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Determination of the Essential Oil Composition and the Antifungal Activities of Bilberry (*Vaccinium myrtillus* L.) and Bay Laurel (*Laurus nobilis* L.)

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Abstract: This study aimed to determine the composition andthe antifungal activity of the essential oils of *Vaccinium myrtillus* and *Laurus nobilis* plants. In the study, 22 components were identified in the essential oil of *V. myrtillus*, which represented 100 % of the total essential oils. Accordingly, 1,8-cineole (41.07 %), β-Linalool (12.72 %), α-Pinene (12.17 %) and Myrtenol (6.48 %) were determined as the main components of the essential oil of *V. myrtillus*. The essential oil of *L. nobilis* consisted of 39 compounds and 1,8-cineole (50.68 %), α-Terpinyl acetate (14.19 %), 4-Terpinenol (4.07 %) and α-Terpineol (2.90 %) were determined as the main components, which represented 100 % of the total essential oils. In the trials, doses of 0 (control) 1, 2, 4, 8 and 10 µL/petri dish were used. *V. myrtillus* essential oil inhibited mycelial growth in *Sclerotinia sclerotiorum (Lib.), Alternaria solani*, *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Sacc.) W.C. Synder & H.N. Hans (FORL) and and *Verticillium dahliae* Kleb by 61.38 %, 100 %, 80.36 % and 57.91 % respectively. Bay laurel essential oil at 10 µL/petri dish dose inhibited the mycelial growth of *A. solani, S. sclerotiorum,* (FORL) by 100 %, whereas it inhibited the mycelial growth in *V. dahliae* by 61.23 %. Study results showed that *V. myrtillus* and *L. nobilis* essential oils have strong antifungal activities.

Key words: Antifungal, *V. myrtillus*, *L. nobilis*, essential oil.

Introductýon

Sclerotinia sclerotiorum, Alternaria solani, *Fusarium oxysporum* f. sp. *radicis-lycopersici* and and *Verticillium dahliae* are plant diseases causing important yield losses. FORL is the disease agent for crown and root rot in tomato. *S. sclerotiorum* is the cause of the disease called white mold in more than 400 plant species. It causes disease in the body, fruits and roots of cucumber. *A. solani* causes the early blight disease, which is very common in tomato. *V. dahliae* causes Verticillium wilt, which causes leaves to curl and discolor. It can cause death in some plants. More than 400 plant species are affected by *Verticillium* complex [1-3].

It is known that, until today, more than 1300 plant species contained compounds that have antimicrobial activity a few of which have been scientifically studied [4,5]. Previous studies have reported that essential oils and plant extracts belonging to many plant species have antibacterial, antifungal, insecticidal and antioxidant properties [6,7]. Essen-

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tial oil compounds are rich sources in terms of biological activity [8]. However, there are very few scientific studies on the activity of these essential oils against plant pathogens. There are 45 genera and 1000 species in the Lauraceae family. There are two species of Laurus genus, as *Laurus nobilis* L. (Laurel Bay) and *Laurus canariensis*. Only *Laurus nobilis* species grow in Turkey [9,10]. The essential oil of bay laurel leaf is used as an aroma in the food and cosmetic industries as well as in soap making. Its dried leaves are also used as tea. It has been reported that essential oil of bay laurel has antimicrobial, analgesic, anti-inflammatory and antitumor properties [11-15]. In Turkey, its wild forms (*Vaccinium vitisidea, Vaccinium-myrtillus, Vaccinium uliginosum* and *Vaccinium-arctostaphyllos*) grow in the Marmara and Eastern Anatolia Regions, especially in the Black Sea Region [16]. Previous studies on *V. myrtillus* have shown that it contains high amounts of anthocyanin and has a high antibacterial effect. Moreover, *V. myrtillus* has high antioxidant content and is used in herbal medicines and in pharmaceuticals [17,18]. However, no scientific studies have been carried out on the antifungal activity of *V. myrtillus* essential oil.

In this study, chemical composition and antifungal activities of the essential oils of *Vaccinium myrtillus* L. and *Laurus nobilis* L. were determined.

Materials and methods

Collection of plant samples and obtaining their essential oils

Plant samples were collected from Alanya in Antalya Province in June of 2016. The plant specimens were identified by Dr. Melih Yilar. The collected leaf samples were dried at room temperature in the shade. Then, 300 g of the dried plant material was placed in a 5 L flat bottom distillation vessel and 2 L distilled water was added. Essential oils were isolated by hydro-distillation for 2 hours using the Neos essential oils system. The hydro-distillation was repeated twice. The obtained essential oils were stored at +4°C until use [19].

