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Original Article

Chemical Composition and Antifungal Effects of *Vitex agnus-castus* L. and *Myrtus communis* L. Plants

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Abstract

The purpose of this study was to assess the effectiveness of essential plant oils from *Vitex agnus-castus* L. (VAC) and *Myrtus communis* L. against the plant pathogens, *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Sacc.) W.C. Synder & H.N. Hans, *Rhizoctonia solani* J.G. Kühn., *Sclerotinia sclerotiorum* (Lib.) de Bary and *Verticillium dahliae* Kleb., and to determine the chemical composition of the compounds in these essential oils. GC/MS analysis was identified 25 different compounds in VAC essential oil, while the main compounds were determined as Eucalyptol (17.75%), β-Caryophyllene (13.21%) and Spathulenol (10.41%). On the other hand, the essential oil of *M. communis*, consisted of 16 different compounds which were Eucalyptol (49.15%), Myrtenol (19.49%) and α-Pinene (8.38%) being its main compounds. An assessment of antifungal activity was performed under in vitro conditions. Plant pathogens were inoculated onto Petri dishes (60 mm) containing PDA medium (10 mL/Petri^{-1}), and plant essential oils were applied at concentrations of 0.5, 1, 1.5, 2, 5 and 10 (µL/Petri^{-1}) into the 5 mm diameter wells opened on the Petri dish surface. After that, the Petri dishes incubated at 22±2 °C. The results of this study, the essential oil of *M. communis*, at a dose of 10 µL/ Petri, inhibited the 100% mycelium growth of *V. dahliae*, *S. sclerotiorum* and *R. solani*. The highest dose of VAC essential oil was also 100% inhibited *V. dahliae* and *S. sclerotiorum*. The LC_{50} and LC_{90} values of *M. communis* and VAC essential oil calculated for *V. dahliae*, FORL, *S. sclerotiorum* and *R. solani*. This plant extracts were shown by in vitro conditions to be potential antifungal agents.

Keywords: chaste tree, common myrtle, essential oil, plant diseases

Introduction

Although compounds with antimicrobial activity have been identified in over 1,340 plant species; about 60 are mentioned by Nychas (*1995*) and Beuchat (*1994*) (Pillai and Ramaswamy, 2012). Previous studies have demonstrated that the essential oils and plant extracts of many plant species exhibit various antibacterial, antifungal, insecticidal and antioxidant activities against plant pathogens and insects (Burt, 2004; Kordali *et al.*, 2005, Polatoğlu *et al.*, 2013). Some of these essential oils are being used in cancer treatment (Sylvestre *et al.*, 2005), while others are being used for food preservation and in the cosmetics industry. Essential oil compounds offer a rich source in terms of biological activity (Prabuseenivasan *et al.*, 2006).

Vitex agnus-castus L. (VAC) is an evergreen shrub plant of the Verbennaceae family, commonly known by names such as chaste tree, chasteberry, or monk's pepper. VAC is naturally distributed in many provinces of Turkey, including Amasya, Antalya, Bursa, Çanakkale, Muğla and Trabzon (Davis, 1972a). The essential oils and plant extracts of VAC has been reported to have antioxidant, antimicrobial and antifungal functions. Essential oils obtained from VAC seeds have demonstrated strong antifungal activity on *Candida* species (Asdadi *et al.*, 2014). However, there are only a few scientific studies investigating whether or not these essential oils are effective against plant pathogens.

Myrtus communis L. is a bush-like perennial plant, naturally distributed in many provinces across Turkey, such as Antalya, Hatay, Istanbul, Ordu and Sinop (Davis, 1972b). There are various studies in the literature investigating the chemical composition of essential oils of *M. communis* (Zomorodian *et al.*, 2013; Hennia *et al.*, 2015). The essential oil of *M. communis* has been investigated efficiency of antimicrobial,

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antibacterial, antiviral, insecticidal and antifungal activities (*Shan et al., 2007*; *Funatogawa et al., 2004*; *Naserian, 1997*). In particular, Antifungal activities of *M. communis* have been determined on the pathogens such as *Candida albicans* (Nejad *et al.*, 2014) and *Rhizoctonia solani* (Curini *et al.*, 2003).

