hexane extract, the ethyl acetate extract demonstrated an ABTS radical scavenging activity with IC₅₀ values ranging from 5.16 to 5.74 µg/mL, while the methanol extract showed an activity ranging from 3.61 to 4.49 µg/mL (IC₅₀ values). It is worth noting that the hexane extract displayed a higher activity compared to the standard compounds Trolox, BHA, and BHT, whereas the other extracts exhibited similar effects to the standards. Furthermore, the *Vaccinium myrtillus* plants collected from Mersin province exhibited higher ABTS radical scavenging activity than those collected from Muğla.

Reducing Power In general, the reducing power of a substance is attributed to its ability to donate electrons and thereby break the oxidative bonds of free radical ions. The electron-binding capacities of the plant extracts were compared to those of BHT and BHA, which are recognized as standards with high reducing power. Among the different plant extracts, the methanol extract exhibited the highest reducing power, ranging from 4.75 to 485 µmol TE/mg extracts. Following the methanol extract, the ethyl acetate

extract displayed a reducing power ranging from 2.65 to 2.81 $\mu\text{mol TE/mg}$ extracts, while the hexane extract demonstrated a reducing power ranging from 0.88 to 1.35 $\mu\text{mol TE/mg}$ extracts (as depicted in Fig. 3).

DPPH The results of the DPPH radical removal analysis are presented in Fig. 4. The DPPH radical scavenging activities of hexane, ethyl acetate, and methanol extracts obtained from *Vaccinium myrtillus* plants collected from different locations were compared to the standards Trolox, BHA, and BHT. In terms of IC50 ($\mu\text{g/mL}$), the highest DPPH radical scavenging activity was observed in the hexane extract, followed by the ethyl acetate and methanol extracts, respectively. The significantly higher activity of the hexane extract compared to the standards Trolox, BHA, and BHT can be attributed to its potent scavenging capability. The best DPPH radical scavenging activity was observed in the methanol extracts, with IC50 values ranging from 5.64 to 6.68 $\mu\text{g/mL}$. The methanol extract exhibited a comparable activity to the second highest standard, BHT, which displayed higher activity than Trolox and BHA, both of which were used as standards.

Biological Activity of *Vaccinium myrtillus*

Allelopathic Effects The allelopathic activity of *Vaccinium myrtillus* L., commonly known as bilberry, was investigated through petri dish experiments conducted in laboratory conditions. Methanol extracts of *V. myrtillus* collected from different locations were tested for their effects on the germination and growth of *Taraxacum officinale* (dandelion) and *Rumex crispus* (curly dock) seeds. The results indicated that the allelopathic effects varied depending on the extract application dose, the collection location of *Vaccinium* species, and the test plant species (refer to Table 3 and Table 4 for details).

Table 3
Effect of plant methanol extracts on plant seed germination, root and shoot development in *Taraxacum officinale*

Doses	Muğla			Mersin		
	GR**	RL	SL	GR	RL	SL
Control	88,0a* $\pm$ 4,6	7,07a $\pm$ 1,2	8,26a $\pm$ 1,2	88,0a $\pm$ 4,6	7,07a $\pm$ 1,2	8,26a $\pm$ 1,2
1000ppm	40,0b $\pm$ 2,3	2,64b $\pm$ 0,1	3,31b $\pm$ 0,1	65,3b $\pm$ 9,3	5,13ab $\pm$ 0,2	4,42b $\pm$ 0,0
2000ppm	4,0c $\pm$ 2,3	0,99b $\pm$ 0,7	1,06b $\pm$ 0,7	32,0c $\pm$ 2,3	3,74b $\pm$ 0,1	3,76b $\pm$ 0,4
3000ppm	0,0c $\pm$ 0,0	0,0bc $\pm$ 0,0	0,0bc $\pm$ 0,0	0,0d $\pm$ 0,0	0,0c $\pm$ 0,0	0,0c $\pm$ 0,0

**GR:Germination, RL:Root length, SL:Shoot length

*Means in the same column with the same letter were not significantly different by ANOVA (a = 0.05)

Table 4

Effect of plant methanol extracts on *Rumex crispus* plant seed germination, root and shoot development