Obtaining fungus cultures

The plant pathogen fungus [*Sclerotinia sclerotiorum*, *Alternaria solani*, *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *Verticillium dahliae* used in the study was obtained from stock cultures found in Ahi Evran University, Faculty of Agriculture, Plant Protection Department, Phytopathology laboratory. Fungus cultures were developed in 900 mm petri dishes containing 20 mL Potato Dextrose Agar (PDA) at 25 ± 2°C for 7 days and, then, used in the study.

Gas chromatography and Gas chromatography-Mass spectrometry (GC-MS)

20 mg essential oil was dissolved in 1.2 mL hexane and made ready for analysis. GC-MS analyses were applied on an Agilent Technologies 7890A GC System, 5975C by Triple-Axis Detector mass spectrometer with a built-in- Autosampler formed with the used of the HP-5MS capillary column (30 m x 0.25 mm x 0.25 µm). For GC-MS detection, electron ionisation system and ionisation energy of 70 eV was used. Helium was the transporter gas at a flow rate of 1 mL min^{-1}. The injector temperature was set at 250°C and the FID was operated at 250°C. An initial column oven temperature of 60°C was elevated to 240°C at a rate of 4°C/min and held for 5 min. As in the gas chromatography, 1.0 L split/splitles (10:1) of the sample diluted with hexane were transferred to the clone.

Identification of oil components was successful by comparison of their mass spectral fragmentation model by the available mass library (Willey and NIST). Relative percentages of the components in the essential oil were calculated by MSD ChemStation (E02.02.1431) software, multiplying the ratio of the peak area of each component to the total peak area by hundred.

In vitro antifungal assay of essential oils

PDA media prepared in 250 mL flasks were autoclaved and transferred to 60 mm diameter petri dishes (forming a 10 mm diameter media disc). Then, 5 mm were diameter papers adhered to covers of the petri dishes containing PDA media. Mycelium discs (5 mm) of the disease agents were placed in the center of these petri dishes. Plant essential oils were dripped onto paper adhered to the micropipette at 0 (control), 1, 2, 4, 8, 10 µL/petri dish concentrations. The petri dishes

were thoroughly coated with parafilm and left to incubate for 7 days at 22°C. At the end of the incubation period, mycelial growth values of diseases in petri dishes were measured with callipers. Trials were carried out in 4 replicates with 2 repetitions. The inhibition in development was compared tothat in the control group, and percentile mycelial growth was calculated according to Pandey *et al.* [20]

$$I = 100 \times (dc \times dt) / dc$$

I: percentile inhibition of mycelial growth dc: mycelial growth in the control group dt: Mycelial growth of the fungus treated with essential oil.

Results and discussions

The chemical composition of the essential oils

Chemical compositions of the essential oils obtained from the aerial part of *Vaccinium myrtillus* L. and *Laurus nobilis L.* by hydro-distillation method were analyzed by GC/MS. The results of the chemical compositions of *V.myrtillus* and *L. nobilis* essential oils are given in Table 1-2. The GC/MS chromatogram of *V. myrtillus* and *L. nobilis* essential oil are given in Figure 1 and 2.

There are no studies in the literature on the chemical composition of *V. myrtillus* essential oil. In this study, 22 components were determined in the essential oil of *V.myrtillus*, corresponding to the 100 % of the total essential oils. In GC/MS analyzes of *V.myrtillus* essential oil were determined as the main components 1,8-cineole (41.07 %), β-linalool (12.72 %), α-pinene (12.17 %) and myrtenol (6.84 %).

Also in the same study, as a result of the GC/MS analyses on the essential oils of *L.nobilis*

Table 1. Chemical composition of essential oil from *Vaccinium myrtillus* L.

No.	Compounds	RT	RI	%
1	Isobutyric acid, isobutyl ester	10.989	880	0.35
2	α-Phellandrene	11.55	900	0.06
3	α-Pinene	11.844	910	12.17
4	β-Pinene	13.218	955	0.14
5	Limonene	14.868	1005	5.49
6	1,8-cineole	15.039	1010	41.07
7	β-Linalool	17.145	1072	12.72
8	Terpinen-4-ol	20.035	1156	0.31
9	α-Terpineol	20.479	1168	5.90
10	Myrtenol	20.726	1175	6.84
11	Linalyl acetate	22.411	1223	5.60
12	Pinocarvyl acetate	24.113	1274	0.19
13	Myrtenyl acetate	24.946	1297	4.60
14	α-Terpineol acetate	25.685	1320	1.28
15	lavandulyl acetate	25.959	1329	0.12
16	Nerol acetate	26.562	1348	0.91
17	Methyleugenol	27.331	1371	1.04
18	Humulene	29.324	1434	0.20
19	7-Isopropyl-7-methyl-nona-3,5-diene-2,8-dione	31.097	1490	0.32
20	Caryophyllene oxide	33.261	1564	0.18
21	Caryophyllene oxide-isomer	34.005	1589	0.24
22	Pulegone	34.691	1613	0.26
	Total			100.00

