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Exploring the potential of bilberry 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 the leaf essential oil and the total phenolic and flavonoid contents as well as the antioxidant activity of the *n*-hexane, ethyl acetate, and methanol extracts of *Vaccinium myrtillus* leaves collected from two different locations (Muğla and Mersin, Turkey). In addition, the antifungal activity against *Sclerotinia sclerotiorum* (SS), *Fusarium oxysporum* f sp. *melonis* (FOM), *Fusarium oxysporum* f sp. cucumerinum (FOC), and *Rhizoctonia solani* (RS) and the bioherbicidal activity against *Taraxacum officinale* and *Rumex crispus* were evaluated for the first time. The methanolic extract inhibited mycelium growth of SS, FOM, FOC, and RS in a dose-dependent manner. No significant difference was observed in antifungal activity between the two different collection sites. In the bioherbicidal activity tests, the methanol extract of the fruits completely suppressed the root-shoot development of *Taraxacum officinale*, while also significantly inhibiting the root-shoot development during the seed germination of *Rumex crispus* compared to the control group at 3 mg/mL concentration for both locations. Based on the results of the GC/MS analysis, the major constituents identified in the leaf essential oils collected from the Muğla and Mersin locations were α-pinene (29.16%/15.75%), eucalyptol (22.19%/26.46%), linalool (12.66%/25.27%), and linalyl acetate (7.43%/2.71%). The total phenolic and flavonoid contents of the plant extracts for the Muğla and Mersin samples were determined as (42.80–157.58 GAE/g extracts), (29.38–151.44 GAE/g extracts) and (10.52–37.88 QE/g extracts), (14.81–44.18 QE/g extracts) respectively. In addition, the plant exhibited significant antioxidant activity. These findings indicate quantitative differences in the chemical composition of the essential oils between the two geographical locations. These findings are significant as they provide insights for the development of new natural antifungal agents with potential applications in agriculture.

Keywords:
Antioxidant, antifungal, *Vaccinium myrtillus*, essential oil, bioherbicidal activity

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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 utilised. 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 & Karabulut 2005; Isik *et al.* 2016). The adverse effects of pesticides have prompted researchers to explore alternative approaches. One such method is the utilisation of natural components which are environmen-

tally friendly and pose no harm to human health. Naturally grown plants contain compounds which possess potential biological effects, with these components being produced in greater quantities under stressful conditions and released into the environment. Plant secondary metabolites, in particular, have been linked to allelopathy and have been the focus of research in various fields, including herbology, ecology, and physiology (Li *et al.* 2020; Emre & Battal 2022). These secondary metabolites, 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 & 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 metabolites on plant pathogenic fungi (Cakir *et al.* 2005; Bayan 2016; Yilar *et al.* 2018). Additionally, the components of plant secondary metabolites possess 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; Sengul *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, the antioxidants derived from medicinal aromatic plants can neutralise these radicals (McCord 2000; Sengul *et al.* 2009). Antioxidants are commonly used as food additives to protect against oxidative degradation caused by free radicals (Akşit *et al.* 2022a; Baltaci *et al.* 2022). It has been reported that natural phenolics, due to their antioxidant activity, reduce the risk of cancer and are particularly effective in preventing the development of Alzheimer's disease, which negatively affects brain function (Akşit 2023).

Bilberry (*Vaccinium myrtillus* L.), a wild fruit, can grow in temperate, Mediterranean and subtropical climates (Altiok *et al.* 2022). *Vaccinium myrtillus* show high levels of biological activity thanks to the high amounts of phenolic compounds and anthocyanins they contain. Ecological, physiological and genetic biotechnological studies have been conducted with numerous blueberry species (Bayar *et al.* 2018).

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

MATERIALS AND METHODS

Plant material and isolation of essential oils. The leaf samples were collected from the Mersin and Muğla provinces during the 2020–2021 vegetation period. The plant specimens were identified by Dr. Melih Yilar. The plant materials were dried in the shade then finely ground. The ground samples were stored +4°C prior to use in the extraction process. The plant essential oils were extracted using the Neo-Clevenger apparatus. 100 g of plant material was weighed, then 300 mL of deionised water was added and the mixture was distilled for 2 hours. The essential oils were separated, dried with anhydrous sodium sulfate, and stored in dark glass bottles at +4°C until analysis (Akşit *et al.* 2022b).