Doses	Muğla			Mersin		
	GR**	RL	SL	GR	RL	SL
Control	100a* $\pm$ 0,0	15,82a $\pm$ 2,8	18,17a $\pm$ 0,9	100a $\pm$ 0,0	15,82a $\pm$ 2,8	18,17a $\pm$ 0,9
1000ppm	96,66a $\pm$ 1,6	2,68b $\pm$ 0,3	5,70b $\pm$ 0,3	98,33a $\pm$ 1,6	5,85b $\pm$ 0,3	13,69a $\pm$ 2,7
2000ppm	96,66a $\pm$ 1,6	1,19b $\pm$ 0,0	4,64b $\pm$ 0,2	95a $\pm$ 0,0	1,3bc $\pm$ 0,1	3,32b $\pm$ 0,0
3000ppm	70b $\pm$ 2,4	1,06b $\pm$ 0,1	3,53b $\pm$ 0,1	85b $\pm$ 2,8	0,61c $\pm$ 0,0	2,13b $\pm$ 0,2
**GR:Germination, RL:Root length, SL:Shoot length						
*Means in the same column with the same letter were not significantly different by ANOVA (α = 0.05)						

A dose of 3000 ppm of *V. myrtillus* methanol extract completely inhibited the germination and growth of *T. officinale* seeds. Doses of 1000 ppm and 2000 ppm resulted in statistically significant decreases in plant growth compared to the control group (Table 3). Similarly, the water and methanol extracts of *V. myrtillus* inhibited the germination and growth of *Rumex crispus* seeds when compared to the control group (Table 4). Previous studies have also reported allelopathic effects of *V. myrtillus* on different plant species. For instance, *V. myrtillus* water extract negatively affected the seed germination and root-shoot development of *Lepidium sativum* (Anonymous, 2017). In another study investigating the effects of *V. myrtillus* water extract on the germination and seedling growth of aspen (*Populus tremula* L.), birch (*Betula pendula* Roth.), Scots pine (*Pinus sylvestris* L.), and Norway spruce [*Picea abies* (L.) Karst.], it was observed that the plant water extract inhibited the germination and growth of *Populus tremula* L. seeds and also caused root damage and growth abnormalities (Jäderlund et al., 1996). Moreover, it has been reported that natural extracts of *V. myrtillus* tea, which are rich in total phenolic compounds, negatively affect root elongation in spruce seedlings (Gallet, 1994). *V. myrtillus* is known to contain germination inhibitors for species within the Ericaceae family, including *V. myrtillus* itself. These inhibitors include phenolic compounds such as tannins, caffeic acid, and p-coumaric acid, as well as flavonoids (Jäderlund et al., 1996). Additionally, air-dried shoot powder of *Vaccinium vitis-idaea* L. has been found to have a significant allelopathic effect on the growth of *Lepidium sativum* (Saario et al., 2002). Wepplo (1987) reported that the leaves of *Vaccinium macrocarpon* contain substances that inhibit plant growth.

Antifungal activity The antifungal activity of the methanol extract of *Vaccinium myrtillus* was investigated against several important soil-borne plant pathogens, namely *Rhizoctonia solani* (RS), *Sclerotinia sclerotiorum* (SS), *Fusarium oxysporum* f. sp. *cucumerinum* (FOC), and *Fusarium oxysporum* f. sp. *melonis* (FOM). The study examined the effect of methanol extracts on the mycelium growth of these pathogens, and the results are presented in Table 1 and Fig. 5.

Table 5
% Inhibition rate of *Vaccinium myrtillus* Mersin population ethanol extract on mycelium growth of pathogens

Doses (ppm)	*** <i>S.S</i>	**** <i>R.S</i>	***** <i>FOM</i>	***** <i>FOC</i>
**Control+	100 ^{a*} ± 0.00	100 ^a ± 0.00	83.88 ^a ± 0.00	78.76 ^a ± 0.00
*Control-	0.00 ^d ± 0.00	0.00 ^d ± 0.00	0.00 ^d ± 0.00	0.00 ^e ± 0.00
1000	19.96 ^d ± 10.50	13.98 ^c ± 7.10	55.09 ^c ± 4.15	21.84 ^d ± 3.08
2000	45.42 ^c ± 8.95	24.75 ^{bc} ± 4.09	61.66 ^c ± 4.92	40.21 ^c ± 1.07
3000	78.00 ^b ± 4.41	31.43 ^b ± 0.97	73.31 ^b ± 1.75	46.33 ^b ± 7.45
*Negatif kontrol; ** Pozitif kontrol; ***Ss <i>Sclerotinia sclerotiorum</i> ****RS <i>Rhizoctonia solani</i> *****FOM <i>Fusarium oxysporum</i> f. sp. melonis *****FOC <i>Fusarium oxysporum</i> f. sp. Cucumerium				

Table 6
% Inhibition rate of *Vaccinium myrtillus* Muğla population ethanol extract on mycelium growth of pathogens