Plant pathogens cause significant yield losses in both Turkey and all around the world. Among these pathogens, *Sclerotinia sclerotiorum* (Lib.) de Bary is to cause white mold disease in 408 plant species belonging to 275 different varieties (Boland and Hall, 1994). Another, *Verticilium dahliae* is a soil-borne pathogen that causes *Verticillium* wilt disease, which affects over 200 plant species, including the tomato (Fradin and Thomma, 2006). *R. solani* is a pathogen that causes various diseases, affecting the roots and tubers of different plant species (Carling *et al.*, 1989), and induces to a significant loss of potato crops (Yanar *et al.*, 2005). *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Sacc.) W.C. Synder & H.N. Hans (FORL) is one of the most important and destructive tomato pathogens (Benhamou *et al.*, 1994). Although numerous studies have conducted over the years into the control and management of plant pathogens, they continue to be a significant cause of crop loss. The persistence of these pathogens has also lead to a growing resistance to commercial synthetic pesticides. For this reason, research is increasingly focusing on new active substances and control methods that could serve as alternatives to commercial fungicides.

This study was examined the antifungal activities of chemical compounds of essential oils from *Vitex agnus-castus* L. and *Myrtus communis* L. against plant pathogens, *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Sacc.) W.C.Synder&H.N. Hans (FORL), *Rhizoctonia solani* J.G. Kühn., *Sclerotinia sclerotiorum* (Lib.) de Bary, and *Verticillium dahliae* Kleb.

Materials and Methods

Plant material

The *Vitex agnus-castus* and *Myrtus communis* were collected from Demre district of Antalya province (Turkey) in 2014.

Fungus cultures

The plant pathogenic fungi have been isolated from different host plants of *Fusarium oxysporum f. sp. radicis-lycopersici* (the cause of Fusarium wilt in tomato), *Rhizoctonia solani* (the cause of root rot in potato), *Sclerotinia sclerotiorum* (the cause of white mold in cucumber) and *Verticillium dahliae* (the cause of *Verticillium* wilt in tomato). The plant pathogenic fungi were growth in the 90 mm of Petri plates including 20 ml potato dextrose agar (PDA) at 22±2 °C for 7 days and later used in the study.

Extraction of essential oils

The plant parts of *V. agnus-castus* (seeds) and *M. communis* (leaf) (100 g) were collected through hydro-distillation using a Schilcher device. After that, the plant samples were weighted, distilled water added to them at a ratio of 1:10 w/v, and boiled for two hours. The obtained essential oils were stored at + 4 °C until used.

Gas chromatography/mass spectrometry (GC/MS) analysis

Compound analysis was performed through 7890 A model GC system with automatic autosampler system, 5975C inert MSD with Triple-Axis Detector. The samples were diluted with hexane in the 1:10 ratio and they were injected in the mode of split (10:1) as HP-5 (5% Phenyl Methyl Siloxan) 1 µL for distinction of compound. The internal pressure of helium used as the carrier gas was set to 5 psi. The temperatures of both injector and detector planned as 250 °C. FID detector used for quantitative values. Clone's starting temperature was 60 °C, its final temperature was 240 °C and it was programmed to increase 4 °C per minute.

For GC/MS distinction, electron ionization system with 70 eV ionization powered was used. The flow rate of helium that used as the carrier gas was 1.0 mL per minute, the clone was HP-5Ms (30m × 0.25mm × 0.25µm film), and the beginning and the final temperatures and works programme were the same with GC. Injector and MS's transfer temperatures were set to be respectively 230 °C and 250 °C.

As in the gas chromatography, 1.0 µL split/splitles (10:1) of the sample diluted with hexane and transferred to the clone. Identification of oil compounds was accomplished by comparison of their mass spectral fragmentation patterns with available mass library (WILLEY and NIST).