The GC/MS analysis. Gas chromatographic (GC) analyses were performed using a Thermo Scientific Trace 1310 GC/MS system, equipped with a DB-5MS capillary column (30 m × 0.25 mm ID and 0.25 μm) according to Akşit *et al.* (2024). Helium was used as the carrier gas with a 1.2 mL/min flow rate. For the GC/MS analysis, a 1.33% w/v solution of the essential oil prepared in acetone and 1 μL was injected into the injection port pre-heated to 280°C. The column oven temperature was programmed as follows: the initial column oven temperature was 60°C, held for 3 min, ramped to 200°C at a rate of 3°C/min and held for 0 min, and then ramped to 240°C at a rate of 5°C/ min and held for 5 min. The mass spectrometer conditions were as follows: the transfer line and ion source temperatures were set at 280°C and the ionization energy was at 70 eV. The percentages of each component were calculated using the peak areas obtained from the GC-FID analysis maintained with the same GC conditions without any correction factors. The retention indexes were calculated for all the components based on the retention times of the homolog n-alkane series (C8–C20). The components were identified by comparing their mass spectral fragmentation patterns with the NIST, Pfleger, and Wiley mass spectral databases and confirmed by comparing the calculated RI values with those previous reports run on the DB-5 column.

The preparation of the plant extracts. The plant material (1 kg) was macerated with 5 litres of each extraction solvent (*n*-hexane, ethyl acetate, and methanol) by shaking 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 concentrations of 1, 2, and 3 mg/mL for the antifungal activity tests, (Kadioğlu & Yanar 2004).

Table 1. The chemical composition of the essential oils of the *Vaccinium myrtillus* populations.

Peak No	RT	RI	RI lit	Components name	% composition 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	*trans*-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	*trans*-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	*trans*-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
				Oxigenated monoterpene hydrocarbons	59.46	62.86
				Sesquiterpene hydrocarbones	0.61	0.39
				Oxygenated sesquiterpene hydrocarbons	0.19	<0.05
					97.87	**97.25**

RI: Retention indices and RT: Retention time, tr: < 0.05

The preparation of the antioxidant extracts. For the antioxidant tests, 1 mg/mL stock solutions were prepared in ethanol from the aforementioned extracts. The stock solutions were stored at +4°C prior to use in the antioxidant activity tests and total phenolic and flavonoid assays.

The free radical scavenging activity (DPPH). The free radical scavenging activity was determined using the method reported by Şımşek *et al.* (2023). Briefly, 1 ml of DPPH (0.26 mM) solution was added to 3 mL of a solution containing the extracts at different concentrations between 5–100 µg/mL. The final mixtures were shaken vigorously, then incubated for 30 minutes in the dark. The absorbances sample and standard test solutions were recorded at 517 nm. The absorbance values were subsequently converted to % activity, and the IC_{50} concentrations were calculated. The DPPH radical scavenging activity was expressed as IC_{50} in µg/mL. The results were compared with BHA and BHT as standard antioxidants.

Table 2. The total phenolic and total flavonoid contents of *Vaccinium myrtillus* leaves extracted with different organic solvents and collected from two different locations.

Locations	Solvents	TPC	TFC
Mersin	*n*-hexane	29.38 ± 0.08^{a}	14.81 ± 0.52^{a}
	EtOAc	76.02 ± 0.19^{b}	42.18 ± 0.71^{b}
	MeOH	151.44 ± 0.37^{c}	19.34 ± 0.34^{c}
Muğla	*n*-hexane	42.80 ± 0.20^{d}	10.52 ± 0.34^{a}
	EtOAc	77.02 ± 0.20^{b}	37.88 ± 0.52^{b}
	MeOH	157.58 ± 0.38^{c}	23.41 ± 0.34^{c}

All analyses are the mean of triplicate measurements ± standard deviation (n = 3). The results are expressed in mg GAE/g extract for the TPC and mg QE/g extract for the TFC. Different letters in each column denote significant differences at $p < 0.05$

The radical cation scavenging activity (ABTS). This analysis was conducted following the method proposed by Akman *et al.* (2024). 2 mM ABTS (2,2'-Azino-bis 3-ethylbenzothiazoline-6-sulfonic acid) and 2.45 mM sodium persulfate ($Na_2S_2O_8$) solutions were prepared in a phosphate buffer (0.1 M, pH 7.4) and mixed in a 1:2 ratio. The mixture was incubated in the dark for 6 hours to form cationic radicals. Different concentrations (5–100 µg/mL) of stock solutions were placed in test tubes, and their volumes were adjusted to 3 mL with a phosphate buffer. Then, 1 mL of the ABTS solution was added to each test tube, and the mixture was vortexed. After incubating at room temperature for 30 minutes, the absorbance was measured at 734 nm. The ABTS radical cation scavenging activity was calculated as the IC_{50} in µg/mL.

