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COMPARATIVE EFFECTS OF SOME EXTRACTION SOLVENTS ON THE ANTIOXIDANT, ANTIFUNGAL AND BIOHERBICIDES ACTIVITY OF *IN VITRO* CONDITIONS OF *CRAMBE ORIENTALIS* CRUDE EXTRACTS

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ABSTRACT

There are important benefits in finding environmentally friendly methods that can replace pesticide in some plant biotic stress factors, by integrated pest management principles. Besides, it is a necessity to improvement and adapt alternative methods or technical to chemical control to sustainable agriculture plant productions. This study was carried out to determine extracts obtained from the *Crambe orientalis* naturally grown in Kırşehir (Turkey) locations, total phenolics, total flavonoids, antioxidant, antifungal and bioherbicidal activity. In the study, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Trolox Equivalent Antioxidant Capacity (TEAC), Ferric ions (Fe^{3+}) reducing antioxidant power assay (FRAP), Copper Reduction Power Activity (CUPRAC), Total phenolic and Flavonoid analyzes of plant extracts were investigated. The antifungal activity of plant extracts was determined against *Alternaria solani, Rhizoctonia solani, Verticillium dahliae* and *Fusarium oxysporm* f. sp. *radicis-lycopersici*. Also, the bioherbicidal activities of the extracts in the *Amaranthus retroflexus* L. and *Rumex crispus* L. seed germination and seedling development were also examined. As a result of the antioxidant study, it was determined that different chemical solvent extracts obtained from the plant had remarkable antioxidant activity. The total phenolic and flavonoid were determined to be limit in terms of *C. orientalis*. On the other hand, methanol extracts decreased mycelial growth of pathogens at different rates due to increasing doses, while the highest doses of sterile distilled water extracts inhibited 100% *in vitro* conditions. In the bioherbicidal study, it was determined that different plant extracts had significant herbicidal effects on *A. retroflexus* and *R. crispus* plants for germination and seedling development *in vitro* conditions.

KEYWORDS:
Antioxidant effect, Antifungal effect, Bioherbicidal effect, *Crambe orientalis*

INTRODUCTION

The genus *Crambe* L., which involves to Brassicaceae family, contains annual or perennial species. This genus has about 30 species in the world. The planting area of this plant is reported to be the Iran-Turanian areas of South-West Asia and the Mediterranean locations [1]. As the *Crambe* species is tolerant of unfavorable climate and soil conditions, it is capable of growing especially in winter and spring seasons. The presence of three *Crambe* species are known in Turkey. These *Crambe* species are *C. tataria*, *C. orientalis* and *C. maritima*. Especially, *C. orientalis*, which is naturally distributed in Central Anatolia Plateau [2].

Secondary metabolites include in some plant parts as essential oils or compounds are known as allelochemicals [3]. This tolerant instead of herbicides effect consisting of allelochemicals has the potential for use in the control of weeds [4]. Researchers on the topic have also reported on weed control as well as essential oils of some plant extracts can be used [5-7]. Indeed, there are research on the effectiveness of leaf extracts of *C. orientalis* L. on cultivated cultivars. For example, lettuce (*Lactuca sativa* L. cv. Varamin) has been reported to be phytotoxic [8]. However, research on germination of weed seeds is limited.

Lipid peroxidation is one of the main factors causing food degradation during storage and processing. Oxidized polyunsaturated fatty acids can trigger aging and cancer formation [9]. Although synthetic antioxidant compounds such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), commonly used in processed some foods, have been reported to have some process effects [10]. Therefore, for some researchers, natural antioxidants are among the remarkable issues. It is known that reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in cells in different ways [11, 12]. ROS and RNS can lead to DNA damage and mutation. Antioxidant may mission an important role in the prevention of oxidative damage that may occur in humans [13, 14]. Natural antioxidants, phenolics, carotenoids, α-tocopherols,

and vitamin C constitutes a wide range of compounds [15, 16]. Medicinal and aromatic plants and different plant (carrot, guava, sugarbeet, berry, etc.) are known to be natural sources of antioxidants. Therefore, the determination of antioxidant properties of medicinal and aromatic plants and weeds are among the most important research areas [17-20].

