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CHEMICAL COMPOSITION AND ANTIFUNGAL ACTIVITY OF Origanum acutidens ESSENTIAL OIL AGAINST *Sclerotinia sclerotiorum* (Lib.) DE BARY AND *Phytophthora infestans* (Mont.) DE BARY

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Abstract: The aim of this study was to investigate the chemical components and evaluate the antifungal activity of *Origanum acutidens* essential oil. The aerial parts of *O. acutidens* were collected, and the hydrodistillation method was used to extract the essential oil. Gas chromatography-mass spectrometry (GC-MS) analysis was performed to determine the chemical composition of the essential oil. The main components identified were α-terpineol (4.76%), *p*-cymene (7.6%), linalool (14.82%), and carvacrol (49.4%). The essential oils were tested against two pathogens *in vitro* experiments to evaluate their antifungal activity. Different concentrations of the essential oil were applied, and the inhibition of mycelial growth was measured. The results demonstrated that the essential oil exhibited antifungal properties against both pathogens. At a dose of 4.8 µL/Petri dish, the mycelial growth of both pathogens was completely inhibited. However, *Sclerotinia sclerotiorum* showed higher tolerance to the essential oil compared to *Phytophthora infestans.* Furthermore, a dose-effect study was conducted as a part of this research. The LC_{50} values (lethal concentration at which 50% of the pathogens' growth is inhibited) for *P. infestans* and *S. sclerotiorum* were calculated as 0.982 µL/Petri and 1.61 µL/Petri, respectively. The study concluded that the essential oil of *O. acutidens* has the potential to be a natural antifungal agent, particularly against *S. sclerotiorum* and *P. infestans*. However, further research is needed to investigate the mechanisms of action and explore potential applications of this essential oil in managing plant diseases.

Keywords: Origanum acutidens, Essential oil, Antifungal activity, Plant pathogens

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1. Introduction

In many agricultural areas around the world, there are significant disease factors that lead to the loss of agricultural products such as fruits and vegetables (Yavuz et al., 2022). Two of these diseases are *Sclerotinia sclerotiorum* (Lib.) de Bary and *Phytophthora infestans* (Mont.) de Bary. *Sclerotinia sclerotiorum* is a necrotrophic phytopathogenic fungus that has been the subject of numerous scientific studies (Otun and Ntushelo, 2020). This pathogen has a wide range of hosts, infecting over 400 species and causing damage to various agricultural products. Due to the durable structures known as sclerotia, *Sclerotinia* species can survive in the soil and their hosts for up to 8 years (Kurt et al., 2011). On the other hand, *Phytophthora infestans* is an oomycete, a fungus-like microorganism that causes late blight, and it is particularly detrimental to potato and tomato plants. It has had significant historical consequences, including the Irish Potato Famine of the 1840s (Blancard, 2012). This pathogen is responsible for numerous epidemics in potatoes and tomatoes, particularly in cool and rainy weather conditions.

Chemical control, specifically the use of synthetic pesticides, is considered the most effective method for combating plant diseases worldwide. However, the extensive use of synthetic pesticides has raised concerns regarding durability issues, environmental problems, and the toxicity and persistence of pesticide residues (Isman, 2000). Consequently, there has been an increased focus on finding alternative methods that are less harmful to human health and the environment, as alternatives to synthetic pesticides.

One such alternative approach involves exploring the effectiveness of naturally occurring compounds found in plants for controlling diseases, pests, and weeds. Vegetable-derived essential oils are among these natural compounds, synthesized as secondary metabolites by aromatic plants (El Ayeb-Zakhama et al., 2017). These essential oils have gained importance as potential alternatives to synthetic pesticides due to their diverse biological effects (Baser and Buchbauer, 2009). Several studies have reported the natural fungicidal properties of essential oils (Bayar, 2018; Affes et al., 2022; Tomić et al., 2023).

The objective of this study was to analyze the chemical components of *O. acutidens* essential oil and evaluate its antifungal activity against the important plant pathogens *S. sclerotiorum* and *P. infestans*.