The ferric reducing antioxidant power activity (FRAP). The FRAP analysis was carried out by modifying the method described by Gözcü *et al.* (2024). A volume of 0.25 mL of plant extracts was adjusted to 1.25 mL with a phosphate buffer (0.2 M, pH 6.6). Then, 1.25 mL of potassium ferric cyanide [$K_3Fe(CN)_6$] solution (1%) was added. The mixture was incubated at 50°C for 20 minutes. Then, 1.25 mL of TCA (10%) and 0.25 mL of $FeCl_3$ (0.1%) solutions were added and cooled at room temperature. The absorbance of the final mixture was recorded at 700 nm. The results were expressed as µmol Trolox equivalent (TE)/mg extract.

The cupric reducing antioxidant capacity (CUPRAC). A volume of 100 µL of the sample solutions (1 mg/mL) was taken, and adjusted to 1 mL with methanol. Then, 1 mL of $CuCl_2$ (0.01 M), neocuproine (7.5×10^{-3} M), and ammonium acetate solutions (0.1 M) were added, and the mixture was vigorously vortexed. After incubating at room temperature for 30 minutes, the absorbance value was measured at 450 nm. The results were expressed as µmol Trolox equivalent /mg extract (Apak *et al.* 2004; Elmastas *et al.* 2018).

The determination of the total phenolic content (TPC). The determination of the TPC was performed using the Folin-Ciocalteu reagent following the method reported by Akman *et al.* (2023). A volume of 0.2 mL of the prepared stock solutions of plant extracts was taken and diluted with 4.4 mL of distilled water. Then, 0.3 mL of Na_2CO_3 solution (2%) and 0.1 mL of Folin-Ciocalteu reagent were added. The final mixture was vortexed and incubated for 2 hours at room temperature. The absorbance was measured at 760 nm. The results were calculated as mg gallic acid equivalent (GAE)/g extract.

The determination of the total flavonoid content (TFC). A volume of 0.2 mL of the stock solutions of plant extracts was taken, and adjusted to 4.8 mL with methanol. Then, 0.1 mL of $AlCl_3$ (10%) and 0.1 mL of NH_4CH_3COO 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 mg quercetin equivalent (QE)/g extract (Köksal *et al.* 2021).

Table 3. The antioxidant activity of *Vaccinium myrtillus* leaves extracted with different organic solvents and collected from two different locations.

Locations	Solvents	DPPH	ABTS	FRAP	CUPRAC
Mersin	*n*-hexane	90.63 ± 3.45^{a}	8.19 ± 1.02^{b}	0.88 ± 0.05^{d}	1.47 ± 0.19^{d}
	EtOAc	17.56 ± 0.39^{c}	5.16 ± 0.57^{c}	2.65 ± 0.12^{c}	3.68 ± 0.89^{c}
	MeOH	6.68 ± 0.87^{d,e}	3.61 ± 0.21^{d}	4.75 ± 0.03^{b}	8.55 ± 1.05^{b}
Muğla	*n*-hexane	46.64 ± 2.21^{b}	17.76 ± 1.98^{a}	1.35 ± 0.02^{d}	1.72 ± 0.28^{d}
	EtOAc	13.97 ± 0.54^{c}	5.74 ± 0.32^{c}	2.81 ± 0.08^{c}	4.04 ± 0.43^{c}
	MeOH	5.64 ± 0.33^{e}	4.49 ± 0.44^{c}	4.85 ± 0.09^{b}	10.33 ± 0.98^{a}
Standards					
BHA		4.73 ± 0.30^{e}	3.86 ± 0.51^{d}	4.73 ± 0.18^{b}	11.18 ± 0.22^{a}
BHT		10.85 ± 0.54^{d}	4.71 ± 0.25^{c}	5.65 ± 0.58 ^{a}	5.68 ± 0.32^{b}
Trolox		4.49 ± 0.28^{e}	6.92 ± 0.85^{b}	-	-

All analyses are the mean of triplicate measurements ± standard deviation (n = 3). The results are expressed as IC_{50} in µg/mL for the DPPH and ABTS tests and µmol TE/g extract for the FRAP and CUPRAC tests. Different letters in each column denote significant differences at $p \leq 0.05$.