Fusarium oxysporum f. sp. *radicis-lycopersici, Verticillium dahliae, Alternaria solani* and *Rhizoctonia solani* are plant fungal diseases that cause significant losses in Turkey and in the world. *F. oxysporum* f. sp. *radicis-lycopersici* is a fungal disease called crown and root rot on tomato [21]. *A. solani*, which is very common in tomato cultivate areas and is called early blight [22]. *R. solani* causes significant crop losses in some field crops and vegetable and is called soft rot on the roots and other subsoil plant structures [23].

There are important benefits in finding environmentally friendly methods that can replace pesticide in some plant biotic stress, by integrated pest management principles. Besides, it is a necessity to develop and adapt alternative methods to chemical control to sustainable agriculture productions. One of these alternative methods is to determine the compounds of plant origin essential oils and to use them in the controls against some plant diseases, pests, and weeds. There is some research on the fungicidal, herbicidal and insecticidal effects of plant compounds and essential oils [24-27]. However, there are different research in which the effects of vegetable oil extracts on different plant pathogens are limited.

This study was carried out to determine extracts obtained from the *Carambe orientalis* naturally grown in Kırşehir (Turkey) locations, total phenolics, total flavonoids, antioxidant, antifungal and bioherbicidal activity.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials. *C. orientalis* samples were collected from Kırşehir locations during the flowering stage in the vegetation period of 2018-2019. The collected plants allowed to dry in an aerated and shadow room for two weeks at room temperature (23±2°C) conditions Kirsehir Ahi Evran University, Agriculture Faculty, Plant Protection Department. A voucher specimen of the plant was authenticated using keys, comparison with authentic herbarium materials and expert determination by Dr. Melih Yilar at the Kırşehir Ahi Evran University.

Preparation of Plant Extracts. Preparation of Water Extract. The plant material, was ground into a powder by grinding with a plant grinding mill. The plant sample previously prepared the plant for use in the study (stem + leaves and shoots + flower), the solid filtered to give 1000 mL of sterile distilled water into 200 g of dried plant sample sterile glass erlenmeyer into placing the orbital shaker at 120 rpm for filter paper. After shaking for 24 hours to remove residues. The resulting solution was centrifuged at 5000 rpm for 15 minutes and kept completely separated from the solid residue. The extracts were stored in a vial under the refrigerator (+ 4°C) conditions until it was used in the research [28].

Preparation of Methanol Extract. 100 grams of each dried plant material (stem + leaf and shoot + flower) were weighed and put into 1 L sterile erlenmeyer and 600 mL of absolute methanol (MeOH) were added. The mixture was stirred at 120 rpm on an orbital shaker for 24 hours at room temperature. The extract obtained at the end of the time was passed through sterile filter (Whatman No:1) paper. The evaporator maintained at 32°C was removed by evaporation until a solid was obtained. The remaining solid was prepared as a stock solution with acetone-sterile distilled water. Four concentrations (250, 500, 1000 and 2000 ppm) were obtained from acetone sterile distilled water mixture from the stock solution. The extracts were stored in a vial under the refrigerator (+ 4°C) under conditions until it was used in the experiment research [28].

Preparation of Antioxidant Extracts. 200 mg were taken from the plant sample put inside hexane, ethyl acetate, and methanol extracts. 10 mL of hexane/chloroform (5/1 v/v) was added to the plant sample for hexane extract, 10 mL of ethyl acetate/chloroform (5/1 v/v) for ethyl acetate extract and 10 mL of methanol/chloroform (5/1 v/v) for methanol extract. After vortex proses the mixture samples, they were kept in an ultrasonic bath at 30°C for 30 minutes. Then the solving extraction solutions were deported by rotary evaporator, stock solutions were prepared to be 1 mg/mL. This stock solution was stored at +4°C for use in antioxidant activity tests and total phenolic and flavonoid analyzes.

Free Radical Scavenging Activity DPPH (1,1-diphenyl-2-picrylhydrazyl) test. The free radical scavenging activity was determined through several modifications in the Liyana-Pathirano method [29]. Stock solutions of different amounts of plant extract were put in test tubes and they were topped up to the volume with the addition of 3 mL ethyl alcohol. Then, 1 mL DPPH solution (0.26 mM) was added and mixed by vortex. After it was kept in dark (room) condition for 30 minutes, the absorbance was determined at 517 nm. DPPH radical scavenging activity was calculated as IC_{50} value.

