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RESEARCH ARTICLE

# Chemical Composition, Antibacterial and Antioxidant Activity of Essential Oils and Extracts of *Ferula orientalis*

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**ABSTRACT**

This study aimed to determine the essential oil content, essential oil and extracts, which are known as *Ferula orientalis* and obtained from naturally grown plants in Narman (Erzurum, Türkiye) province, antioxidant effects and antimicrobial effects. *F. orientalis* essential oils were isolated by hydrodistillation and analyzed using gas chromatography-mass spectrometry to identify their components. The antimicrobial activity was measured by the disc diffusion methods and minimal inhibitory concentration (MIC) methods against *Chryseobacterium indologenes* which cause soft rot in certain vegetables and fruits. Total antioxidant and phenolic contents were analyzed by 2,2′-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging, 2,2'-azino-bis(3-ethylbenzothiazoline-6 sulphonic acid (ABTS), thiobarbituric acid reactive species (TBARS), β-carotene bleaching test (BCB) and Folin-Ciocalteu method. According to the results obtained; *F. orientalis* essential oil exhibited a high content of δ-3-Carene (40.38%) as major compound over 14 identified components by GC-MS analysis followed by γ-Terpinene (17.24%), (E)-β-Ocimene (10.51%), and β-Phellandrene (8.49%). The essential oil and extracts was evaluated for its antimicrobial activity against *C. indologenes* showed significant antibacterial activities with MIC values of 9-21 mm and 62.5 µg/mL, respectively, but extracts and antibiotics have no effect against *C. indologenes*. Hexane extract had the highest ABTS free radical scavenging activity with 14.2 ($IC_{50}$ g/l), acetone extract had the highest DPPH capacity with 24.2 ($IC_{50}$ g/l), and water extract had the highest amount of total phenolic compound with 15.13±3.82 mg GAE/g. In the TBARS test antioxidant activity increased as the amount of essential oil increased. The antioxidant capacity of *F. orientalis* essential oil exhibited reduction when evaluated by β-carotene bleaching assay. As a result, it is thought that *F. orientalis* essential oils and extracts can be used as an alternative natural antioxidant source for potential applications.

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## 1. Introduction

Synthetic pesticides are widely used to control plant diseases and pests. Due to the negative effects of synthetic pesticides on the environment and human healths, organic agriculture has gained very crucial and the use of bioagents and natural chemicals as an alternative to synthetic pesticides for disease and pest control has come into use. Numerous studies have been carried out worldwide to control plant, food, and clinical saprophytic and pathogenic microorganisms using vegetable oils, some of their components, and plant extracts (Okeke et al., 2001; Sechi et al., 2001; Abu-Shanab et al., 2004; Adebolu & Oladimeji, 2005; Iroegbu & Nkere, 2005; Rojas et al., 2006; Maggi et al., 2016; Nguir et al., 2016). The number

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of studies on plant extracts and oils in our country has been increasing rapidly in recent years (Meral & Karabay, 2002; Yegen et al., 2002; Sahin et al., 2003, Adıgüzel et al., 2005; Tepe et al., 2006; Kotan et al., 2010; Kotan et al., 2014; Dadasoglu et al., 2015; Görmez et al., 2015; Dadasoglu et al., 2016).

*Ferula* L. is the largest genus in the Apiaceae family, with ~213 species (POWO, 2002). *Ferula* species are widespread in the temperate regions of the Euro-Asian continent, surrounded by the Canary Islands in the West, North Africa in the South, China and India in the East, and Central Europe in the North. *Ferula orientalis* L. grows on the rocky steps of the Eastern Anatolia region of Türkiye at 1600-2900 m altitude (Tubives, 2019). *Ferula* species are known for their medicinal and aromatic properties worldwide and they can be used as precious sources in drug development due to their promising bioactive components (Iranshahy & Iranshahi, 2011; Mahendra & Bisht, 2012; Mohammadhosseini et al., 2019). In recent years, the antioxidant activities, antimicrobial potentials of *Ferula* species were also studied (Kartal et al., 2007; Dadasoğlu et al., 2018; Topdas et al., 2020). Antioxidants prevent and limit the rate of oxidation through one or more mechanisms that involve inhibiting or reducing the effects of free radicals and oxidising compounds on oxidisable substrates. These compounds produced by plants have been the target of research into their antioxidant potential. Among these compounds, essential oils play an important role because many of their components can replace or be combined with synthetic substances. Consumer acceptance of these products has led to the widespread use of essential oils from various plants as raw materials in the food, pharmaceutical, and cosmetic industries (Miranda et al., 2014).