The in vitro antifungal activity. Plant pathogenic fungi were obtained from the 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 25 ± 2°C in 90 mm Petri dishes containing 20 mL of potato dextrose agar (PDA).

The methanolic plant extract was mixed with the sterile melted PDA to give 1, 2, and 3 mg/mL concentrations. The mixture was transferred to 60 mm diameter Petri dishes. Mycelium discs (5 mm) including 7-day-old fungal cultures were transferred to the centre of the Petri dishes. The fungi cultures were incubated for 7 days at 25 ± 2°C after inoculation. The mycelium growth status was recorded at the end of each day for seven days. The percentages of mycelium growth were calculated according to Pandey *et al.* (1982) by comparing the growth inhibition of the control group. % mycelium growth was calculated using the following formula: I = 100 × (dc - dt) / dc (where: I = Percent mycelium growth, dc = Mycelium

growth in the control, and dt = Mycelium development in the treated samples).

Thiram 80% (Thiram), a standard fungicide, was used as the positive control. The experiment was conducted with 3 replications and 2 repetitions.

The allelopathy study. 25 seeds from the test plants, *Rumex crispus* L. and *Taraxacum officinale* F.H. Wigg, were evenly distributed on two layers of blotting paper placed in 9 cm diameter Petri dishes. The prepared methanol extracts were then moistened by adding 5 mL to the Petri dishes using distilled water at 1, 2, and 3 mg/mL concentrations, in addition to the control. Subsequently, the Petri dishes were incubated for 4 weeks at an average temperature of 25°C, following a 12-hour light and 12-hour darkness cycle. At the end of the incubation period, the germination rate, and the root and shoot lengths of the test plant seeds were determined. The experiment was conducted with 4 replications and repeated 2 times (Yilar *et al.* 2014).

The data evaluation. The data obtained from the bioactivity tests were subjected to variance analysis using the SPSS 15 statistical package programme, and the differences between the means were determined using Duncan's test.

RESULTS AND DISCUSSION

The composition of the essential oils of the *Vaccinium myrtillus* populations. According to the GC/MS analysis, the major components in both populations were the same but with significant differences in composition: α-pinene (29.16%), eucalyptol (22.19%), and linalool (12.66%) in the Muğla population, with eucalyptol (26.46%), linalool (25.27%), and α-pinene (15.75%) in the Mersin population, (Table 1). The essential oil of *V. myrtillus* collected from the Black Sea Region (Sinop) was reported to contain eucalyptol (38.6%), α-pinene (21%), linalool (19.5%), and α-terpineol (5.8%) as the major components (Elkiran & Avsar 2020). The essential oils of *V. myrtillus* samples collected from the Mediterranean region (Antalya) were reported to include eucalyptol (41.07%), linalool (12.72%), α-pinene (12.17%), and myrtenol (6.48%) (Bayar *et al.* 2018). Qualitative differences between the collection areas may suggest that geographical and climatic conditions significantly affect the chemical composition of *V. myrtillus* essential oil.

The total phenolic and total flavonoid content of the *Vaccinium myrtillus* leaf extracts. The total phenolic (TPC) and total flavonoid contents (TFC) of different solvent extracts of *V. myrtillus* populations are given in Table 2. The total phenolic and flavonoid content of *V. myrtillus* varied greatly depending on the extraction solvent, although there was no significant difference in the collecting locations ($p < 0.05$). The TPC was found in the methanol extracts (151.44 and 157.58 mg GAE/g extract) for the Mersin and Muğla locations, respectively, followed by ethyl acetate (76.02 and 77.02 mg GAE/g extract) and *n*-hexane (29.38 and 42.80 mg GAE/g extract).

The Table 2 presents the total amounts of flavonoid substances obtained from the *n*-hexane, ethyl acetate, and methanol extracts of *Vaccinium myrtillus*. The ethyl acetate extract from the samples collected in Mersin exhibited the highest amount of flavonoid content, 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. Recent studies have been conducted on *V. myrtillus* grown in different regions to determine the total phenolic and flavonoid contents of the plant. The *V. myrtillus* plant collected from the Eastern Black Sea Region in Turkey exhibited a total phenolic content of 1035 ± 16 mg GAE/100 g fresh weight (fw) and a total flavonoid content of 150 ± 4 mg QE/100 g fw (Altiok *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 dry weight (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 in varying years varied between 144.50 and 796.30 mg GAE/100 g FW, depending on the location and year (Ciulca *et al.* 2021). While significant changes were observed in the total phenolic and flavonoid amounts of *V. myrtillus* plants collected from different geographical regions, no statistically significant differences were observed in the current study. This can be explained by the fact that the collection regions (Muğla and Mersin) are located in similar climate zones.