Doses (ppm)	*** <i>S.S</i>	**** <i>R.S</i>	***** <i>FOM</i>	***** <i>FOC</i>
**Control+	100 ^{a*} ± 0.00	100 ^a ± 0.00	83.88 ^a ± 0.00	78.76 ^a ± 0.00
*Control-	0.00 ^d ± 0.00	0.00 ^d ± 0.00	0.00 ^d ± 0.00	0.00 ^e ± 0.00
1000	39.02 ^c ± 6.83	19.87 ^c ± 1.77	53.73 ^c ± 3.26	36.96 ^d ± 0.27
2000	73.66 ^b ± 6.59	21.37 ^c ± 3.56	60.73 ^b ± 2.07	46.71 ^c ± 0.08
3000	84.77 ^b ± 2.30	34.77 ^b ± 2.81	62.62 ^b ± 1.78	53.60 ^b ± 0.97
*Negatif kontrol; ** Pozitif kontrol; ***Ss <i>Sclerotinia sclerotiorum</i> ****RS <i>Rhizoctonia solani</i> *****FOM <i>Fusarium oxysporum</i> f. sp. melonis *****FOC <i>Fusarium oxysporum</i> f. sp. cucumerium				

The effectiveness of the extract varied depending on the specific extract, application dosage, and pathogen. Higher dosages were associated with increased inhibitory effects. For instance, the methanol extract of *V. myrtillus* from Mersin showed significant inhibition of mycelium growth in *S. sclerotiorum*, *F. oxysporum* f. sp. melonis, *F. oxysporum* f. sp. cucumerinum, and *Rhizoctonia solani*, with inhibitory percentages of 78.00%, 73.31%, 46.33%, and 31.43%, respectively, compared to the control (Table 5). Similarly, the extract from the Muğla location inhibited mycelium growth of plant pathogenic fungi to varying extents, ranging from 34.77–84.77% (Table 6).

Additionally, *V. myrtillus* essential oil demonstrated inhibitory effects on mycelial growth in *Sclerotinia sclerotiorum*, *Alternaria solani*, *Fusarium oxysporum* f. sp. radicle-lycopersici (FORL), and *Verticillium dahliae*, with inhibitory percentages of 61.38%, 100%, 80.36%, and 57.91%, respectively (Bayar et al.,

2018). Stefănescu et al. (2020) also reported the antifungal activity of leaf extracts from *V. myrtillus* and *Vaccinium vitis-idaea* against *Candida zeylanoides*.

Conclusion

Based on the findings of this study, it can be concluded that the methanol extracts of *V. myrtillus*, found in Turkey, exhibit significant levels of total phenolic content, total flavonoid content, and antioxidant activity. These effects have been attributed to the presence of phenolic and flavonoid compounds in the plant. Furthermore, the current research has demonstrated that the methanol extract of *V. myrtillus* possesses allelopathic effects on two important weed species, namely *Rumex crispus* and *Taraxacum officinale*. Additionally, the extract has shown antifungal activity against plant pathogenic fungi, including *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Fusarium oxysporum* f. sp. *cucumerinum*, and *Fusarium oxysporum* f. sp. *melonis*. Specifically, it has been observed that the extract inhibits the growth of mycelium in these fungi. These findings suggest that the biological activity of *V. myrtillus* extract is likely attributed to its phenolic and flavonoid components, which have been previously reported to possess similar biological activities. The identification of these active constituents holds significance in the development of novel natural antifungal agents.

References

1. Arslan Ü, Karabulut ÖA (2005) Antifungal Effect of Spice Plants Against Plant Pathogenic Fungi. Atatürk University Journal of the Faculty of Agriculture, 36 (2): 131–135 <https://agriculture-ataunipress.org>
2. Işık D, Mennan H, Cam M, Tursun N, Arslan M (2016) Allelopathic potential of some essential oil bearing plant extracts on Common Lambsquarters (*Chenopodium album* L.). Revista de Chimie(Bucharest), 67(3):455–459 <https://doi.org/10.37358/Rev.Chim.1949>
3. Li JX, Ye J W, Liu D H (2020) Allelopathic effects of *Miscanthus foridulus* on seed germination and seedling growth of three crops. Chinese Journal of Applied Ecology, 31, 2219–2226 <https://doi.org/10.13287/j.1001-9332.202007.006>
4. Erez ME, Battal, P (2022) Determination of Allelopathic and Antimicrobial Effects of Four Different Plant Species. International Journal of Nature and Life Sciences, 6(2):79–89 <https://doi.org/10.47947/ijnls.1100720>
5. Singh NB, Thapar R (2003) Allelopathic influence of *Cannabis sativa* on growth and metabolism of *Parthenium hysterophorus*. Allelopathy Journal, 12, 61–70
6. Santonja M, Bousquet-Mélou A, Greff S, Ormeño E, Fernandez C (2019) Allelopathic effects of volatile organic compounds released from *Pinus halepensis* needles and roots. Ecol Evol. 9:8201–8213 <https://doi.org/10.1002/ece3.5390>
7. Sudatti DB, Duarte HM, Soares AR, Salgado LT, Pereira RC (2020) New Ecological Role of Seaweed Secondary Metabolites as Autotoxic and Allelopathic. Front Plant Sci. 25;11:347