It is known that bacteria, as a disease agent, cause significant losses in the yield and quality of cultivated plants. They have a very wide host potential both in the world and in our country (Agrios, 2005). Among these, the bacteria that cause soft rot cause damage to many economically important plants (Perombelon & Kelman, 1980; Agrios, 2005). Soft rot diseases are caused by different numbers of bacteria belonging to the genera *Erwinia*, *Pseudomonas*, *Enterobacter*, *Chryseobacterium* and *Bacillus* (Dadaşoğlu, 2013). Pathogenic bacteria pose a major problem due to the proliferation of resistant microorganisms, in particular the contamination of various foods. Outbreaks of food poisoning are becoming increasingly common. Therefore, there is a need for compositions that can control antibiotic-resistant bacterial strains to reduce contamination and degradation of food products (Gomes et al., 2014).

The aim of this study was to chemically characterise the essential oil and extract of *F. orientalis*, a medicinal plant and to evaluate their antioxidant and antibacterial activities.

## 2. Materials and Methods

### *2.1.* Plant Material and Plant Pathogenic Bacterial Strains

The aerial parts of *F. orientalis* were collected (10 kg) at the flowering stage in Erzurum, province of Türkiye in July-September 2017 and dried in the shade. The plant is conserved at Ataturk University, Faculty of Agriculture, Plant Protection Department, Plant Clinical Laboratory.

Eight bacterial strains (Table 1) were used, which were previously tested and are highly virulent. These strains had been identified as pathogens of different host plants and were stored at -80 °C in 15% glycerol and Luria Broth (LB) until used.

**Table 1.** Plant pathogenic bacterial strains used in the study.

| Strains | MIS identification* | Host | SIM* | HR* |
|---|---|---|---|---|
| F-408 | *Chryseobacterium indologenes* | Cucumber | 0.84 | + |
| F-491 | *Chryseobacterium indologenes* | Cucumber | 0.78 | + |
| F-492 | *Chryseobacterium indologenes* | Cucumber | 0.82 | + |
| F-502 | *Chryseobacterium indologenes* | Tomato | 0.35 | + |
| F-544 | *Chryseobacterium indologenes* | Cucumber | 0.89 | + |
| F-709 | *Chryseobacterium indologenes* | Tomato | 0.86 | + |
| F-713 | *Chryseobacterium indologenes* | Pepper | 0.60 | + |
| F-723 | *Chryseobacterium indologenes* | Zucchini | 0.79 | + |

*__MIS:__ Microbial identification system, __SIM:__ Similarity index, __HR:__ Hypersensitive response.

### *2.2.* Preparation of the Essential Oil (EO) and Other Extracts

The dried aerial parts of *F. orientalis* plant samples (500 g) were subjected to hydrodistillation using a Clevenger-type apparatus for 4 h. The EO was extracted with $CHCl_3$ and then dried over anhydrous $Na_2SO_4$ and stored under $N_2$ atmosphere at 20 ℃ in a sealed vial until required (Kotan et al., 2010).

The dried plant samples were powdered in a blender and then 50 g of each plant sample was extracted individually with

*n*-hexane, chloroform, acetone, and methanol at room temperature.

After filtration, the organic solvents were evaporated under reduced pressure and temperature. For the methanol extract of the plant sample, the concentrated methanol extract was dissolved individually in distilled water (60 ℃) and then filtered. The solutions were extracted three times with *n*-hexane to remove lipophilic compounds. The water solutions were then lyophilized in a Labconco 117 freeze dryer (Labconco Company, Kansas City, MO, USA) at 5 µg Hg and -50 °C. The extracts were stored at -20 °C until required (Kotan et al., 2010).