Trolox Equivalent Antioxidant Capacity (TEAC). This analysis was performed according to the method used by Re et al. [30]. Solutions of 2 mM ABTS (2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid) and 2.45 mM sodium persulfate

($Na_2S_2O_8$) prepared with 0.1 M phosphate buffer with a pH value of 7.4 M were mixed at the ratio of 1:2 and kept in the dark for 6 hours. Stock solutions of different amounts of plant extracts were poured in test tubes and their volumes were topped up to 3 mL with a 0.1 M phosphate buffer (pH 7.4). Then 1 mL ABTS solution was added and mixed through vortex, kept in room temperate conditions for 30 minutes, and the absorbance value was determined at 734 nm. ABTS cation radical scavenging activity was calculated as the IC_{50} value.

Ferric ions (Fe3+) reducing antioxidant power assay (FRAP). FRAP analysis was performed by a modified method [31]. Plant extract of 0.25 mL was topped up to 1.25 mL with a 0.2 M phosphate buffer (pH 6.6). Then, 1.25 mL potassium ferricyanide $K_3Fe(CN)_6$ solution (1%) was added. This mixture was kept at 50°C for 20 minutes. Once the mixture was cooled arrange to room temperature, Trichloroacetic acid (TCA) (1.25 mL, 10%) and $FeCl_3$ (0.25 mL, 0.1%) were added and vortex was applied to mix, followed with an absorbance determining at 700 nm. The results obtained were calculated as a Trolox Equivalent (TE).

Copper Reducing Power Activity (CUPRAC). An amount of 0.1 mL was taken from the resulting sample solutions and the volume was topped up to 1 mL with methanol. Following the addition $CuCl_2$ (0.01 M), neocuprin ($7.5x10^{-3}$ M) and ammonium acetate solutions of 1 mL each, it was mixed with vortex. After waiting for 30 minutes at room temperature, the absorbance value was determined at 450 nm. The results obtained were calculated as a Trolox Equivalent (TE) [32, 33].

Total Phenolic Determination. The total phenolic compound was determined using the Folin-Ciocalteu reagent [34]. An amount of 0.2 mL was taken from the stock solutions prepared with the plant extracts and topped up to 4.6 mL with sterile distilled water. With the addition of 0.3 mL Na_2CO_3 solution (2%) and 0.1 mL Folin-Ciocalteu reagent it was placed in the vortex. Thereafter, it was piped in room temperature conditions for 2 hours and the absorbance was determined by a spectrometer at 760 nm. Results were calculated as Gallic Acid Equivalent (GAE).

Total Flavonoid Determination. An amount of 0.2 mL was taken from the resulting sample solutions and the volume was topped up to 4.8 mL with methanol. Then 0.1 mL of $Al(NO_3)$ (10%) and 0.1 mL NH_4CH_3COO solutions (1 M) were added. After vortexing, it was kept at room temperature conditions for 40 minutes and absorbance measurement was determined at 415 nm. The results obtained were calculated as Quercetin Equivalent (QE) [35].

Obtaining Fungus Cultures. The fungal pathogens used for *invitro* testing were *A. solani*, *R. solani*, *F. oxysporum* f. sp. *radices-lycopersici*, *V. dahliae* provided by Phytoclinical Laboratory of Kirsehir Ahi Evran University, Agriculture Faculty, Plant Protection Department. In the research, the factors prepared from these stock cultures; Fresh fungus cultures developed for 7 days at 25±2° C in 90 mm sterile petri dishes containing approximative 20 mL potato dextrose agar (PDA) medium were used.

***In vitro* condition Antifungal Effect of *C. orientalis* Extracts.** The prepared PDA medium (40 grams PDA in 1 L sterile distilled) was autoclaved and cooled to 40°C and was added approximately 10 mL in sterile 90 mm diameter sterile petri dishes. Stored methanol and water plant extracts were completed at a final concentration of 250, 500, 1000 and 2000 ppm on the liquid volume to form a thickness. As diseases positive control group, PDA medium containing 80% thiram and PDA containing sterile distilled water were used as diseases negative control group. Test fungus cultures were incubated for 7 days at 25±2 ° C after transfer to PDA medium and fungal development was recorded for all medium at the end of the period. Inhibition in development was calculated according to the formula given below by comparing the positive and negative diseases control groups to development [36]. All research tests were conducted with 4 replications and 3 biological replications.