<https://doi.org/10.3389/fpls.2020.00347>

8. Cakir A, Kordali S, Kilic H, Kaya E (2005) Antifungal properties of essential oils and crude extracts of *Hypericum linarioides*. Biochemical Systematics and Ecology, 33: 245–256
<https://doi.org/10.1016/j.bse.2004.08.006>
9. Bayan Y (2016) Chemical Composition and Antifungal Activity of the Plant Extracts of Turkey *Cardaria Draba* (L.) Desv. Egyptian Journal of Biological Pest Control. 26(3), 579–581
<https://ejbpc.com/>
10. Yilar M, Kadioglu I, Telci I (2018) Chemical composition and antifungal activity of *Salvia officinalis* (L.), *S. cryptantha* (Montbret et Aucher ex Benth.), *S. tomentosa* (Mill.) plant essential oils and extracts. Fresenius Environmental Bulletin, 27(3): 1695–1706 https://www.prt-parlar.de/download_feb_2018/
11. McCord JM (2000) The evolution of free radicals and oxidative stress. Am J Med., 108: 652–659
[https://doi.org/10.1016/s0002-9343\(00\)00412-5](https://doi.org/10.1016/s0002-9343(00)00412-5)
12. Sengul M, Yildiz H, Gungor N, Cetin B, Eser Z, Ercisli S (2009) Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. Pak. J. Pharm. Sci. 22, No.1, 102–106
<https://www.pjps.pk/>
13. Benhammou N, Bekkara FA, Panovska TK (2008) Antioxidant and antimicrobial activities of the Pistacia lentiscus and Pistacia atlantica extracts. Afr J Pharm Pharmacol., 2: 22–28
<http://www.academicjournals.org/ajpp>
14. Wu N, Fu K, Fu YJ, Zu YG, Chang FR, Chen YH, Liu XL, Kong Y, Liu W, Gu B (2009) Antioxidant Activities of Extracts and Main Components of Pigeonpea [*Cajanus cajan* (L.) Millsp.] Leaves. Molecules. 14, 1032–1043 <https://doi.org/10.3390/molecules14031032>
15. El Ouariachi E, Bouyanzer A (2014) Antioxidant activity of solvent extracts of *Inula viscosa* from Morocco. Arabian Journal of Chemical and Environmental Research, Vol.1 No 1, 33–40
<http://www.mocedes.org/ajcer/>
16. Çelik H (2006) A New Fruit Type Blueberry (Likapa) for Black Sea Fruit. 2nd National Symposium on berry fruits, September, Tokat
17. Aksit H, Bayar Y, Simsek S, Ulutas Y (2022) Chemical Composition and Antifungal Activities of the Essential Oils of Thymus species (*Thymus pectinatus*, *Thymus convolutus*, *Thymus vulgaris*) Against Plant Pathogens, Journal of Essential Oil Bearing Plants, 25:1, 200–207
<https://doi.org/10.1080/0972060X.2022.2043189>
18. Alkan M, Güzel M, Akşit H, Bağdat RB, Alkan FR, Evlice E (2021) Chemical components and insecticidal effects of essential oils from three lavender cultivars against adult Sitophilus granarius (L., 1758) (Coleoptera: Curculionidae). Turk Entomol. Derg. 45(4): 405–416
<https://doi.org/10.16970/entoted.988985>
19. Kadioğlu İ, Yanar Y (2004) Allelopathic Effects of Plant Extracts Against Seed Germination of Some Weeds. Asian J. of Plant Sciences, 3 (4): 472–475 <https://doi.org/10.3923/ajps.2004.472.475>

