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Chemical Composition and Antifungal Activity of the Plant Extracts of Turkey *Cardaria Draba* (L.) Desv.

Bayan, Y.
Dep. of Plant Protection, Fac. of Agric., Ahi Evran University, 40100, Kirsehir, Turkey, yusufbayan@gmail.com.
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ABSTRACT

The main components of *Cardaria draba* (L.) Devs. essential oils were chemically analysed and identified by GC-MS. They were: 5-(methylthio)-pentanenitrile (41.13%), decane (11.40%) and nonane (10.93%). Antifungal activity of *C. draba* methanol and aquatic extracts against the plant pathogens *Fusarium oxysporum* f. sp. *lycopersici* (*FOL*), *Alternaria solani*, *Verticilium dahliae*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum in vitro* was determined. Various volumes of methanol and aquatic extracts were mixed with the sterile PDA to obtain different concentrations. In order to examine the used plant extracts, percentages of mycelium inhibition (MGI) values were calculated to compare with the positive control (80% Thiram), which is a standard fungicide. The used plant extract were showed significant antifungal activity against plant pathogenic fungi.

Key words: *Cardaria draba*, Composition, Antifungal, Essential oil, Plant pathogens.

INTRODUCTION

Cardaria draba (Brassicaceae) mostly known as break watercress, is one year herb. The roots derive by seed and by flat reptile. The leaves are wavy, simplistic, and mostly serrated. White top has lightly domic flower group in which individual flower stalks grows upward from diverse points off the substation to roughly the same height (Anonymous, 2016).

Usually, plant pathogenic fungi are checked up by synthetic fungicides. But, use of these is progressively limited because of the deleterious effects of pesticides on human health and the environment (Harris *et al*., 2001). Plant metabolites, herbal-based medicines are think to be less harmful to human health as well as the environment compared to synthetic pesticides (Kordali *et al*., 2009). Consequently, works on the effects of different plant essential oils and extracts are acquired to be used against plant diseases.

This study aimed to detect the *in-vitro* antifungal potential of the methanol and aquatic extract obtained from *C. draba* against *Alternaria solani, Verticilum dahliae, Rizoctania solani, Fusarium oxysporum* f. sp *lycopersici* and *Sclerotinia sclerotiorum* under laboratory conditions.

MATERIALS AND METHODS

Plant materials

The *C. draba* plant material used in the test was picked up from the province of Kirsehir in Turkey during year 2016. The plant was identified by Dr. M. Yilar.

Extraction of essantial oils

The air-dried plant material was exposed to hydro distillation for 2 h using a Neos essential oils system. The essential oil from aerial part samples were preserved in a sealed vial at 4°C until further analysis**.**

GC-MS analysis

Essantial oil composition of plant material was determined using Perkin Elmer Clarus 500 GC-MS. Elution of components was done over BPX-20 capillary column (30 m x 0.25 mm and 0.25 m ID). Enjection part and GC transfer line temperature were both 250ºC. Oven temperature program was as follows: intial oven temperature: 50ºC, ramp to 120ºC with 3ºC/ minute heating rate, ramp from 120 to 220ºC with 5ºC/minute heating rate and finally hold for 0.67 min. Total run time was 44 min. Helium was used as carrier gas at 1.0 ml/minute flow rateat the split mode (50:1). For MS detection, EI ionization system was used at 70 eV energy. Identification of oil components was accomplished by comparison of their mass spectral fragmentation patterns with available mass library (WILLEY and NIST). For analysis: 20 mg of essential oils were diluted with 1.2 ml acetone and 1 µl of final solution directly was injected to instrument.

Plant Extracts

Methanol extracts:

100 g of plant materials were placed into a 1 liter erlenmayer so 600 ml methanol. Mixture was left for 24 hrs at room temperature, later at 120 rpm in an orbital shaker. Extract was filtered using filter paper. The solvent was remoted using a rotary evaporator at 40°C in solvents. The residual, extract was used to prepare a stock solution with 1% DMSO identified. Several concentrations (100, 200 and 400 mg /100 ml PDA) were prepared (Onaran, 2016).