The antioxidant activities of *Vaccinium myrtillus*. The antioxidant activity of three different extracts of *V. myrtillus* leaf extracts are given in Table 3. The CUPRAC activity results indicate significant variations in the CUPRAC depending on the polarity of the extraction solvent. Specifically, the methanol extracts of plants collected from different locations exhibited comparable activity with synthetic antioxidants, BHA and BHT, compared to the other solvents. However, the collecting site did not affect CUPRAC activities.

The ABTS radical scavenging activities of the extracts obtained from *V. myrtillus* plants collected from different locations were compared with Trolox, BHA, and BHT. In contrast to the TPC, TFC, and CUPRAC findings, the ABTS radical scavenging activity of the extracts differed significantly depending on the collecting location and extraction solvent. The methanol extracts of two collection sites were found to have the highest

Table 4. The effect of the plant methanol extracts on plant seed germination, root and shoot development in *Taraxacum officinale*.

	Muğla			Mersin		
Doses	GR**	RL	SL	GR	RL	SL
Control	88.0 ± 4.6[a*]	7.07 ± 1.2[a]	8.26 ± 1.2[a]	88.0 ± 4.6[a]	7.07 ± 1.2[a]	8.26 ± 1.2[a]
1000 ppm	40.0 ± 2.3[b]	2.64 ± 0.1[b]	3.31 ± 0.1[b]	65.3 ± 9.3[b]	5.13 ± 0.2[ab]	4.42 ± 0.0[b]
2000 ppm	4.0 ± 2.3[c]	0.99 ± 0.7[b]	1.06 ± 0.7[b]	32.0 ± 2.3[c]	3.74 ± 0.1[b]	3.76 ± 0.4[b]
3000 ppm	0.0 ± 0.0[c]	0.0 ± 0.0[bc]	0.0 ± 0.0[bc]	0.0 ± 0.0[d]	0.0 ± 0.0[c]	0.0 ± 0.0[c]

*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)

Table 5. The effect of the plant methanol extracts on *Rumex crispus* plant seed germination, root and shoot development.

	Muğla			Mersin		
Doses	GR**	RL	SL	GR	RL	SL
Control	100 ± 0.0[a*]	15.82 ± 2.8[a]	18.17 ± 0.9[a]	100 ± 0.0[a]	15.82 ± 2.8[a]	18.17 ± 0.9[a]
1000 ppm	96.66a ± 1.6[a]	2.68 ± 0.3[b]	5.70 ± 0.3[b]	98.33 ± 1.6[a]	5.85 ± 0.3[b]	13.69 ± 2.7[a]
2000 ppm	96.66a ± 1.6[a]	1.19 ± 0.0[b]	4.64 ± 0.2[b]	95.0 ± 0.0[a]	1.3 ± 0.1[bc]	3.32 ± 0.0[b]
3000 ppm	70b ± 2.4[b]	1.06 ± 0.1[b]	3.53 ± 0.1[b]	85.0 ± 2.8[b]	0.61 ± 0.0[c]	2.13 ± 0.2[b]

**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)

Table 6. The inhibition rate (%) of the *Vaccinium myrtillus* Mersin population methanol extract on the mycelium growth of pathogens.

Doses (ppm)	***S.S	****R.S	*****FOM	******FOC
**Control+	100 ± 0.00[a1]	100 ± 0.00[a]	83.88 ± 0.00[a]	78.76 ± 0.00[a]
*Control-	0.00 ± 0.00[d]	0.00 ± 0.00[d]	0.00 ± 0.00[d]	0.00 ± 0.00[e]
1000	19.96 ± 10.50[d]	13.98 ± 7.10[c]	55.09 ± 4.15[c]	21.84 ± 3.08[d]
2000	45.42 ± 8.95[c]	24.75 ± 4.09[bc]	61,66 ± 4.92[c]	40.21 ± 1.07[c]
3000	78.00 ± 4.41[b]	31.43 ± 0.97[b]	73.31 ± 1.75[b]	46.33 ± 7.45[b]

*Negative control; ** Positive control; ***Ss *Sclerotinia sclerotiorum* ****RS *Rhizoctonia solani* *****FOM *Fusarium oxysporum* f. sp. *melonis* ******FOC *Fusarium oxysporum* f. sp. *cucumerium*

[1]Means in the same column with the same letter were not significantly different by ANOVA (α = 0.05)

Table 7. The inhibition rate (%) of the *Vaccinium myrtillus* Muğla population methanol extract on the mycelium growth of pathogens.