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

I %: Percent inhibition compared to negative control (Mycelium development)
DC: Mycelium development in positive control
DT: Mycelium development in plant extract applications

Effect of Plant Extracts on Seed Germination and Seedling Development of Test Plants *in vitro* Condition. The bioherbicidal activities of the extracts in the *Amaranthus retroflexus* and *Rumex crispus* germination and seedling development were also examined. The research investigating the effect of plant extracts on seed germination and seedling growth of test plants were also carried out in plant extracts of sterile 90 mm diameter petri dishes. The seeds of the test plants (25 pieces) were homogeneously placed in sterile petri dishes with sterile blotting paper placed in 2 layers. Water and methanol plant extracts of the prepared plant samples were mixed with different concentrations (1%, 5%, 10%) and 6 mL were added to the petri dishes using sterile distilled water for the control group. Petri dishes were kept at 24±2 ºC for 4 weeks. At the end of the this period, germination rate and root and shoot lengths of seeds of test plants were determined [37].

The experiments were conducted with 4 replications and repeated 2 times.

Analysis of Data. The significance of the differences between the applications in the experiments was determined by analysis of variance (ANOVA) and the means were compared using DUNCAN test [38]. Statistical analyses were performed using SPSS 15.0 version program.

RESULTS

Total Phenolic and Total Flavonoid Contents. The total phenolic content of methanol, ethyl acetate and hexane extracts from *C. orientalis* was determined (Table 1). Significant differences were determined between different solvent extracts in terms of total phenolic matter. Among these solvents, the phenolic content was determined in methanol (15.32±0.17 mg GAE/g extracts) and ethyl acetate (10.66±0.07 mg GAE/g extracts) and hexane (8.34±0.06 mg GAE/g extracts) respectively.

In Table 1 shows the total flavonoid content of methanol, ethyl acetate and hexane extracts obtained from *C. orientalis*. Significant differences were found between different solvent extracts in terms of total flavonoid content. The highest flavonoid content was determined in methanol (11.42±0.11 mg QE/g extracts), followed by ethyl acetate (7.12±0.19 mg GAE/g extracts) and hexane (1.92±0.11 mg GAE/g extracts) respectively.

DPPH Free Radical Scavenging Activity Tests. According to the ability to extract hydrogen DPPH free radical scavenging activity was determined. DPPH free radical removal activities of plant extracts as determined in Table 2, respectively ethyl acetate (IC_{50}, 302.43±0.66 µg/mL), methanol (IC_{50}, 125.28±0.29 µg/mL) and hexane (IC_{50}, 365.47±1.26 µg/mL) compared to the standard antioxidant BHT (IC_{50}, 10.85±0.19µg/mL), BHA (IC_{50}, 4.73±0.08 g/mL) and Trolox (IC_{50}, 4.48±0.05 µg/mL) a limited free radical removal activity.

ABTS Radical Scavenging Activity. The values determined for ABTS radical scavenging activity are given in Table 2. ABTS removal activities of the extracts were determined as ethyl acetate (IC_{50}, 24.07±0.14 µg/mL), methanol (IC_{50}, 13.72±0.27 µg/mL) and hexane (IC_{50}, 41.95±0.16 µg/mL). Antioxidant BHT (IC_{50}, 4.71±0.08 µg/mL), BHA (IC_{50}, 3.86±0.08 µg/mL) and Trolox (IC_{50}, 6.92±0.06 µg/mL) compared to a remarkable ABTS radical removal activity.

CUPRAC Radical Scavenging Activity. The results of CUPRAC radical scavenging activity are reported in Table 2. CUPRAC radical scavenging activity of extracts had the highest level methanol (5.86±0.09 TEmol TE/mg extracts) compared to standard antioxidants BHT (5.68±0.09 TEmol TE/mg extracts) and BHA (11.18±0.20 µmol TE/mg extracts). This was followed by ethyl acetate (0.68±0.48 µlmol TE/mg extracts) and hexane (0.098±0.05 µmol TE/mg extracts).

Determination of Reducing Power. The reducing power of the compounds is indicative of the antioxidant capacity of those compounds. The reduction strengths of the extracts are given in Table 2 and BHT (4.73±0.08 TEmol TE/mg extracts) and BHA (5.63±0.13 µmol TE/mg extracts) were

TABLE 1
Total phenol contents of *C. orientalis* with different extraction solvents.

