Antifungal Activity and Chemical Composition of the Essential Oil of *Heracleum platytaenium* Boiss's

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ABSTRACT

This study was performed to identify effects of the essential oil from *Heracleum platytaenium* Boiss*'s* plant on the fungi, *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) (Sacc.) W.C. Snyder & H. N. Hans., *Rhizoctonia solani* Kühn, *Sclerotinia sclerotiorum* (Lib.) de Bary, *Botrytis cinerea* Pers. Fr and *Verticillium dahliae* Kleb. Chemical composition of the plant essential oil was identified. According to GC/MS analysis, a total of 12 components were identified, including mainly myristicin (27.47%), octyl acetate (25.10%), 1-octanol (16.90%) and octyl 2-methylbutyrate (9.88%) as basic components. Antifungal activities of the essential oil were determined by using the agar well diffusion method. Each well contained different concentrations [0 (control), 0.5, 1, 1.5, 2, 5 and 10 µl/well^{-1}] of essential oil. As a result, 10 µl of *H. platytaenium* essential oil inhibited 100% the growth of mycelium of FORL, *R. solani* and *S. sclerotiorum* pathogens reduced significantly the growth of mycelium of other pathogens as well. In addition, the concentration-effect tests of the essential oil of *H. platytaenium* against these plant pathogens were estimated as LC_{90} obtained in: FORL, (17.53 µl) *R. solani,* (15.52 µl), *S. sclerotiorum* (8.83 µl), *B. cinerea* (15.58 µl) and *V. dahliae* (149.54 µl). These results could suggest using the essential oil of *H. platytaenium* plant for controlling some plant pathogens.

Key words: *Heracleum platytaenium*, Essential oil, Plant pathogens, Antifungal activity.

INTRODUCTION

Heracleum spp. plants belonging to family Apiaceae, have about 125 species. Fourteen species of them is represented in Turkey of which seven are endemic (Kocak and Ozdemir, 2012). *Heracleum platytaenium* Boiss's is spread in the Black Sea, the Central Anatolia and the Aegean coasts. In general, it has occupied mixed forests, rocky slopes, stream sides and coasts. It has spread at the areas with height between 0 and 1500 m (Anonymous, 2016). *H. platytaenium* has been used in treatments for high blood pressure, epilepsy and diarrhea (Sayyah *et al.*, 2005).

The species of *Heracleum* have been found to be biologically active against insects and bacteria (Kocak and Ozdemir, 2012 and Akcin *et al.*, 2013). Also, it has been known that the essential oils of *H. platytaenium* are active against *Candida* species (İscan *et al.*, 2004). However, there is lack of studies on the activity of the essential oils of *H. platytaenium* on plant pathogenic fungi.

Plant pathogens cause a loss of major agricultural products (tomato, potato, cucumber and strawberry) all around the world. Root and crown rot of greenhouse-grown tomato caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) (Agrios, 2005). Stem canker and black scurf diseases of potato caused by *Rhizoctonia solani* (Yanar *et al.*, 2005). *Sclerotinia sclerotiorum* affects more than 400 plant species and causes the white mold on cucumber stem and fruit parts (Onaran and Yanar, 2011). Gray mold of strawberry is caused by *Botrytis cinerea* (Sallato *et al.*, 2007). *Verticillium dahliae* causes symptoms of *Verticillium* wilt in tomato (Robison *et al.*, 2001). Synthetically produced pesticides are used to control plant diseases but using pesticides is unfavorable for environment and humans. Consequently, studies of essential oils have been conducted to determine alternative control compounds against plant diseases.

Some scientific articles revealed that the essential oils of these plants had antioxidants, relaxing and antimicrobial effects (Adams, 2004). Antioxidant activity is demonstrated in *Origanum vulgare, O. dictamnus* and *Thymus vulgaris* L. (Proestos *et al.,* 2013). Forty-five different species of plants have anti-fungal activity (Duraipandiyan and Ignacimuthu, 2011). It was also reported that plant extracts and particularly essential oils of many medical aromatic plants constituted antimicrobial activity against food-borne human and plant pathogens (Isman, 2000; Kalemba and Kunicka, 2003; Burt, 2004 and Soylu *et al.*, 2007).

Therefore, this study was planned to assay the chemical components of the essential oil of *H. platytaenium* and its antifungal activity against some plant pathogenic fungi.

MATERIALS AND METHODS

Plant material

H. platytaenium plant material used was collected from the province of Gümüşhane in Turkey during the year 2014.

Extraction of essential oils

Essential oil of *H. platytaenium* used was derived through hydro-distillation method using the Schilcher device. A certain amount of plant sample was taken and mixed with distilled water (1:10 w/v), then boiled for a period of 2 hrs. The boiled crude extract was maintained in dark glass bottles at 4°C until being used in tests (Telci *et al.*, 2006).