RI: Retention index; RT: Retention time

Table 2. Chemical composition of essential oil from *Laurus nobilis* L

No.	Compounds	RT	RI	%
1	α-Pinene	11.803	909	1.40
2	β-Pinene	12.999	949	2.74
3	β-Pinene-isomer	13.186	954	1.46
4	2.3-Dehydro-1.8-cineole	13.544	966	0.47
5	α-Phellandrene	14.000	979	0.51
6	α-Terpinene	14.399	991	0.20
7	*o*-Cymene	14.671	999	1.05
8	1,8-Cineole	14.946	1007	50.68
9	γ-Terpinene	15.793	1033	0.61
10	Sabinene hydrate	16.124	1043	0.53
11	Sabinene hydrate-isomer	17.211	1074	0.76
12	2.8-p-Mentha-dien-1-ol	18.018	1096	0.30
13	Pinocarveol	18.744	1118	1.43
14	α-Terpineol	19.591	1143	2.19
15	4-Terpinenol	20.002	1155	4.07
16	α-Terpineol-isomer	20.436	1167	2.90
17	Myrtenal	20.693	1174	2.11
18	Bornyl acetate	23.642	1260	0.37
19	Limonene	24.615	1288	1.54
20	2-Oxabicyclo[2.2.2]octan-6-ol. 1.3.3-trimethyl-. acetate	25.410	1311	0.42
21	α-Terpinyl acetate	25.659	1319	14.19
22	α-Ylangene	26.585	1348	0.27
23	β-Elemene	27.162	1366	0.98
24	Methyleugenol	27.339	1371	0.89
25	Caryophyllene	28.206	1397	0.22
26	Aromandendrene	28.650	1411	0.12
27	α-Gurjunene	28.814	1417	0.09
28	β-copaene	30.083	1458	0.35
29	γ-Gurjunene	30.297	1465	0.14
30	Spathulenol	30.746	1479	0.15
31	γ-Cadinene	31.026	1488	0.47
32	δ-Cadinene	31.210	1494	0.29
33	4-*epi*-Cubedol	31.520	1504	0.62
34	Dodecanoic acid	31.967	1520	1.15
35	Caryophyllene oxide	33.233	1563	2.21
36	β-Guaiene	34.009	1589	0.60
37	Androstan-17-one-3-ethyl-3-hydroxy	34.685	1612	0.88
38	Verrucarol	42.937	1926	0.14
39	Eremanthin	44.090	1973	0.53
	Total			100.00