2. Materials and Methods

2.1. Sample Collection

The aerial parts of *O. acutidens* (1.1 kg) were collected at 32nd kilometers of Gümüşhane/Kelkit road, Türkiye. Systemically identification of the plant samples was carried out by Prof. Dr. Ali Kandemir, at the Biology Department, University of Erzincan Binali Yıldırım. The voucher specimens were deposited under the EBYU 1378 number.

2.2. Essential Oil Extraction

A total of 350 grams of dried aerial parts from *O. acutidens* were blended and subjected to hydrodistillation for 3 hours using a Clevenger apparatus. The extraction process was performed in triplicate. Subsequently, the resulting oils were carefully collected and stored in sealed sample tubes, which were then kept at 4 °C until the analyses were conducted.

2.3. GC-MS Analysis Conditions

GC-MS analyses were conducted utilizing a Thermo Scientific Trace 1310 GC-MS system, which was outfitted with an HP-5MS capillary column (30 m x 0.25 mm and 0.25 µm ID) according to previously published methods (Aksit et al., 2022; Alkan et al., 2021). A carrier gas, helium, was employed at a constant flow rate of 1.2 mL/min in split mode with a ratio of 50:1. The injection site and mass transfer line temperature were both set to 280 °C. The column oven temperature a programmed as initially held at 60 °C for 3 minutes, then increased to 200 °C at a rate of 3 °C/min and held for 0 minutes, and finally ramped up to 240 °C at a rate of 5 °C/min and held for 5 minutes. The mass spectrometer parameters were set as follows: the ion source temperature was maintained at 280 °C, and electron ionization (EI) mode with an ionization energy of 70 eV was employed. Retention indexes (RI) for all components were determined by calculating the retention times using the Van den Dool and Kratz equation, based on a homolog *n*-alkane series (C8-C40). To confirm compound identities, Wiley and NIST2004 MS libraries were utilized. The relative peak area percentages of each compound were calculated based on the peak areas obtained from MS chromatograms.

2.4. Obtaining fungal cultures

In this study, the fungi *P. infestans* and *S. sclerotiorum* were obtained from stock cultures maintained in the Phytoclinical laboratories of Ahi Evran University, Faculty of Agriculture, and Department of Plant Protection. The experiments involved using young fungal cultures derived from these stocks, which were grown on 90 mm petri dishes containing 20 ml of potato dextrose agar (PDA) at a temperature of 25±2 °C for 7 days. The aim was to investigate the in vitro fumigant effect of *O. acutidens* essential oil. To set up the experiment, the prepared PDA was sterilized and cooled to 40 °C before being transferred to 60 mm diameter petri dishes with a depth of 10 mm. Sterile filter paper with a diameter of 5 mm was attached to the lids of the petri dishes containing PDA. Mycelium obtained from 7-day-old fungal cultures was transferred onto the PDA plates. Different concentrations of *O. acutidens* essential oil (0 µl/petri dish for control, and 0.6, 1.2, 2.4, 4.8, and 9.6 µl/petri dish) were applied to the filter papers on the lids of the petri dishes using a micropipette. The petri dishes were then sealed, and the fungal cultures were incubated at a temperature of 25±2 °C for 7 days. At the end of the incubation period, the fungal growth was measured, and the degree of growth inhibition was calculated using the following formula: % Inhibition = [(C − T)/C]× 100, where C represents the mean radial mycelial growth of the pathogen in the control samples, and T represents the mean radial mycelial growth of the treated samples. The experiments were performed with 4 replications and 2 repetitions to ensure statistical reliability.

2.5. Statistical analysis

The significance of differences between treatments in trials was determined through analysis of variance (ANOVA), and means were compared using the Duncan test (Genç and Soysal, 2018). Statistical analyses were conducted using the SPSS 15 computer program.

3. Results and Discussion

3.1. Chemical Composition of Essential Oil of *O. acutidens*

The hydrodistillation process applied to the aerial parts of *O. acutidens* resulted in an essential oil yield of 0.65±0.2% (w/w). This yield was found to be lower compared to the samples collected from Bayburt (Baser et al., 1997) and Sivas (Sökmen et al., 2004), higher compared to Tunceli (Gulec et al., 2014) and Ankara (Cosge et al., 2009) while it was comparable to the samples collected from Erzurum (Kordali et al., 2008) depending on the climate and environmental conditions of collection sites.