In vitro antifungal effect of the essential oils

The antifungal activities of the compounds were determined by the agar well diffusion method (Tepe *et al.*, 2005). The PDA was autoclaved and then cooled to 40 °C, after which they were transferred to 60 mm diameter Petri dishes (10 ml Petri^{-1}) and then, 5 mm diameter wells were opened on the PDA within the Petri dishes. The plant essential oils were added to the wells at concentrations of 0.5, 1, 1.5, 2, 5 and 10 µl/ Petri. Mycelium discs of 5 mm were placed at equal distances to these wells. The Petri dishes were incubated at 22 ± 2 °C. The Petri dishes were evaluated by based on the growth observed in the control group. The inhibition zone between the wells and the mycelium discs were measured by using a compass. The measured values were compared with the controls, and the percent (%) inhibition was calculated by using the formula below (Pandey *et al.*, 1982). Experiment was performed in two repeats and four duplicates.

$I=100\times(DC-DT)/DC$

I=Inhibition (%)

DC: Radial development in the control

DT: Radial development in applications

Statistical analysis

Data were analysed by using One Way procedure of ANOVA (Windows version of SPSS, release 15.00). Differences among concentrations were compared with using DUNCAN Multiple Range Test of $p<0.05$. The probity analysis of the data derived in consequence of the tests was performed through SPSS 15 computer program and the values of LC_{50} and LC_{90} were calculated.

Results and Discussion

Chemical analyses

Chemical composition and GC/MS chromatograms of the *Myrtus communis* L. and *Vitex agnus-castus* L. (VAC)

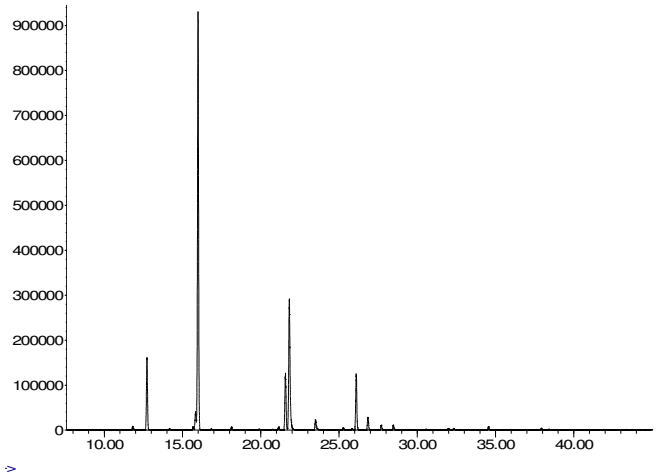

Fig. 1. GC/MS chromatogram of the *Myrtus communis* essential oil

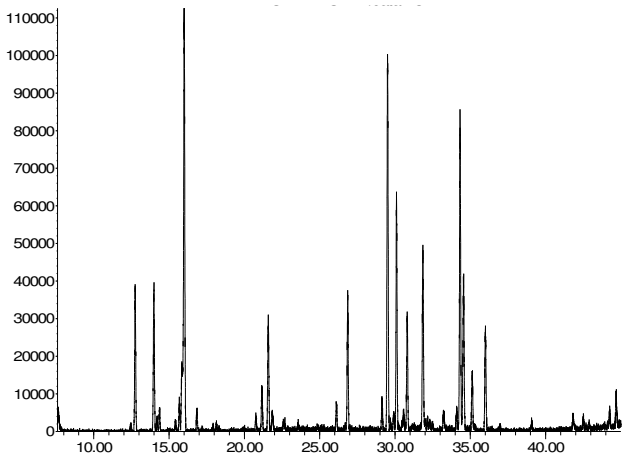

Fig. 2. GC/MS chromatogram of *Vitex agnus-castus* essential oil

Table 1. Chemical composition of *Myrtus communis* L. essential oil

No	RT	RI	Compounds	%
1	11.819	909	Isobutyl isobutyrate	0.44
2	12.727	940	α-Pinene	8.38
3	15.675	1030	m-Cymol	0.40
4	15.831	1035	Limonene	2.05
5	15.990	1039	Eucalyptol	49.15
6	18.131	1099	beta-Ocimene	0.37
7	21.150	1186	4-Terpinenol	0.40
8	21.574	1198	alpha-Terpineol	7.41
9	21.823	1205	Myrtenol	19.49
10	23.498	1256	trans-Geraniol	1.54
11	25.268	1307	Myrtenyl acetate	0.26
12	26.101	1333	Myrtenyl acetate	6.85
13	26.847	1356	Terpinolene	1.64
14	27.692	1382	cis-geraniol	0.61
15	28.471	1405	Methyleugenol	0.62
16	34.549	1607	D-Germacrene	0.39
		Total		100