Gas chromatography/mass spectrometry (GC/MS) analysis

Component analysis was performed through a GC model 7890 system with automatic auto sampler system, 5975C inert MSD with Triple-Axis Detector. The samples were diluted by hexane at (1:10 ratio) and were injected in the mode of split (10:1) as HP-5 (5% Phenyl Methyl Siloxan) 1 µl for distinction of components. Internal pressure of helium used as a gas carrier was set to 5 psi. Temperatures of both injector and detector were adjusted at 250°C. FID detector was used for quantitative values. Clone's starting temperature was 60°C. Its final temperature was 240°C. It was programmed to be increased 4°C per minute. A model GC system 7890 with auto-sampler system was executed with 5975C inert MSD with Triple-Axis Detector. For GC/MS distinction, electron ionization system with 70 eV ionization powered was used. Flow rate of helium that was used as gas carrier was 1.0 ml per minute. The clone used was HP-5Ms (30m x 0.25mm x 0.25µm film) and its standard final temperatures and work program was the same as GC. Injector and MS's transfer temperatures were set to be 230 and 250°C, respectively. As in the gas chromatography, 1.0 µl split/splitles (10:1) of the sample diluted with hexane were transferred to the clone.

Fungal cultures

Plant pathogenic fungi used in the study were acquired from the stock cultures available at the Ahi Evran University, Plant Protection Department, Phytoclinic Laboratory in Kırşehir, Turkey (Table 1). The fungal cultures were developed 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.

Table (1): List of plant pathogens, diseases, host plants and isolate places tested in the study

Pathogens	Common name	Origin	Isolation Place
V. dahliae	Verticillium wilt	Tomato	Antalya, Turkey
FORL	Fusarium wilt	Tomato	Antalya, Turkey
S. sclerotiorum	White mold	Cucumber	Antalya, Turkey
R. solani	Root rot	Potato	Kırşehir, Turkey
B. cinerea	Gray mold	Strawberry	Antalya, Turkey

In vitro antifungal activity of the essential oil

Antifungal activity of the essential oil of *H. platytaenium* on mycelia growth of the plant pathogens; *F. oxysporum* f. sp. *radicis-lycopersici*, *R. Solani*, *S. sclerotiorum*, *B. cinerea* and *V. dahliae* was investigated and determined by the agar well diffusion method (Grammar, 1976). The essential oil at the concentrations of [0 (control), 0.5, 1, 1.5, 2, 5 and 10 µl/well $^{-1}$] was used. The PDA was poured into 60 mm Petri plates (~10ml/plate^{-1}). Wells (5mm diameter) were opened by sterile cork borer, at equal distances far from centre, on the PDA plate. Each well contained different concentration [0 (control), 0.5, 1, 1.5, 2, 5 and 10 µL/well^{-1}] of the essential oil. Each concentration was added into the well and kept at a refrigerator for 2 hrs for diffusing. The PDA plates were inoculated (in the centre of PDA) by 5 mm plugs from 7-day-old cultures. The plates were incubated at 22±2°C for 5 days. Experiment was set up as 4 replicates and repeated 2 times. All data were recorded. All antifungal activities values were determined by measuring inhibition zone distance between pathogen and well (essential oil filled). The mycelial growth inhibition was calculated as percentage of inhibition of radial growth relative to the control, using the following formula (Kordali *et al.*, 2013).

$$\text{Inhibition } (\%) = 100 \times (C - T)/C$$

Where: **C** = mean of the 4 replicates of hyphal extension (mm) of the controls and **T** = mean of the 4 replicates of hyphal extension (mm) of the plates treated with the essential oil.

Statistical analysis

Data were analyzed using One Way procedure of ANOVA (Windows version of SPSS, release 15.00). Differences among concentrations were compared using DUNCAN Multiple Range Test of $p<0.05$. The probit analysis of the data derived in consequence of the tests was performed through SPSS 15 computer program and the values of LC_{10}, LC_{50} and LC_{90} were calculated.

RESULTS AND DISCUSSION

Chemical composition of *H. platytaenium* essential oil

Chemical components of the essential oil of *H. platytaenium* plant were identified through GC/MS (Table 2). A total of 12 components including the basics of Myristicin (27.47%), Octyl acetate (25.10%) and 1-Octanol (16.90%) were identified in the essential oil. Previous researches showed that essential oils isolated from *H. platytaenium* plant growing in different regions of the world were characterized by high contents of esters, alcohols and aldehydes (Iscan *et al.*, 2004). The basic components in *H. platytaenium* essential oil were found as octyl acetate (85.53%), octyl hexanoate (3.06%), (Z)-4-octenyl acetate (1.60%) (Akcin *et al.*, 2013).