The chemical composition of *O. acutidens* is given in Table 1 showing the percentage of each component, retention time (RT), and retention indices (RI). Table 1 shows 27 compounds were identified representing 97.9 % of the essential oil. Oxygenated monoterpenoids (86.64%) were the major class of the oil while

hydrocarbon monoterpenoids constituted 8.1% of the essential oil. *p*-cymene (7.6%), linalool (14.82%), α-terpineol (4.76%), linalyl acetate (%4.16), and carvacrol (49.4%) were the principal components of the essential oil. Previous studies have demonstrated that the carvacrol content in various regions of Türkiye ranged from 61.8% to 87.0% (Baser et al., 1997; Cosge et al., 2009; Gulec et al., 2014; Kordali et al., 2008; Sökmen et al., 2004). However, the current study revealed that the carvacrol content of *O. acutidens* was found as 49.4%. Additionally, this study is the first to report the presence of the carvacrol/*p*-cymene/linalool chemotype in *O. acutidens*.

3.2. Antifungal Activity of Essential Oil of *O. acutidens*

The study investigated the fumigant activity of essential oils obtained from the *O. acutidens* plant against two plant pathogens, *P. infestans* and *S. sclerotiorum.* The results are presented in Table 2-3, which includes the IC_{50} values and the effect of different doses of the essential oil on the mycelial growth of the pathogens. Table 2 shows the inhibitory effect of the essential oil on the mycelial growth of *P. infestans* and *S. sclerotiorum* at various doses. The lowest dose tested, 0.6 µL/Petri dish, did not show any inhibition compared to the control group for both pathogens. However, at a higher dose of 4.8 µL/Petri dish, the essential oil completely inhibited the mycelial growth of both tested fungi. Based on these results, it can be concluded that the essential oil of *O. acutidens* exhibits antifungal properties against *P. infestans* and *S. sclerotiorum*. However, the tolerance of the two pathogens to the essential oil differs. *S. sclerotiorum* showed greater tolerance to the essential oil compared to *P. infestans*, as it required a higher dose (4.8 µL/Petri dish) to completely inhibit its mycelial growth. On the other hand, *P. infestans*, being the more susceptible pathogen, was completely inhibited at a lower dose (4.8 µL/Petri dish) of the essential oil. These findings suggest that the essential oil from the *O. acutidens* plant has potential as a natural antifungal agent, particularly against *P. infestans*, which is known for causing devastating late blight disease in plants. Further studies may be warranted to explore the specific mechanisms of action and the potential application of this essential oil in plant disease management.

Table 1. Chemical composition of *Origanum acutidens* essential oil

RT	RI	Compounds	% Composition
3,68	846	Diketone alcohol	0.19
6,93	952	1-Octen-3-ol	0.53
7,1	969	3-octanone	0.43
7,22	982	α-Myrcene	0.28
8,18	1008	*p*-cymene	7.6
8,3	1019	Limonene	0.14
8,85	1034	*trans*-*β*-ocimene	0.08
9,57	1060	*cis*-linalool oxide	1.98
10,39	1080	Linalool	14.82
11,52	1152	Pinocarveol	0.17
11,68	1125	Verbenol	0.12
12,31	1150	Borneol	4.2
12,62	1165	4-terpineol	1.78
13,02	1172	α-Terpineol	4.76
13,17	1219	Dihydrocarveol	0.55
14,04	1229	Nerol	0.64
14,76	1237	Linalyl acetate	4.16
15,77	1259	Thymol	0.51
16,23	1278	Carvacrol	49.4
17,67	1342	Neryl acetate	1.2
18,17	1358	Geranyl acetate	1.97
19,17	1424	Caryophyllene	0.39
19,66	1441	Aromadendrene	0.41
21,7	1488	Cadinene	0.16
23,03	1572	Spathulenol	0.17
23,14	1580	Caryophyllene oxide	1.23
		Monoterpenes	8.10
		Oxygenated monoterpenes	86.64
		Sesquiterpenes	0.80
		Oxygenated sesquiterpenes	1.32
		Total	97.87

Compounds were listed in order of elution on the HP-5MS column, RT= retention time, RI= retention Indices, Bolded name = Chemotype.