Table 2. Chemical composition of the *Vitex agnus-castus* L. essential oil

No	RT	RI	Compounds	%
1	12.749	941	α-Pinene	4.40
2	13.993	979	sabinene	4.60
3	14.363	990	beta-Myrcene	0.64
4	15.685	1030	p-Cymene	0.87
5	16.005	1040	Eucalyptol	17.75
6	16.850	1064	Crithmene	0.68
7	21.163	1187	4-Terpinenol	1.64
8	21.586	1198	alpha-Terpineol	3.70
9	21.856	1206	Myrtenol	0.86
10	26.111	1334	Myrtenyl acetate	0.99
11	26.862	1357	Terpinolene	4.66
12	29.140	1428	Neoisolongifolene	1.07
13	29.508	1440	beta-Caryophyllene	13.21
14	30.101	1459	beta-Farnesene	7.32
15	30.570	1474	alpha-Caryophyllene	0.87
16	30.812	1482	Alloaromadendrene	3.93
17	31.851	1516	γ-Elemene	6.88
19	34.098	1592	1-Oxaspiro[4.5]dec-3-ene, 6,6-dimethyl-10-methylene-	0.89
20	34.322	1599	Spathulenol	10.41
21	34.552	1607	Caryophyllene oxide	5.54
22	35.129	1629	not detected	2.33
23	36.007	1660	Viridiflorol	3.66
24	44.256	1980	Cadinol	0.92
25	44.690	1997	14-Cedrandiol	1.23
	Total			99.05

essential oils are shown in Tables 1-2 and Figs. 1-2. The essential oil of *M. communis* consisted of 16 compounds. The main compounds of this essential oil were Eucalyptol (49.15% RT: 15.990), Myrtenol (19.49% RT: 21.823) and α-Pinene (8.38% RT: 12.727) (Table 1).

Various studies have conducted in different regions (e.g. Saudi Arabia, Greece, and Tunisia) for determining the chemical composition of the essential oil of *M. communis*. These studies identified Eucalyptol (1.8-cineole), Myrtenol and α-Pinene as the primary constituents. Khan *et al.* (2014) identified 65 compounds in the essential oil of *M. communis* distributed across the central region of Saudi Arabia, while the main compounds were determined as Eucalyptol (26.5%), linalool (18.0%), α-pinene (11.6%), α-terpineol (8.9%) and limonene (4.0%). In the essential oil of *M. communis* collected from different regions of Greece, the main compounds were identified as a-pinene, Eucalyptol and linalool, and limonene (Koutsaviti *et al.*, 2015). Seventeen compounds were identified in the essential oil of *M. communis* collected in Kef, Tunisia, with myrtenylacetate (20.75%), Eucalyptol (16.55%), a-pinene (15.59%), linalool (13.30%), limonene (8.94%), linalyl acetate (3.67%), geranyl acetate (2.99%) and a-terpineol (2.88%) as the main compounds (Hsouna *et al.*, 2014).