20. Liyana-Pathirana CM, Shahidi F (2005) Antioxidant activity of soft commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. J. Agric. Food Chem; 53:2433–2440
<https://doi.org/10.1021/jf049320i>
21. Re R, Pellegrini N, Protrggente A, Pannala A, Yang, M, Rice-Evans C(1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26(9–10): 1231–1237 [https://doi.org/10.1016/s0891-5849\(98\)00315-3](https://doi.org/10.1016/s0891-5849(98)00315-3)
22. Oyaizu M(1986) Studies on product of browning reaction prepared from glucoseamine. Japan Journal of Nutrition, 44, 307–315 <https://doi.org/10.5264/eiyogakuzashi.44.307>
23. Apak R, Güçlü K, Özyürek.M, Karademir SE(2004) Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. Journal of Agricultural and Food Chemistry 52: 7970–7981
<https://doi.org/10.1021/jf048741x>
24. Elmastas M, Celik SM, Genc N, Aksit H, Erenler R, Gulcin İ(2018) Antioxidant activity of an Anatolian herbal tea—*Origanum minutiflorum*: isolation and characterization of its secondary metabolites. International Journal of Food Properties 21: 374–384
<https://doi.org/10.1080/10942912.2017.1416399>
25. Singleton VL, Orthofer R, Lamuela-Raventos RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folinciocalteu reagent. Methods in Enzymology, 299: 152–178 [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1)
26. Chang C-C, Yang, MH, Wen HM, Chern JC (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Analysis, 10, 178–182
<https://doi.org/10.38212/2224-6614.2748>
27. Pandey DK, Tripathi NN, Tripathi RD, Dixit SN(1982) Fungitoxic and phytotoxic properties of essential oil of *Hyptis suaveolens*. Z. Pflanzenkrankheiten Pflanzenschutz, 89, 344–349
28. Yılar M, Onaran A, Yanar Y, Belgüzar S, Kadioğlu I(2014) Herbicidal and Antifungal Potential of *Trachystemon orientalis* (L.) G. Don (Kaldırık). Journal of the Institute of Science and Technology, 4(4), 19–28 <https://dergipark.org.tr/en/pub/jist/issue/7940/388671>
29. Elkiran O, Avşar C(2020) Chemical Composition and Biological Activities of the Essential Oil from the Leaves of *Vaccinium myrtillus* L.. *Bangladesh J. Bot.* 49(1): 91–96
<https://doi.org/10.3329/bjb.v49i1.49098>
30. Bayar Y, Onaran A, Yılar M, Gul F(2018) Determination of the essential oil composition and the antifungal activities of Bilberry (*Vaccinium myrtillus* L.) and Bay Laurel (*Laurus nobilis* L.). J. Essent. Oil Bear. Plants, 21, 548–555 <https://doi.org/10.1080/0972060X.2017.1417060>
31. Altıok E, Kacmaz S, Altıok D (2022) Extraction of Bioactive Compounds in Wild Bilberry (*Vaccinium Myrtillus* L.) in The Eastern Black Sea Region With Different Techniques. Türk Doğa ve Fen Dergisi, 11 (2), 130–136 <https://doi.org/10.46810/tdfd.1001501>
32. Seyis F, Yurteri E, Özcan A (2019) Phenolic Content and Antioxidant Activity of *Vaccinium myrtillus* Collected from Rize Highlands, Turkey. International Journal of Crop Science and Technology,

5(1):14–19 <https://dergipark.org.tr/en/pub/ijcst/issue/48349/465682>

33. Ciulca S, Roma G, Alexa E, Radulov I, Cocan I, Madosa E, Ciulca A (2021) Variation of Polyphenol Content and Antioxidant Activity in Some Bilberry (*Vaccinium myrtillus* L.) Populations from Romania. *Agronomy*, 11, 2557 <https://doi.org/10.3390/agronomy11122557>
34. Anonymous (2017) The allelopathic effect of extracts of *Vaccinium myrtillus*, *Empetrum nigrum* and *Sphagnum girgensohnii* on the germination and growth of *Lepidium satvium* http://datasaab.se/Stipendium/2017/Beskrivningar/Isabelle_May2017_Biology_EE_20170213.pdf
35. Jäderlund A, Zackrisson O, Nilsson M-C (1996) Effects Of Bilberry (*Vaccinium myrtillus* L.) Litter on Seed Germination and Early Seedling Growth of Four Boreal Tree Species. *Journal of Chemical Ecology*, 22(5): 973–986 <https://doi.org/10.1007/BF02029948>
36. Gallet C(1994) Allelopathic Potential In Bilberry-Spruce Forests: Influence of Phenolic Compounds on Spruce Seedlings. *Journal of Chemical Ecology*, Vol. 20, No. 5 <https://doi.org/10.1007/BF02059738>.
37. Saario M, Koivusalo S, Laakso I, Autio J (2002) Allelopathic Potential of Lingonberry (*Vaccinium vitis-idaea* L.) Litter for Weed Control. *Biological Agriculture and Horticulture*, Vol. 20, pp.11–28. <https://doi.org/10.1080/01448765.2002.9754946>
38. Wepplo P(1987) Isolation and identification of plant growth inhibitors from leaves of the american cmwberry (*Vaccinium macrocarpon*), pp. 328–333, in G.R. Waller (ed.). *Allelopathy: Role in Agriculture and Forestry*. ACS Symposium Series 330. American Chemical Society, Washington, D.C.
39. Ștefănescu B-E, Călinoiu LF, Ranga F, Fetea F, Mocan A, Vodnar DC, Crișan G (2020) Chemical Composition and Biological Activities of the Nord-West Romanian Wild Bilberry (*Vaccinium myrtillus* L.) and Lingonberry (*Vaccinium vitis-idaea* L.) Leaves. *Antioxidants*, 9, 495 <https://doi.org/10.3390/antiox9060495>