RI: Retention index; RT: Retention time

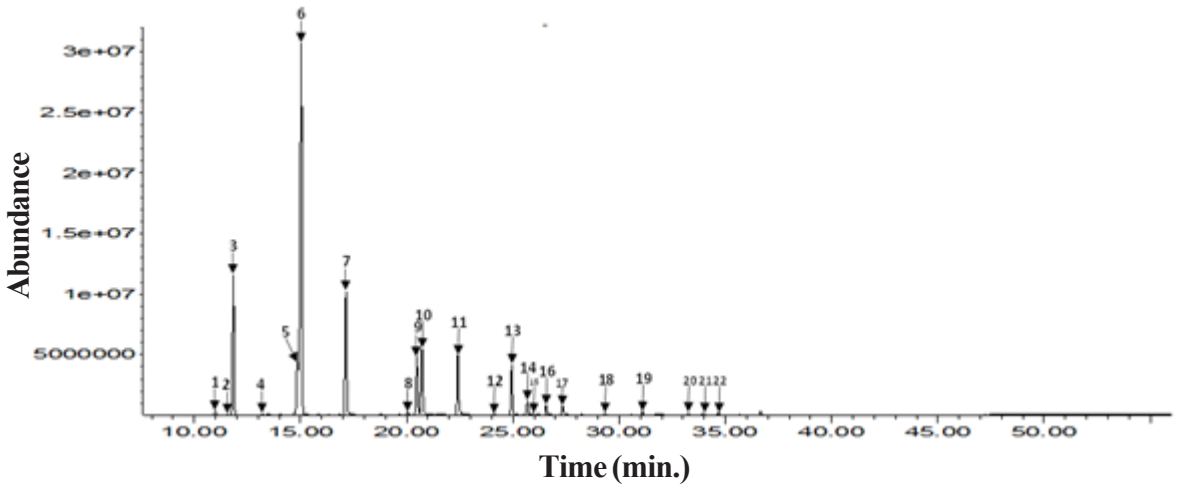

Fig. 1. GC-MS chromatogram of *Vaccinium myrtillus* essential oil

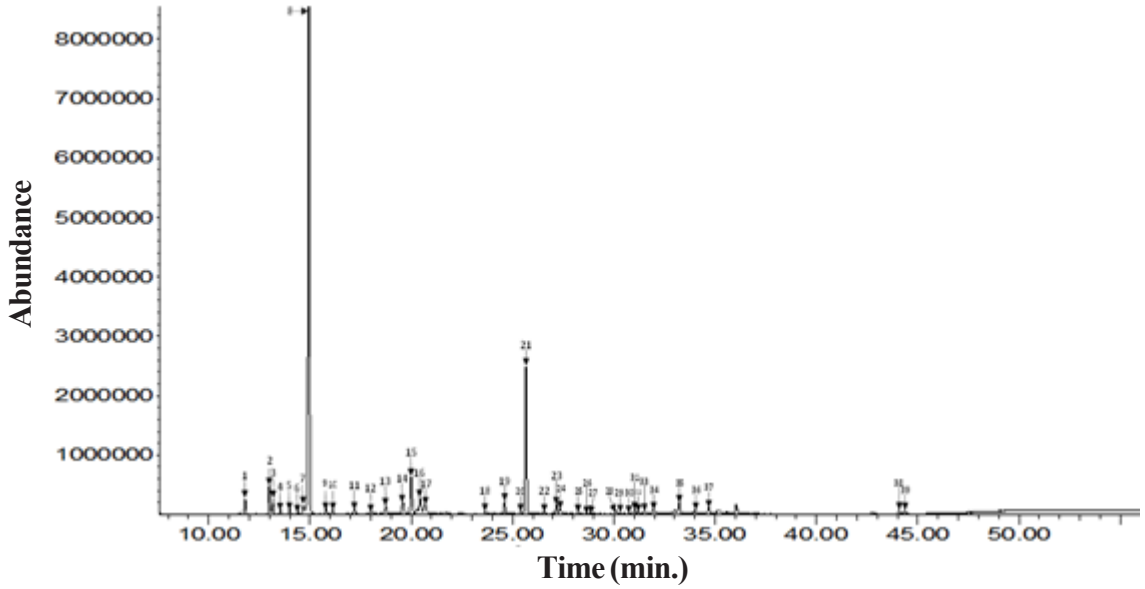

Fig. 2. GC-MS chromatogram of *Laurus nobilis* essential oil

leaves, a total of 39 components were determined, corresponding to the 100 % of the total essential oils. In the GC/MS, 1,8-cineole (50.68 %), α-terpinyl acetate (14.19 %), 4-terpinenol (4.07 %) and α-Terpineol (2.90 %) were determined as the main components of *L.nobilis* essential oil. Marzouki *et al.* [21], in their study on the essential oil compositions of leaves, shoots, flowers and buds of wild-grown *L. nobilis* plant in Tunisia, determined that the most important component, depending on the plant parts, was 1,8-cineole. Also, 1,8-cineole (% 51.73-68.48), α-terpinyl acetate (% 4.04-9.87), sabinene (% 4.44-7.75), α-pinene (% 2.93-4.89), β-pinene (% 2.58-3.91), terpinene-4-ol (% 1.33-3.24) and α-terpineol (% 0.95-3.05) were determined as the main components in the essential oil composition of the leaves of *L. nobilis* wildly growing in different locations in Turkey [22].

In vitro antifungal activity

The results of the antifungal activity tests of *V. myrtillus* and *L. nobilis* essential oils on plant pathogen fungi are given in Table 3 and 4.

V. myrtillus and *L. nobilis* essential oils inhibited the mycelial growth of *A. solani, S. sclerotiorum*. FORL and *V. dahliae* plant pathogens at a high level compared to the control. V.