Similarly, 25 compounds were identified in VAC essential oil collected in province of Antalya, Turkey, while the main compounds were determined as Eucalyptol (17.75% RT: 16.005), β-Caryophyllene (13.21% RT: 29.508) and Spathulenol (10.41% RT: 34.322) (Table 2). Comparisons were drawn with previous studies determining the chemical content of VAC-derived essential oil. Stojkovic *et al.* (2011) identified 46 compounds in VAC essential oil, while 34 were identified in the leaves of VAC growing in the North-Central region of Nigeria, and 32 were identified in a study conducted by Katiraee *et al.* (2015). The main compounds in VAC essential oil were determined as α-Pinene (19.48%), Cyclohexene, 1-methyl-4-(1-methylethenyl) (13.37%) and Sabinene (6.89%) (Katiraee *et al.*, 2015). In essential oil of VAC seeds, the main compounds were determined as

Table 3. Antifungal activity values – Inhibition (I - %) and Inhibition zone (Iz - mm) – for *Vitex agnus-castus* essential oil

Dose	Vd		FORL		Ss		Rs	
(µl/Petri)	I (%)	Iz (mm)	I (%)	Iz (mm)	I (%)	Iz (mm)	I (%)	Iz (mm)
Control	0.00g*	0.00	0.00e	0.00	0.00e	0.00	0.00e	0.00
0.5	37.33f	14.93	50.48d	20.19	0.00e	0.00	38.68d	15.47
1	45.75e	18.30	50.63d	20.25	15.85d	6.34	43.35dc	17.34
1.5	55.63d	22.25	52.83dc	21.13	18.13dc	7.25	44.125c	17.65
2	65.03c	26.01	56.75cb	22.70	21.43c	8.57	46.40c	18.56
5	77.45b	30.98	60.65b	24.26	54.05b	21.62	54.85b	21.94
10	100a**	40.00	70.70a	28.28	100a**	40.00	65.35a	26.14

*Means in the same column with the same letter were not significantly different by ANOVA (p = 0.05).
**Fungicidal effect; Vd=*Verticillium dahlia*, FORL=*Fusarium oxysporum f. sp. radicis- lycopersici*, Ss=*Sclerotinia sclerotiorum*, Rs=*Rhizoctonia solani*, I=Inhibition, Iz=Inhibition zone.

Table 4. Antifungal activity values – Inhibition (I - %) and Inhibition zone (Iz - mm) – for *Myrtus communis* essential oil

Dose	Vd		FORL		Ss		Rs	
(µl/Petri)	I (%)	Iz (mm)	I (%)	Iz (mm)	I (%)	Iz (mm)	I (%)	Iz (mm)
Control	0.00f*	0.00	0.00f	0.00	0.00e	0.00	0.00f	0.00
0.5	39.48e	15.79	60.30e	24.12	0.00e	0.00	38.68e	15.47
1	72.68d	29.07	63.325e	25.33	0.00e	0.00	49.28d	19.71
1.5	76.63dc	30.65	69.68d	27.87	25.65d	10.26	56.15dc	22.46
2	81.10c	32.44	75.38c	30.15	31.98c	12.79	58.90c	23.56
5	90.23b	36.09	84.68b	33.87	55.68b	22.77	70.70b	28.28
10	100a**	40.00	93.00a	37.20	100a**	40.00	100a**	40.00

*Means in the same column with the same letter were not significantly different by ANOVA (p = 0.05).
**Fungicidal effect; Vd=*Verticillium dahlia*, FORL=*Fusarium oxysporum f. sp. radicis- lycopersici*, Ss=*Sclerotinia sclerotiorum*, Rs=*Rhizoctonia solani*, I=Inhibition, Iz=Inhibition zone.

Table 5. Results of the dose-effect experiments between plant essential oils and plant pathogen fungi

Pathogens	*Myrtus communis* L.		*Vitex agnus-castus* L.	
	LC_{50} (µL/Petri)	LC_{90} (µL/Petri)	LC_{50} (µL/Petri)	LC_{90} (µL/Petri)
V. dahliae	0.607	3.302	1.063	7,313
FORL	0.344	8.691	0.738	11,026
S. sclerotiorum	3.207	8.269	3.322	9,729
R. solani	1.072	9.765	2.355	7,864

caryophyllene oxide (24.9%), n-hexadecane (12.5%) and α-terpinyl acetate (11.6%) (Ghannadi *et al.*, 2012). The main compounds in essential oil from VAC growing in the Northern Brazil were determined as Eucalyptol, trans-β-farnesene, sabinene, α-pinene, α-terpenyl acetate, β-caryophyllene and bicyclogermacrene (Zoghbi *et al.*, 1999). These differences stem from various ecological characteristics, such as the part of the plant being analyzed, the region where the plants are collected, and the soil. It has reported that the essential oil composition may also differ, according to the flowering period of the plants, the region where they are cultivated and climatic factors (Senatore *et al.*, 1997).