Solvent of extracts	Total phenolic content (mg GAE/g extracts)	Total flavonoid content (mg QE/g extracts)
Ethyl acetate	$10.66±0.07^{b*}$	$7.12±0.19^{b}$
Methanol	$15.32±0.17^{a}$	$11.42±0.11^{a}$
Hexane	$8.34±0.06^{c}$	$1.92±0.11^{c}$

*: a–c; Column with different superscripts indicating a significant difference (P < 0.05).

TABLE 2
The antioxidant activities of different solvent extracts determined with DPPH, ABTS, CUPRAC and TEAC radicals

Solvent of extracts	DPPH (IC_{50}, µg/mL)	ABTS (IC_{50}, µg/mL)	CUPRAC (µmol TE/mg extracts)	TEAC µmol TE/ mg extracts
Ethyl acetate	$302,43±0.66^{b*}$	$24,07±0.14^{b}$	$0,68±0.48^{c}$	$0.58±0.03^{d}$
Methanol	$125,28±0.29^{c}$	$13,72±0.27^{c}$	$5,86±0.09^{b}$	$0.93±0.04^{c}$
Hexane	$365,47±1.26^{a}$	$41,95±0.16^{a}$	$0.098±0.05^{d}$	$0.30±0.01^{e}$
**BHT	$10.85±0.19^{d}$	$4,71±0. 08^{e}$	$5.68±0.09^{b}$	$4.73±0.09^{b}$
**BHA	$4.73±0.08^{e}$	$3.86±0.08^{f}$	$11.18±0.20^{a}$	$5.63±0.13^{a}$
**Trolex	$4.48±0.05^{e}$	$6.92±0.06^{d}$		

Results are expressed as half inhibitory concentration (IC_{50}) of each antioxidant activity.
*a–f Column with different superscripts indicating a significant difference (P < 0.05).
** Used as standard antioxidant.

determined in the highest level methanol (0.93±0.04 μmol TE/mg extracts) extract compared to standard antioxidants. The followed by ethyl acetate (0.58±0.03 μmol TE/mg extracts) and hexane (0.30±0.01 μmol TE/mg extracts), respectively. It was determined that plant extracts obtained from *C. orientalis* had a limit level reducing power.

Antifungal Effect *in vitro* Conditions to Test Pathogens. *C. orientalis* methanol (stem+leaf and shoots+flower) and water (stem+leaf and shoots+flower) extract were found to have antifungal potential, although they differed according to application dose and plant fungal pathogen. It was determined that the stem+leaf part methanol extract of *C. orientalis* plant *A. solani V. dahliae* and *F. oxysporm* f. sp. *radicis-lycopersici* pathogens could significantly limit the development of mycelium in the petri dishes compared to the positive control group. On the hand, stem+leaf part methanol extract not affect the development of mycelium for *R. solani* (Table 3).

The shoots+flower part methanol extract of *C. orientalis* was also determined that *A. solani, V. dahliae* and *F. oxysporm* f. sp. *radicis-lycopersici* pathogens could significantly limit the development of mycelium compared to the positive control group. On the other hand, the shoots+flower part methanol extract not affect mycelium development for *R. solani*. The methanol extract at the dose of 2000 ppm obtained from the flower was prepared by *A. solani, F. oxysporm* f. sp. *radices-lycopersici* and *V. dahliae*, mycelium development limited of the plant pathogens 20.74%, 28.13% and 31.37% respectively (Table 4).

The stem+leaf part water extract of *C. orientalis* significantly inhibited the mycelium development of *A. solani R. solani*, *V. dahliae* and *F. oxysporm* f. sp. *radicis-lycopersici* compared to the positive control group. It was determined 20% compared with the control group that *A. solani, R. solani* and *V. dahliae* inhibited mycelium development 100%, while *F. oxysporm* f. sp. *radicis-lycopersici* was able to limit mycelium development 72.93%. Water extract at a dose of 20% *A. solani R. solani*, *V. dahliae* and *F. oxysporm* f. sp. *radicis-lycopersici* determined inhibited the development of mycelium of 100% of all pathogens (Table 5).