Aquatic extracts:

Dried plant materials were pulverized by grinding with a plant grinding mill. Twenty g of ground plant

material were placed in a glass vessel containing 100ml of distilled water and shacked for 24 hrs at 120 rpm in an orbital shaker. Residues were remoted using filter paper. Several concentrations (5, 10 and 20% ml/100 ml PDA) used in the study were prepared.

Fungal cultures

The plant pathogenic fungi used in this study were obtained from the stock cultures of the Dep. of Plant Prot, Fac. of Agric, Phytopathol. lab, Ahi Evran Univ. Fungal cultures used were recultured and kept for 7 days at 23±2°C in 90 mm plates containing 20 ml of potato dextrose agar (PDA).

***In- vitro* antifungal activity of plant extracts**

These assays were carried out to determine the effect of *C. draba* methanol and aquatic extracts against *A. solani*, *F. oxysporum* f. sp *lycopersici*, *S. sclerotiorum*, *R. solani* and *V. dahliae*. Different volumes of methanol (100, 200 and 400 mg/ml and 5, 10 and 20) aquatic extracts of plant extract doses were used, respectively. PDA was poured into 60-mm Petri plates (10 mL plate-1). Agar disc (5 mm in diameter) of the desired plant pathogenic fungi was inoculated on the medium in each plate and the plates were incubated for 7 days at 25 °C. Fungal development daily observed was assesed for 7 days. Inhibition in the development in fungal growth was calculated, using the following formula (Pandey., 2009). PDA synthetic Thiram 80% (hektas) was used as a positive control. All experiments were repeated twice and each in four replicates.

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

Where: I: Inhibition percentage compared to the control (mycelium development), DC: Mycelium development in the control., DT: Mycelium development in plant extracts applications.

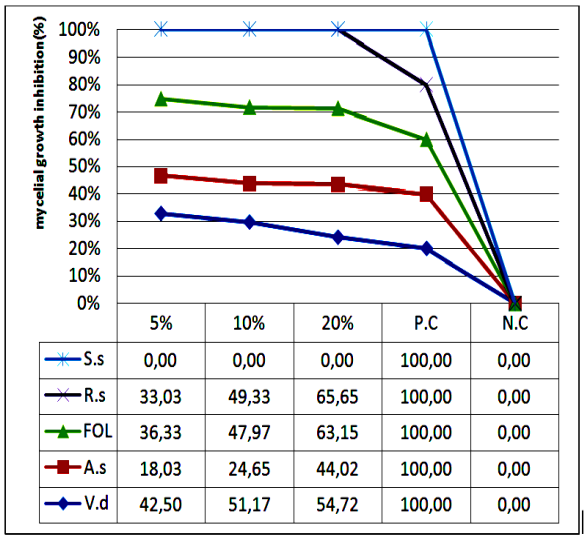
RESULTS AND DISCUSSION

Chemical composition of the volatiles

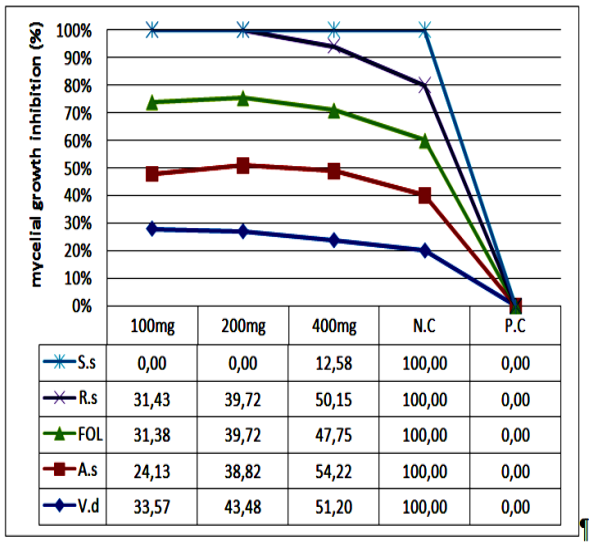
GC-MS analyses of *C. draba* revealed that the essential oils was composed of 19 various components, (96,04%) of the total oil. The identified components of the essential oil are listed in table (1). The basic components of *C. draba* essential oils were 5-(methylthio)-pentanenitrile (41.13%), decane (11.40%) and nonane (10.93%).