Figures

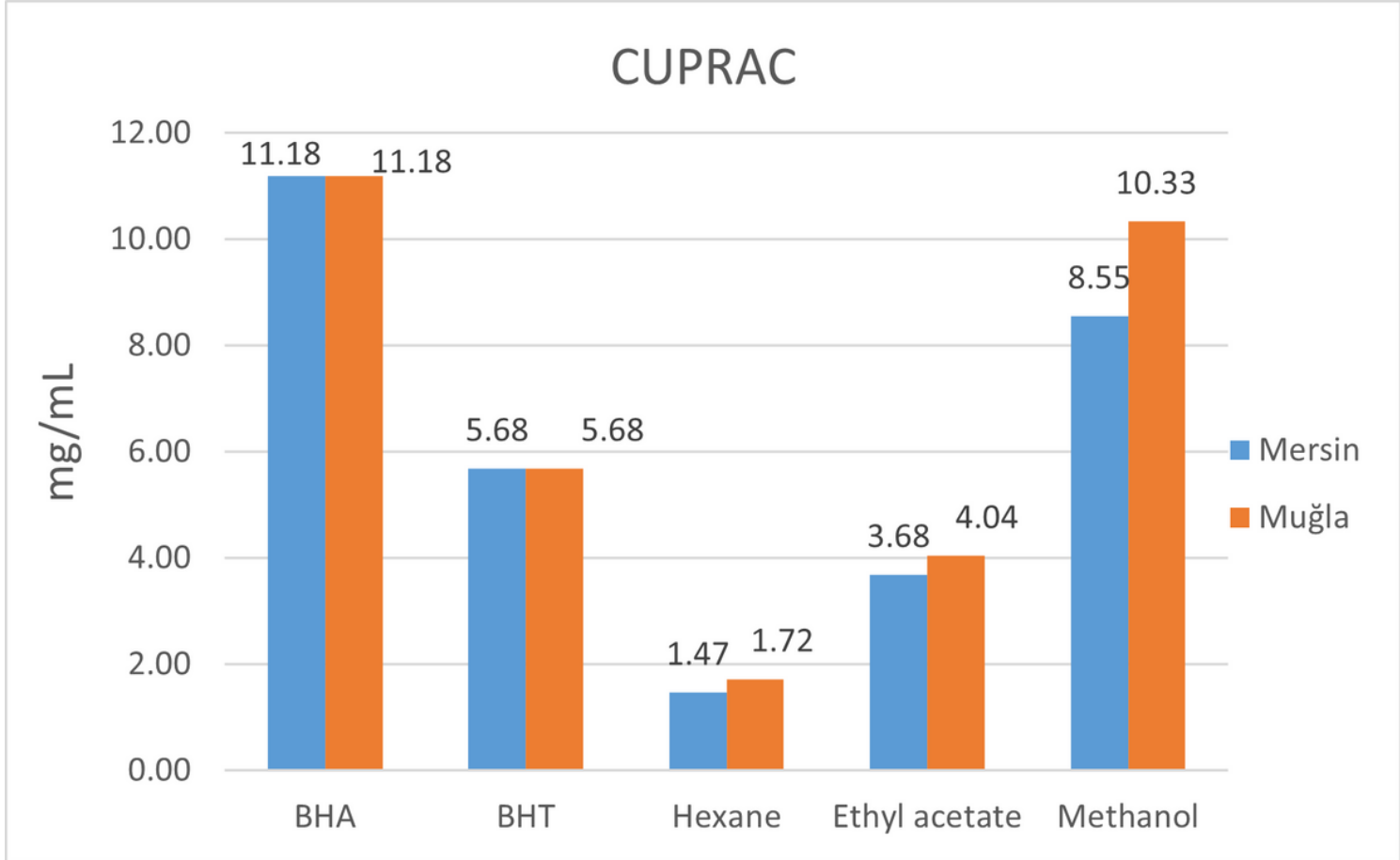


Figure 1

Copper reducing power activity

ABTS (Radical cation assay)