The flower+shoot part water extract obtained from *C. orientalis* fungal pathogens *A. solani R. solani, V. dahliae,* and *F. oxysporm* f. sp. *radicis-lycopersici* were determined to significantly inhibited mycelium development compared to the positive control group. It was determined that the factors of *A. solani, R. solani, F. oxysporm* f. sp. *radicis-lycopersici* and *V. dahliae* 100% inhibition of mycelium development by 20% dose concentration water extract obtained from flower+shoot part. While *F. oxysporm* f. sp. *radicis-*

TABLE 3
***C. orientalis* stem+leaf parts methanol extract % effect development on fungal pathogens.**

Doses (ppm)	*A. solani*	*R. solani*	*F. oxysporm* f. sp. *radicis lycopersici*	*V. dahliae*
Control+	100±0.00 ^{a*}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}
Control-	0.00±0.00 ^{d}	0.00±0.00 ^{b}	0.00±0.00 ^{d}	0.00±0.00 ^{e}
250	0.00±0.00 ^{d}	0.00±0.00 ^{b}	21.24±1.01 ^{c}	8.58±4.29 ^{d}
500	0.00±0.00 ^{d}	0.00±0.00 ^{b}	22.18±0.51 ^{c}	15.66±1.86 ^{c}
1000	11.19±5.63 ^{c}	0.00±0.00 ^{b}	22.94±0.86 ^{c}	16.48±1.29 ^{c}
2000	46.45±5.01 ^{b}	0.00±0.00 ^{b}	29.18±1.17 ^{b}	23.91±1.72 ^{b}

*: a–e Column with different superscripts indicating significant difference (P < 0.05).

TABLE 4
***C. orientalis* shoots + flower parts methanol extract % effect on fungal pathogens.**

Doses (ppm)	*A. solani*	*R. solani*	*F. oxysporm* f. sp. *radicis-lycopersici*	*V. dahliae*
Control+	100±0.00 ^{a*}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}
Control-	0.00±0.00 ^{d}	0.00±0.00 ^{b}	0.00±0.00 ^{e}	0.00±0.00 ^{e}
250	0.00±0.00 ^{d}	0.00±0.00 ^{b}	11.01±2.13 ^{d}	16.85±2.33 ^{d}
500	0.00±0.00 ^{d}	0.00±0.00 ^{b}	20.01±1.20 ^{c}	17.15±0.57 ^{d}
1000	15.35±0.62 ^{c}	0.00±0.00 ^{b}	20.66±0.16 ^{c}	23.72±2.41 ^{c}
2000	20.74±8.29 ^{b}	0.00±0.00 ^{b}	28.13±0.75 ^{b}	31.37±3.87 ^{b}

*: a–e Column with different superscripts indicating significant difference (P < 0.05).

TABLE 5
***C. orientalis* stem + leaves parts water extract inhibited on fungal pathogens % effect.**

Doses (%)	*A. solani*	*R. solani*	*F. oxysporm* f. sp. *radicis-lycopersici*	*V. dahliae*
Control+	100±0.00 ^{a*}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}
Control-	0.00±0.00 ^{d}	0.00±0.00 ^{c}	0.00±0.00 ^{e}	0.00±0.00 ^{d}
1%	67.43±4.11 ^{c}	67.51±1.15 ^{b}	9.60±3.29 ^{d}	80.15±0.57 ^{c}
5%	80.45±7.08 ^{b}	100±0.00 ^{a}	42.72±0.10 ^{c}	83.89±0.43 ^{b}
10%	100±00 ^{a}	100±0.00 ^{a}	72.93 ±0.17 ^{b}	100±0.00 ^{a}
20%	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}

*: a–e Column with different superscripts indicating significant difference (P < 0.05).

TABLE 6
***C. orientalis* plant flower+shoot water extracts developed on plant pathogens % effect.**

Doses (%)	*A. solani*	*R. solani*	*F. oxysporm* f. sp. *radicis-lycopersici*	*V. dahliae*
Control+	$100±0.00^{a*}$	$100±0.00^{a}$	$100±0.00^{a}$	$100±0.00^{a}$
Control-	$0.00±0.00^{c}$	$0.00±0.00^{c}$	$0.00±0.00^{e}$	$0.00±0.00^{e}$
1%	$39.93±4.11^{b}$	$8.22±8.22^{c}$	$12.86±0.93^{d}$	$50.72±3.47^{d}$
5%	$45.01±4.55^{b}$	$59.35±3.29^{b}$	$24.56±0.61^{c}$	$74.33±0.40^{c}$
10%	$100±0.00^{a}$	$100±0.00^{a}$	$52.28±0.18^{b}$	$83.90±0.22^{b}$
20%	$100±0.00^{a}$	$100±0.00^{a}$	$100±0.00^{a}$	$100±0.00^{a}$

*: a–e Column with different superscripts indicating significant difference (P < 0.05).