Table (1): Chemical composition of *Cardaria draba* L. essential oils and their (%)

Peak no.	RT	Component	(%)
1	3.15	o-xylene	0.78
2	3.34	p-xylene	3.20
3	3.50	Nonane	10.93
4	3.72	Styrene	3.71
5	3.90	Hexane-3,3,4-Trimethyl	1.13
6	4.09	Octane-2,6-Dimethyl	2.66
7	4.20	Heptane,3-Methyl-2-Ethyl	2.55
8	4.37	Decane	0.55
9	4.74	Nonane-4-Methyl	2.25
10	4.97	Nonane-2-Methyl	2.17
11	5.17	Benzene-1-Ethyl-2-Methyl	2.07
12	5.46	Cyclohexen-2methylpropyl	1.57
13	5.76	Decane	11.40
14	6.25	Mesitylene	1.91
15	6.34	Dodecane	1.27
16	6.85	Limonen	3.23
17	8.53	Benzeneacetaldehyde	2.43
18	9.07	Tetradecane	1.12
19	16.33	5-(Methylthio)-Pentanenitrile	41.13
Total			96.04



	5%	10%	20%	P.C	N.C
S.s	0,00	0,00	0,00	100,00	0,00
R.s	33,03	49,33	65,65	100,00	0,00
FOL	36,33	47,97	63,15	100,00	0,00
A.s	18,03	24,65	44,02	100,00	0,00
V.d	42,50	51,17	54,72	100,00	0,00



	100mg	200mg	400mg	N.C	P.C
S.s	0,00	0,00	12,58	100,00	0,00
R.s	31,43	39,72	50,15	100,00	0,00
FOL	31,38	39,72	47,75	100,00	0,00
A.s	24,13	38,82	54,22	100,00	0,00
V.d	33,57	43,48	51,20	100,00	0,00

Figs. (1, 2): Effect of *C. draba* aquatic and methanol extract on the mycelial growth inhibition of different plant pathogenic fungi.

Plant pathogens; *F. oxysporum* f. sp *lycopersici* = **FOL**, *S. sclerotiorum* = **S.s**, *A. Solani* = **A.s**, *R. solani* = **R.s**. P.C=Positive control,

The essential oil components from the aboveground portion of *C. draba* were detected by GC-MS and GC-FID. The major essential oil components obtained by hydrodistillation were 4-methyl sulfanyl butyl isothiocyanate (28.0 %) and 5-methyl sulfanyl pentanenitrile (13.8 %) (Radonic *et al*., 2011).

***In- vitro* antifungal activity of plant extracts**

The obtained methanol and aquatic extracts were added to PDA at 40ºC to the final concentrations of 100, 200 and 400 mg/ml and 5, 10 and 20% for each extract, respectively. The antifungal activity of plant extracts inhibition against *A. solani*, *F. oxysporum* f. sp *radicis- lycopersici*, *S. sclerotiorum*, *R. solani* and *V. dahliae* was determined as mycelial growth inhibition (MGI) (Figs. 1, 2).

Results showed that the methanol and water extracts blocked fungal mycelium development by 100%. However, compared to control, MGI was increased as the concentration increased. Antifungal effect of *C. draba* aquatic extracts against *R. solani*, *F. oxysporumf sp lycopersici*, *V. dahliae*, and *A. solani* was recorded by different MGI rates (65.65, 63.15, 54.72 and 44.02%), respectively. No effect was observed on *S. sclerotiorum*. Correspondent antifungal effect of *C. draba* methanol extracts were; (54.22, 51.20, 50.15, 47.75 and 12.58%), respectively.

Family Brassicaceae has an antimicrobial potancy of members against bacterial isolates. The antimicrobial potential of *Brassica oleracea* L., *Raphanus sativus* L. and *Brassica rapa* L. showed significant antimicrobial activity against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (FNSST 982) and *Pseudomonas aeruginosa* (FNSST 014), the clinical bacterial isolates (Panghal *et al*., 2011). The antibacterial, antioxidant, anti-inflammatory from diverse seed and leaf extracts of *C. draba* were investigated (Sharifi-Rad *et al*., 2015). However, further studies are needed on the potential of *C. draba* extracts against plant pathogens. The anti-fungal activity the of *C. draba* extracts on plant pathogens has been determined in the present for the first time in Turkey.

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