Doses (ppm)	***S.S	****R.S	*****FOM	******FOC
**Control+	100 ± 0.00[a1]	100 ± 0.00[a]	83.88 ± 0.00[a]	78.76 ± 0.00[a]
*Control-	0.00 ± 0.00[d]	0.00 ± 0.00[d]	0.00 ± 0.00[d]	0.00 ± 0.00[e]
1000	39.02 ± 6.83[c]	19.87 ± 1.77[c]	53.73 ± 3.26[c]	36.96 ± 0.27[d]
2000	73.66 ± 6.59[b]	21.37 ± 3.56[c]	60.73 ± 2.07[b]	46.71 ± 0.08[c]
3000	84.77 ± 2.30[b]	34.77 ± 2.81[b]	72.62 ± 1.78[b]	53.60 ± 0.97[b]

*Negative control; ** Positive control; ***Ss *Sclerotinia sclerotiorum* ****RS *Rhizoctonia solani* *****FOM *Fusarium oxysporum* f. sp. *melonis* ******FOC *Fusarium oxysporum* f. sp. *cucumerium*

[1]Means in the same column with the same letter were not significantly different by ANOVA (α = 0.05)

activity. The hexane extract exhibited the lowest ABTS radical scavenging activity with IC_{50} values ranging from 8.19 to 17.76 µg/mL. Furthermore, the *V. myrtillus* plants collected from the Mersin province exhibited higher ABTS radical scavenging activity than those collected from Muğla. It may be concluded from the results that the individual plant polyphenols from the Muğla and Mersin locations differ in their reactions with the ABTS radical.

According to the results (Table 3), the methanol extract exhibited the highest reducing power (FRAP activity), ranging from 4.75 to 485 µmol TE/mg extract. Following the methanol extract, the ethyl acetate extract displayed a reducing power within the range of 2.65 to 2.81 µmol

TE/mg extract, while the hexane extract demonstrated a reducing power ranging from 0.88 to 1.35 µmol TE/mg extract. This indicated that the methanol extracts of the plant samples from the Muğla and Mersin locations exhibited similar activity to BHA and BHT. While there was a statistically significant variation in activity trends between extraction solvents, no significant differences were detected based on their different locations.

The results of the DPPH radical scavenging activity are also presented in Table 3. The DPPH radical scavenging activities of the hexane, ethyl acetate, and methanol extracts obtained from *V. myrtillus* plants collected from different locations were compared to the standards Trolox, BHA, and BHT. In terms of IC_{50} (µg/mL), the highest DPPH radical scavenging activity was observed in the methanol extracts, followed by the ethyl acetate and *n*-hexane extracts, respectively. It can be said that the active polyphenols in the methanol extracts contribute to its higher activity than BHT and comparable activity to BHA and Trolox.

In general, the antioxidant capacity of natural products is attributed to their ability to donate electrons and thereby break the oxidative bonds of free radical ions. The electron-binding capabilities of the plant extracts were compared to those of BHT and BHA, which are generally acknowledged as the criteria for identifying natural antioxidants which can replace synthetic antioxidants. It is worth noting that the samples collected from both locations exhibited strong activity in all the assays, particularly in the high methanol extracts.

The allelopathic effects. The allelopathic activity of *V. myrtillus* was investigated through Petri dish experiments conducted under 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 the *Vaccinium* species, and the test plant species (refer to Tables 4 and 5 for details).

A dose of 3 mg/mL of *V. myrtillus* methanol extract completely inhibited the germination and growth of the *T. officinale* seeds. Doses of 1 mg/mL and 2 mg/mL 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 the *R. crispus* seeds when compared to the control group (Table 4). Previous studies have also reported the 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*. 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). Also, Wepplo (1987) reported that the leaves of *Vaccinium macrocarpon* contain substances which inhibit plant growth.