Table (2): Chemical composition of the *H. platytaenium* essential oil

RT	RI	Components	%
12.706	939	Octanal	1,98
14.719	1000	alpha-Pinene	2,10
15.967	1039	Eucalyptol	2,61
16.974	1068	1-Octanol	16,90
21.874	1207	Octyl acetate	25,10
26.393	1343	Isobutyric acid, octyl ester	1,71
29.276	1432	Octyl 2-methylbutyrate	9,88
31.087	1490	Curcumene	1,15
32.320	1532	Myristicin	27,47
33.747	1580	Hexanoic acid, octyl ester	3,76
38.887	1766	8,10-Hexadecadien-1-ol	6,40
39.157	1776	Octanoic acid, octyl ester	0,95
TOTAL			100

Table (3): Mycelial growth inhibition (%) of *H. platytaenium* against tested plant pathogens

Essential oil	Plant pathogens				
Conc.(µl/well $^{-1}$)	V. d*	Forl*	S. s*	R. s*	B. c*
0 (Control)	00.00^{g**}	00.00^{e**}	00.00^{f**}	00.00^{e**}	00.00^{e**}
0.5	43.42^{f}	53.12^{d}	00.00^{f}	38.67^{d}	0.00^{e}
1.0	50.85^{e}	55.02^{d}	5.47^{e}	42.42^{d}	0.00^{e}
1.5	56.62^{d}	56.02^{d}	14.68^{d}	47.90^{c}	17.45^{d}
2.0	60.07^{c}	59.27^{c}	19.32^{c}	52.02^{c}	34.80^{c}
5.0	67.77^{b}	68.27^{b}	56.92^{b}	65.00^{b}	56.37^{b}
10.0	73.25^{a}	100^{a***}	100^{a***}	100^{a***}	76.03^{a}

*V.d: *Verticillium dahlia;* Forl: *Fusarium oxysporum f. sp. radicis- lycopersici;* S.s: *Sclerotinia sclerotiorum;* R.s: *Rhizoctonia solani;* B.c: *Botrytis cinerea* **According to DUNCAN, the averages with different letters in the same column are different at the significance level of $p<0.05$. *** Fungicidal effect

Table (4): Concentration-effect (µl/fungi) test results between essential oils and plant pathogens

Pathogens	LC_{10}	LC_{50}	LC_{90}
V. dahliae	0.005	0.87	149.54
FORL	0.033	0.75	17.53
S. sclerotiorum	1.46	3.59	8.83
R. solani	0.12	1.35	15.52
B. cinerea	1.16	4.25	15.58

Antifungal Activity of *H. platytaenium* essential oil

The essential oil of *H. platytaenium* plant was significantly effective on the tested fungi. Inhibition effect on the growth of mycelium of the fungi increased depending on concentration of the essential oil of *H. platytaenium* plant. While the concentrations of 0 (control), 0.5, 1, 1.5, 2, 5 and 10 µl/well^{-1} prevented the growth of mycelium. The concentration of 10 µl showed highest mycelium inhibition effect than the others. Also, the concentration of 10 µl/well^{-1} prevented the growth of mycelium (100%) of FORL, *R. solani* and *S. sclerotiorum* pathogens (Table 3).

Probit analysis of the concentration–effect of the essential oil of *H. platytaenium* on FORL, *R. solani*, *S. sclerotiorum*, *B. cinerea* and *V. dahliae* was performed through SPSS 15 computer program and the values of LC_{10}, LC_{50} and LC_{90} were calculated and presented in table (4).

Recent studies have shown that antibacterial activities of the members of the genus *Heracleum* are good known (Koush and Bayat, 2012), have documentary effects over fungal growth (Iscan *et al.*, 2004 and Ciesla *et al.*, 2008) and demonstrate growth inhibitory activities of diverse *Heracleum* species against a widespread order of pathogenic fungi. Antifungal activities of the methanol extract of *Heracleum persicum* against *Aspergillus niger* and *Candida albicans* was recorded (Kousha and Bayat, 2012).

Usually, the cytotoxic activity of essential oil is due to existence of aldehydes, alcohols, methylene dioxy compounds and phenols. An inhibitory effect of *H. platytaenium* against ochratoxigenic *Penicillium verrucosum* was recorded (Ozcakmak, 2012). Antifungal and antibacterial activities of *H. siamicum* essential oil were examined against five bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Esherichia coli*, *Pseudomonas aeruginosa*) and two fungi (*Candida albicans* and *Microsporum gypseum*) (Kuljanabhagavad *et al.*, 2010). Aqueous extracts of *H. persicum* showed also highest activities over *Bacillus megaterium*, *Micrococcus* sp., *Pseudomonas* sp. and *Staphylococcus aureus* (Kousha and Ringo, 2014).

Obtained results showed an antifungal activity on plant pathogens mycelial growth inhibition of *H. platytaenium* against FORL, *R. solani*, *B. cinerea, S. sclerotiorum* and *V. dahlia*. Our study performed so far was *in vitro* level. Also, the relevant to the concentration identified as effective in practice should be performed *in vivo* level.

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