TABLE 7
***Rumex crispus* seed germination of *C. orientalis* water and methanol extracts, the effect of development.**

Doses (%)	Shoots+Flower						Stem+Leaves					
	Water			Methanol			Water			Methanol		
	SG**	RL	SL	SG	RL	SL	SG	RL	SL	SG	RL	SL
Control	66.66^{a*}	9.36^{a}	3.97^{a}	66.66^{a}	9.36^{a}	3.97^{a}	66.6^{6a}	9.36^{a}	3.97^{a}	66.66^{a}	9.36^{a}	3.97^{a}
1%	40.00^{b}	2.52^{b}	2.94^{b}	66.66^{a}	1.44^{b}	1.79^{b}	52.00^{ab}	9.09^{a}	3.68^{b}	61.33^{a}	1.38^{b}	0.8^{5b}
5%	28.00^{b}	1.60^{c}	1.57^{c}	38.66^{b}	0.78^{c}	0.00^{c}	45.33^{bc}	5.50^{b}	3.05^{c}	42.66^{b}	1.11^{c}	0.09^{c}
10%	0.00^{c}	0.00^{d}	0.00^{d}	37.33^{b}	0.60d	0.00^{c}	28.00^{c}	1.73^{c}	1.59^{d}	0.00^{c}	0.00^{d}	0.00^{c}

*: a–d Column with different superscripts indicating a significant difference (P < 0.05).
**Seed Germination (SG), Root Length (RL) and Shoot Length (SL)

TABLE 8
Effect of *C. orientalis* water and methanol extracts on *A. retroflexus* seed germination, root and shoot growth.

Doses	Shoots+Flower						Stem+Leaves					
	Water			Methanol			Water			Methanol		
	SG**	RL	SL	SG	RL	SL	SG	RL	SL	SG	RL	SL
Control	78.66^{a*}	3.34^{a}	6.11^{a}	78.66^{a}	3.34^{a}	6.11^{a}	78.66^{a}	3.34^{a}	6.11^{a}	78.66^{a}	3.34^{a}	6.11^{a}
1%	54.66^{b}	1.90^{b}	3.12^{b}	0.00^{b}	0.00^{b}	0.00^{b}	42.66^{b}	3.05b	4.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
5%	33.33^{c}	1.52^{c}	1.26^{c}	0.00^{b}	0.0^{0b}	0.00^{b}	29.33^{b}	1.92^{c}	2.35^{c}	0.00^{b}	0.00^{b}	0.00^{b}
10%	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{b}	0.00^{b}	0.00^{b}	26.66^{b}	1.18^{d}	1.46^{d}	0.00^{b}	0.00^{b}	0.00^{b}

*: a–d Column with different superscripts indicating significant difference (P < 0.05).
**Seed Germination (SG), Root Length (RL) and Shoot Length (SL)

lycopersici and *V. dahliae* mycelium development inhibited 20% dose concentration respectively 52.28% and 83.90% (Table 6). Obtained flower+shoot part water extract 20% dose was determined that all test pathogens 100% mycelium growth inhibited.

Bioherbicidal Effect in *in vitro* Conditions to Test Plant. Different extracts of *C. orientalis* have been determined to have significant herbicidal effects on *A. retroflexus* and *R. crispus* germination and seedling development, root and shoot *in vitro* condition (Table 7 and 8). It has been determined that this negative effect increase and decrease on *A. retroflexus* and *R. crispus* may different in the extract depending on the application dose. *C. orientalis* flower+leaf parts on water and methanol extracts were determined to limit seed germination (SG), root length (RL) and shoot length (SL) of *R. crispus* at a dose of 10% compared to the positive control group. Methanol extracts on of *A. retroflexus* were more effective on seed germination (SG) and seedling growth compare to negative control group. Shoot+flower water and methanol and stem+leaf methanol extracts were determined to limit seed germination completely by seed germination when compared with 10% application dose negative control group (Table 8).