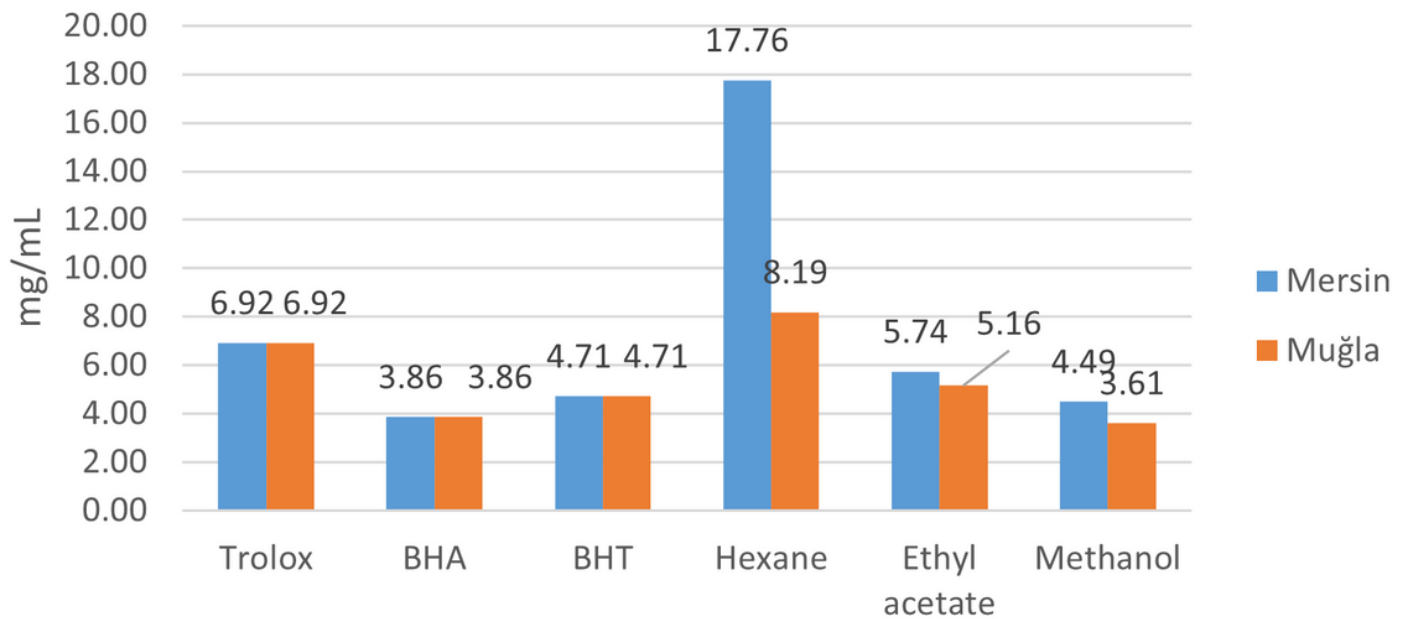


Figure 2

ABTS radical scavenging activity

Reducing power

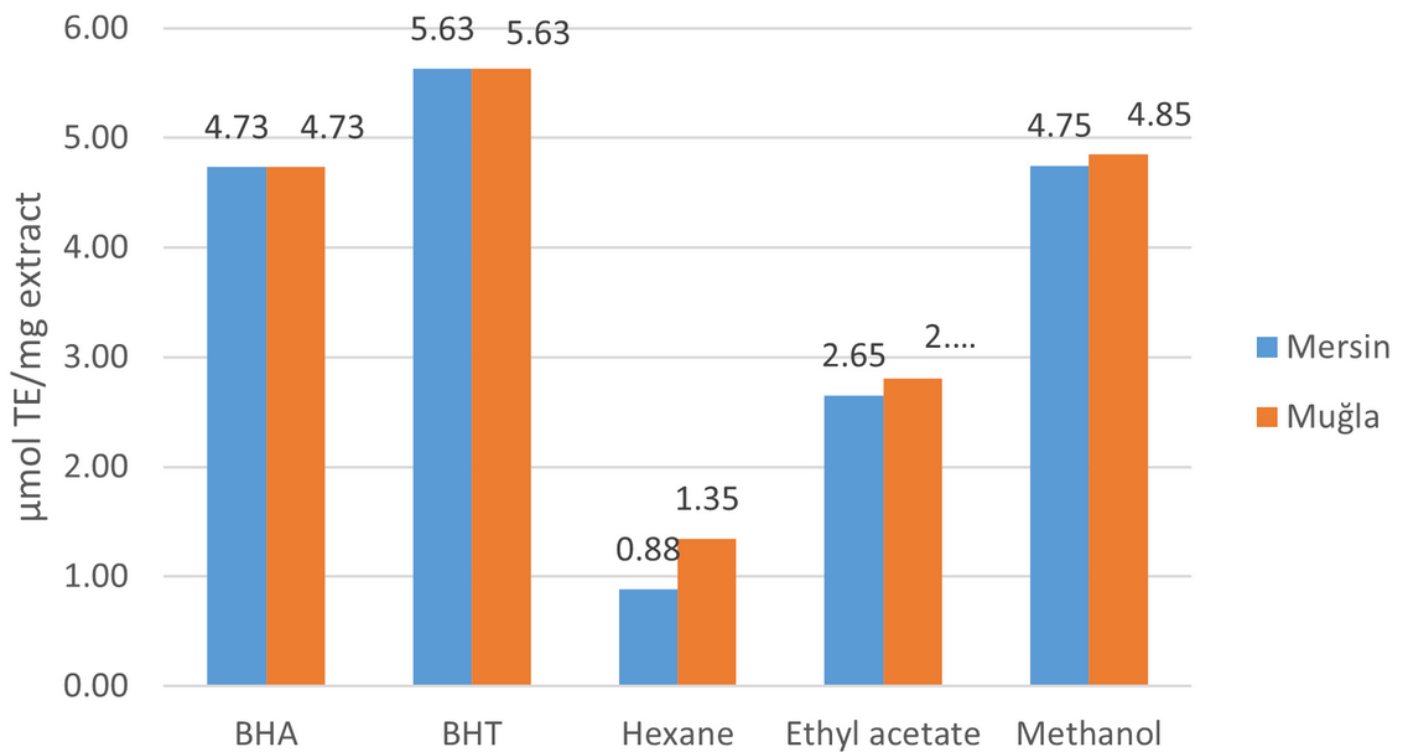