Table 3. Antifungal activity of essential oils of *Vaccinium myrtillus* against some plant pathogens

Doses	*A. solani*		*S. sclerotiorum*		FORL		*V. dahliae*	
	MG (mm)	I %	MG (mm)	I %	MG (mm)	I %	MG (mm)	I %
Control	60.00±0.00^{a*}		60.00±0.0^{a}		60.00±0.0^{a}		60.00±0.0^{a}	
1 µl	54.75±5.43^{b}	8.75	60.00±0.0^{a}	0	45.51±0.56^{b}	24.15	46.44±0.72^{b}	22.60
2 µl	48.36±0.89^{c}	19.40	60.00±0.0^{a}	0	42.42±0.21^{c}	29.30	42.19±1.14^{c}	29.68
4 µl	45.11±1.96^{c}	24.81	39.34±1.98^{b}	34.43	40.39±1.16^{c}	32.68	37.98±1.92^{d}	36.70
8 µl	35.01±2.39^{d}	41.65	0.00±0.0^{c}	100	30.49±2.84^{d}	49.18	34.90±1.98^{e}	41.83
10 µl	23.17±1.67^{e}	61.38	0.00±0.0^{c}	100	11.78±0.73^{e}	80.36	25.25±1.89^{f}	57.91

* Means in the same column with the same letter were not significantly different by ANOVA (a = 0.05)
Inhibition (I): (MG): Mycelium growth

Table 4. Antifungal activity of essential oils of *Laurus nobilis* against some plant pathogens

Doses	*A. solani*		*S. sclerotiorum*		FORL		*V. dahliae*	
	MG (mm)	I %	MG (mm)	I %	MG (mm)	I %	MG (mm)	I %
Control	60.00±0.00^{a*}		60.00±0.00^{a}		60.00±0.00^{a}		60.00±0.00^{a}	
1 µl	44.21±2.22^{b}	26.32	60.00±0.00^{a}	0.00	40.92±0.39^{b}	31.80	47.07±1.17^{b}	21.55
2 µl	39.33±1.42^{c}	34.45	47.22±1.57^{b}	21.30	38.59±1.33^{b}	35.68	44.57±1.61^{c}	25.72
4 µl	31.52±4.92^{d}	47.47	40.60±3.41^{c}	32.33	30.87±3.96^{c}	48.55	38.87±1.30^{d}	35.22
8 µl	14.18±1.66^{e}	76.37	26.06±2.56^{d}	56.57	12.42±1.27^{d}	79.30	34.79±1.87^{e}	42.02
10 µl	0.00±0.00^{f}	100.00	0.00±0.00^{e}	100.00	0.00±0.00^{e}	100.00	23.25±1.04^{f}	61.25

* Means in the same column with the same letter were not significantly different by ANOVA (a = 0.05)
Inhibition (I): (MG): Mycelium growth

myrtillus essential oil significantly inhibited the mycelial growth of plant pathogen fungus at a P<0.005 significance level. *S. sclerotiorum* inhibited mycelial growth by 100 % at 8 µl/petri dose. *V. myrtillus* inhibited mycelial growth in *A. solani*. FORL and *V. dahliae* at a dose of 10 µl/petri dish, by 41.65 %, 49.18 % and 41.83 % respectively (Table 3). There are no studies conducted on the biological activity of *V. myrtillus* essential oil. However, there are studies on the antioxidant, antimicrobial, antifungal and antibacterial effects of the extracts obtained from the plant. In these studies, it has been reported that lyoniside compound obtained from the stems and rhizomes of *V. myrtillus* had allelopathic and antifungal [23] activity, in addition to antibacterial and antioxidant activity against 30 clinical isolates [24].

L. nobilis essential oil has statistically shown an inhibitory effect on mycelial growth of plant pathogenic fungi. *L. nobilis* essential oil inhibited the mycelial growth of *A. solani*, *S. sclerotiorum* and FORL pathogens at 10 µl/petri dose at 100 % level, whereas it inhibited the mycelial growth of *V. dahliae* at 61.25 % level at the same dose (Table 4). Similar studies conducted on *L. nobilis* have also reported biological activity. It has been reported that *L. nobilis* essential oil has antifungal activities against *Candida* spp. [25], and mycorrhizal fungi *Glomus deserticola* and *G. intraradices* [26]. Antimicrobial and antioxidant activities of *L. nobilis* were also determined [27-29].

Conclusions

This study and similar studies have shown that

Vaccinium myrtillus and *Laurus nobilis* have antifungal activities against plant pathogen fungi. The studies on the biological activities of plant extracts and essential oils have become more important as a result of the proven harmful effects of fungicides on the environment and human health.

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