In contrast, leaf+stem water extract was determined to significantly reduce *A. retroflexus* seed germination (SG), root length (RL) and shoot length (SL) compared to the negative control group.

DISCUSSION

Polyphenolic compounds are known to have antioxidant activity, and the activity of plant parts extracts are likely to arise from these compounds [39, 40]. This activity is believed to be mainly due to its redox properties, which play an important role in absorbing and neutralizing free radicals, single and triple oxygen or in the separation of peroxides [41]. Many medicinal and aromatic plants contain large amounts of antioxidant substances such as polyphenols. Many of these phytochemicals have antioxidant properties that play an important role in reducing the occurrence of diseases caused by disease organisms in humans [42, 43]. Antioxidant properties are very important in protecting against free radical damage in foods and biological systems [44]. DPPH is a reliable free radical scavenger that demonstrates

the activity of antioxidants. A low IC_{50} value indicates that the extract has a stronger capacity in terms of DPPH removal [45]. Factors such as solubility of the extract in different test systems or stereoselectivity of radicals have been reported to affect the ability of the extracts to react and remove different radicals [46]. In another study found that some compounds with ABTS removal activity do not show DPPH removal activity [47]. Ferric reductant, antioxidant power (FRAP) is widely used in the evaluation of antioxidant component in dietary polyphenols. [48]. Antioxidant activity increases in direct proportion with polyphenol content. A positive correlation was determined between total phenols and antioxidant activity. It is conceivable that many plant species have this positive relationship [49]. In this research, a positive relationship was determined between total phenols and antioxidant activity.

The results of this study are consistent with similar studies. *C. orientalis* methanol extract showed strong antibacterial activity against *Bacillus subtilis* (PTCC 1207), *Bacillus cereus* (PTCC 1247), *Staphylococcus epiderrnidis* (PTCC 1114), *Escherichia coli*. However, *C. orientalis* hexane and dichloromethane extract did not show any antibacterial effect and plant extracts were reported to have a significant antifungal effect on *Candida kefyr* and *Candida krusei* [8]. It has been reported by some researchers that the glucosinolates determined in the leaves of different *Crambe* species and the isothiocyanates formed by their degradation can also be determined in the essential oil of these isothiocyanates [50, 51, 8]. It is assumed that isothiocyanates in the genus *Crambe* may be responsible for some biological activities such as cytotoxic and phytotoxic effects [8]. It can be assumed that the strong antifungal effect of methanol and water extracts of *C. orientalis* is due to these effects of isothiocyanates. It was determined that the extracts obtained from the flowers part of the *C. orientalis* were more effective than the extracts obtained from the leaf part. It has been reported in different researches that the difference determined is due to the differences in the composition and composition of the components present in the flower and leaf parts [51]. In the same study, it was reported that the essential oil obtained from the leaves and flower parts of *C. orientalis* inhibited the germination of lettuce seeds, seedling root and shoot growth compared to the positive control group at a dose of 1.60 mg mL^{-1}. It has been reported that different extracts obtained from *C. orientalis* plant have phytotoxic effect on lettuce s seeds, seedling root and shoot growth and besides, it has cytotoxicity, antioxidant and antimicrobial activity [8]. *C. orientalis* water extract has been reported to completely limit root-shoot development by seed germination of wheat [52]. Similar studies on *C. orientalis* show that the species has different biological activities. This activity is associated with the presence of bioactive compounds possessed by the plant which can easily penetrate through the biomembranes [53].

CONCLUSION

As a result of the research conducted, it was determined that different solvent extracts obtained from *C. orientalis* shoots+flower and stem+leaves part showed remarkable antioxidant, and antifungal, and bioherbicidal activity *in vitro* condition. In the study, it was determined that the different level antifungal activity of the shoots+flower and stem+leaves methanol and water extracts obtained from different parts of the plant on *A. solani R. solani, V. dahliae* and *F. oxysporm* f. sp. *radicis-lycopersici*, which are some plant fungal pathogens previously reported, has been determined on this pathogen. Research on different extracts of *C. orientalis* shoots+flower and stem+leaves have also been determined to have different antioxidant capacities. The fact that *C. orientalis* species can be widely distributed, ease of cultivation and similar species are widely used all over the world. With the work carried out; It will future studies to develop new products as herbal essential oils that can be used in biopesticides that can be used in plant production with different extras obtained from *C. orientalis*.

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