The antifungal activity. The antifungal activity of the methanol extracts of *V. 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 the methanol extracts on the mycelium growth of these pathogens, and the results are presented in Tables 6 and 7.

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 6). Similarly, the extract from the Muğla location inhibited the mycelium growth of plant pathogenic fungi to varying extents, ranging from 34.77% to 84.77% (Table 7).

Additionally, *V. myrtillus* essential oil demonstrated inhibitory effects on mycelial growth in *Sclerotinia sclerotiorum*, *Alternaria solani*, *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL), and *Verticillium dahliae*, with inhibitory percentages of 61.38%, 100%, 80.36%, and 57.91%, respectively (Bayar *et al.* 2018). Ştefănescu *et al.* (2020) also reported the antifungal activity of leaf extracts from *V. myrtillus* and *V. vitis-idaea* against *Candida zeylanoides*.

CONCLUSION

Based on the findings of this study, it can be concluded that the methanol extracts of *V. myrtillus* exhibit significant levels of total phenolic content, total flavonoid content, and

antioxidant activity. These effects are attributed to the presence of phenolic and flavonoid compounds in the plant. Furthermore, the current research has demonstrated that the methanol extracts of *V. myrtillus* exhibit allelopathic effects on two important weed species, namely *Rumex crispus* and *Taraxacum officinale*. In addition, the extracts also showed antifungal activity against plant pathogenic fungi, including *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Fusarium oxysporum* f.sp. *cucumerinum*, and *Fusarium oxysporum* f. sp. *melonis*. Specifically, the extracts were shown to inhibit the growth of mycelium in these fungi. These findings suggest that the biological activity of *V. myrtillus* extracts 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 for the development of novel natural antifungal agents.

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REZIME

Botanica SERBICA

Istraživanje potencijala ekstrakta borovnice kao prirodnih antifungalnih i bioherbicidnih agenasa u poljoprivredi: sastav i antioksidativna aktivnost

Yusuf Bayar, Melih Yilar, Hüseyin Akşit i Nusret Genç

Ova studija ima za cilj da istraži sastav eteričnog ulja lista i ukupan sadržaj fenola, flavonoida i antioksidativnu aktivnost *n*-heksana, etil acetata i metanolnih ekstrakata listova *Vaccinium myrtillus* sakupljenih sa dve različite lokacije (Muğla and Mersin, Turska). Pored toga, prvi put je procenjena antifungalna aktivnost protiv *Sclerotinia sclerotiorum* (SS), *Fusarium oxysporum* f sp. *melonis* (FOM), *Fusarium oxysporum* f sp. cucumerinum (FOC), i *Rhizoctonia solani* (RS) i bioherbicidna aktivnost protiv *Taraxacum officinale* i *Rumex crispus*. Metanolni ekstrakt je inhibirao rast micelija SS, FOM, FOC i RS na način zavisan od doze. Nije primećena značajna razlika u antifungalnoj aktivnosti između dva različita mesta sakupljanja. U testovima bioherbicidne aktivnosti, metanolni ekstrakt plodova je potpuno potisnuo razvoj korena *Taraxacum officinale*, dok je takođe značajno inhibirao razvoj korena tokom klijanja semena *umex crispus* u poređenju sa kontrolnom grupom pri koncentraciji od 3 mg/mL za obe lokacije. Na osnovu rezultata GC/MS analize, glavni sastojci identifikovani u eteričnim uljima lista sakupljenim sa lokacija Muğla i Mersin su bili α-pinen (29,16%/15,75%), eukaliptol (22,19%/26,46%), linalool (12,66%/25,27%), i linalil acetat (7,43%/2,71%). Ukupni sadržaj fenola i flavonoida u biljnim ekstraktima za uzorke Muğla i Mersin je određen (42,80–157,58 GAE/g ekstrakta), (29,38–151,44 GAE/g ekstrakta) i (10,52–37,88 QE/g ekstrakta), (14,81–44,18 QE/g ekstrakta). Pored toga, biljka je pokazala značajnu antioksidativnu aktivnost. Ovi nalazi ukazuju na kvantitativne razlike u hemijskom sastavu eteričnih ulja između dve geografske lokacije. Ovi nalazi su značajni jer pružaju uvid u razvoj novih prirodnih antifungalnih agenasa sa potencijalnom primenom u poljoprivredi.

Ključne reči: antioksidativni, antifungalni, *Vaccinium myrtillus*, esencijalno ulje, bioherbicidna aktivnost

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