Figure 3

Reducing power activity

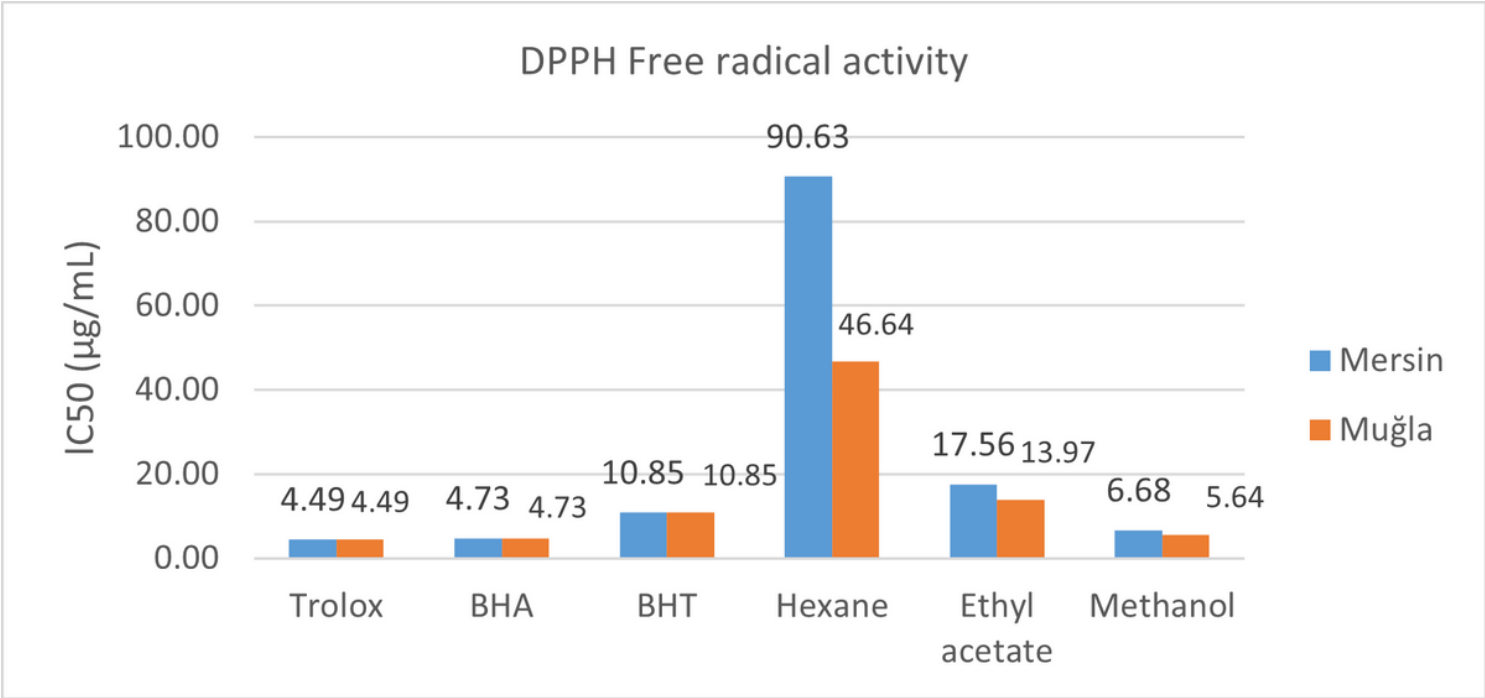


Figure 4

DPPH radical scavenging activity