Table 2. Antifungal effects (%) of the *O. acutidens* essential oil

Doses (µL/ Petri dish)	*Phytophthora infestans*	*Sclerotinia sclerotiorum*
Negative Control (0)	0.0±0.00c*	0.0±0.00d
0.6	0.0±0.00c	0.0±0.00d
1.2	53.21±15.60b	8.57±3.15c
2.4	100.00±0.00a	72.08±14.91b
4.8	100.00±0.00a	100.00±0.00a
9.6	100.00±0.00a	100.00±0.00a

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

Table 3. Lethal concentration values (µL/Petri) of *O. acutidens* essential oil against test microorganisms

Plant	LC Values	Test microorganisms	
		P. infestans	*S. sclerotiorum*
O. acutidens	LC_{50} (µL/ Petri dish)	0.982 µL/ Petri dish	1.61 µL/ Petri dish
	Slope	11.55±4.02	6.77±0.65
	Chi-square	0.06	0.576

LC= effective dose (Lethal concentration).

It appears that the essential oil of *O. acutidens* exhibited higher toxicity towards the plant pathogen *P. infestans* compared to *S. sclerotiorum*. The IC_{50} value, which represents the concentration required to inhibit 50% of the growth of *P. infestans*, was determined to be 0.982 µL/Petri. On the other hand, the LC_{50} value, representing the concentration lethal to 50% of the test organisms, was calculated as 1.61 µL/Petri for *S. sclerotiorum*.

Therefore, based on these values, it can be concluded that the essential oil of *O. acutidens* showed a higher level of toxicity against *P. infestans* compared to *S. sclerotiorum*, indicating its potential as a more effective treatment option against *P. infestans*.

A previous report was reported that the essential oil of *O. acutidens* exhibited antifungal activity against several fungal species, including *Absidia repens, Aspergillus ochraceus*, *Penicillium jensenii, Aspergillus niger, Scopulariopsis chartarum*, and *Cladosporium herbarum* (Çetin et al., 2011). Another study by Kordali et al. (2008) found that *O. acutidens* essential oil demonstrated strong antifungal activity against 17 plant pathogenic fungi. Furthermore, this study also examined the antifungal effects of carvacrol and p-cymene, which are the main components of *O. acutidens* essential oil, on the same fungi. Carvacrol completely inhibited the mycelial growth of all tested fungi, while *p*-cymene exhibited weak antifungal activity against *Fusarium acuminatum* and *Pythium ultimum*, but significantly increased the mycelial growth of *F. culmorum*, *F. equiseti*, *F. nivale, F. oxysporum*, and *Sclerotinia minor*.

In another study by Sökmen et al. (2004), it was found that the essential oil of *O. acutidens* demonstrated remarkable antifungal activity and inhibited the growth of 12 out of 18 tested fungi. Overall, previous studies were suggested that essential oils rich in carvacrol and thymol exhibit the highest antifungal activity against various phytopathogenic fungi. Consequently, the potent antifungal activity of *O. acutidens* essential oil is attributed to its key components.

4. Conclusion

This study primarily focused on investigating the chemical components of the essential oil extracted from *O. acutidens*, as well as its antifungal properties against pathogenic fungi. The emergence of naturally derived pesticides has provided a viable solution to counter the negative impacts associated with synthetic agents, such as the persistence of residues, the development of resistance, and the induction of environmental pollution. Consequently, the utilization of natural pesticides and herbicides offers numerous advantages, including effectiveness, selectivity, biodegradability, and reduced toxicity to towards the environment. Given the well-documented harmful effects of fungicides on both the environment and human well-being, research into the biological activities of essential oils holds great significance.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	Y.B.	Z.A.	S.Ş.	A.K.
C	50	50		
D	25	25	25	25
S		100		
DCP	50	50		
DAI		50	25	25
L	25	25	25	25
W	60	20	10	10
CR		20	20	60
SR	100			
PM	60	40		

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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