In vitro antifungal activity

The results regarding the antifungal activity of VAC essential oil are shown in Table 3. VAC essential oil exhibited a high degree of fungicidal activity against the tested fungi. Compared with the control, VAC essential oil at a dose of 10 µL/ Petri reduced the mycelium growth of *V. dahliae* and *S. sclerotiorum* by 100%, the mycelium growth of FORL by 70.70%, and the mycelium growth of *R. solani* by 65.35% (Table 3). According to the dose-effect experiments, the LC_{50} and LC_{90} values of VAC essential oil were 1.063 and 7.313 µL/Petri for *V. dahliae*; 0.738 and 11.026 µL/ Petri for FORL; 3.322 and 9.729 µL/ Petri for *S. sclerotiorum*; 2.355 and 7.864 µL/Petri for *R. solani*, respectively (Table 5). Katiraee *et al.* (2015) reported that VAC essential oil exhibits antifungal effects against *Alternaria* spp., *Penicillium* spp., *Aspergillus niger* and *Aspergillus flavus,* and that its MIC values against these pathogenic fungi were 6.3, 12.5, 0.8 and 12.5 µL/Petri dish respectively. Studies have also demonstrated that VAC methanol extract exhibits a strong antifungal effect against *Pythium ultimum*, a tomato pathogen, under both in vivo and in vitro conditions (Svecova *et al.*, 2013) and that essential oil of VAC fruits and leaves reveals antifungal and antibacterial effects in vitro, while also being effective against *Aspergillus niger* on apple fruits under in vivo conditions (Stojkovic *et al.*, 2011).

The essential oil of *M. communis* displayed a strong fungicidal effect against the pathogens used in the experiment. Results concerning this fungicidal effect summarized in Table 4. The effect of *M. communis* essential oil on mycelium growth varies depending on the targeted pathogen and increasing dose. The dose of 10 µL/Petri dish, *M. communis* essential oil inhibited 100% mycelium growth of *V. dahliae, S. sclerotiorum* and *R. solani*, and inhibiting FORL mycelium growth by 93.00% compared with the control.

The values of LC_{50} and LC_{90} were calculated for plant diseases. According to the dose-effect experiments, the LC_{50} and LC_{90} values of essential oil of *M. communis* were 0.607 and 3.302 µL/Petri dish for *V. dahliae;* 0.344 and 8.691 µL/Petri dish for *F. lycopersici*; 3.207 and 8.269 µL/Petri dish for *S. sclerotiorum* and 1.072 and 9.765 µL/Petri dish for *R. solani* respectively (Table 5).

There are various studies in the literature investigating the biological activity of *M. communis* essential oils and extracts. It has reported that essential oil of *M. communis* exhibits antibacterial effects against pathogenic bacteria, such as *Staphylococcus aureus, Proteus mirabilis* and *Kleibsiella pneumonia* (Hennia *et al.*, 2015). Cannas *et al.* (2014), found

the antifungal effects of *M. communis* essential oil against *Candida* species. It has also reported that *M. communis* essential oil at a dose of 1600 ppm inhibits the growth of the *R. solani* pathogen by 60% (Curini *et al.*, 2003). In the present study, mycelium growth of *R. solani* was inhibited 65.35% by 10 ml dose of VAC essential oil, and 100% by the essential oil of *M. communis*.

In this study, antifungal activity of essential oils of *Myrtus communis* and *Vitex agnus-castus* on *Fusarium oxysporum f. sp. radicis-lycopersici*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Verticillium dahliae* were investigated. The essential oils were showed antifungal activity against phytopathogenic fungi. Inconclusion, according to the results, essential oils of *Myrtus communis* and *Vitex agnus-castus* present a potential alternative to commercial fungicides for the management of plant pathogen fungi.

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