### *2.3.* Gas Chromatography-Mass Spectrometry (GC-MS) Analyses

The oil composition was analysed by gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis was performed using a Thermo Finnigan Trace GC/Trace DSQ/A1300, (E.I. Quadrapole) equipped with an SGE-BPX5 MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). An electron ionisation system with an ionisation energy of 70 eV was used for GC-MS detection. The carrier gas was helium at a flow rate of 1 ml/min. Injector and MS transfer line temperatures were set at 220 and 290 ℃, respectively. The oven temperature was programmed from 50 to 150 °C at 3 ℃/min, then held isothermally for 10 min and finally increased to 250 °C at 10 ℃/min. Diluted samples (1/100 v/v, in methylene chloride) of 1 µl were injected manually in the splitless mode. The relative percentage of the oil constituents was expressed as percentages by peak area normalisation. The identification of individual compounds of essential oils was based on the comparison of their relative retention times with those of authentic samples on the SGE-BPX5 capillary column, and on the comparison of the mass spectra of their peaks with those of authentic samples and/or the spectra of the Wiley 7N and TRLIB libraries and published data (Jennings & Shibamoto, 1980; Adams, 2017).

### *2.4.* Antibacterial Activity

The *in vitro* antimicrobial activities of the essential oil and extracts (methanol, ethanol, acetone, chloroform, n-hexane) of *F. orientalis* were evaluated by the disc diffusion method (Murray et al., 1995) with a slight modification using Tryptic Soy Agar (TSA, Merck, Germany) medium with the determination of inhibition zones (IZ). Essential oil and extracts were prepared by dissolition with 10% dimethyl sulfoxide (DMSO), and then were sterilised by filtration through 0.45 µm Millipore filters. Bacterial cultures were grown in Tryptic Soy Broth (TSB, Merck, Germany), and their suspension (100 µl) containing $1\times10^8$ colony-forming units/ml (cfu/ml) of bacteria spread was plated on TSA medium using a sterile swab. Disks (6 mm in diameter) containing 12.5 µl of essential oil and 10.0 mg/ml suspensions of the extracts were used and placed in the centre of the inoculated plates. The diameters of the inhibition zones around the discs were measured in millimetres (mm) after 24 and 48 hours of incubation at 25±2 °C. All studies were performed in triplicate. Standard antibiotic discs (6 mm in diameter) of penicillin and kanamycin (1 µg/disc) were used as positive controls for comparison. 10% DMSO solvent was also tested as a negative control.

### *2.5.* Determination of Minimal Inhibition Concentration (MIC)

The minimum inhibitory concentrations of the extracts and essential oils were determined. Tenfold serial dilutions of the extracts and essential oils were prepared with 10% DMSO solution with an initial dilution of 1/1 v/v (concentration range from 500 µl/ml to 3.125 µl/ml). The concentrations of the bacterial strains grown in TSB were adjusted to $1x10^8$ cfu/ml and 100 µl of bacterial suspension was plated on TSA plates. Then, the blank discs (Oxoid) were directly impregnated with essential oil at different concentrations (12.5 µl and 1.25 mg of the extracts) by placing 6 pieces in each petri dish at equal intervals and were incubated at 25±2 °C for 48 h. The inhibition zone was checked and the lowest concentration of the essential oil showing a clear zone of inhibition was considered as the MIC. All tests were performed in triplicate.

### *2.6.* Determining the Amount of Total Phenolic Compounds

The total amount of phenolic compounds in the extracts was measured according to the method (Singleton et al., 1999). Briefly, 1 ml of plant extract dissolved in ethanol (1 mg/ml) was mixed with 0.5 ml of Folin-Ciocalteu reagent (diluted 10 times in distilled water) in test tubes in triplicate. After 3 minutes, 3 ml of $Na_2CO_3$ (2% w/v) solution was added, and the test tubes were incubated for 2 hours in the dark with continuous shaking. After this time the absorbance of the samples was measured at a wavelength of 760 nm using a spectrophotometer. Gallic acid was used as a standard curve and distilled water was used as a blank.

### *2.7.* Determination of Antioxidant Activity

Due to the complex reactive facets of phytochemicals, the antioxidant activities of plant extracts cannot be evaluated by only a single method, but at least two test methods have been recommended for the determination of antioxidant activity (Rodriguez et al., 1997). For this reason, the antioxidant capacity of *F. orientalis* essential oil and extracts was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6 sulphonic acid (ABTS), thiobarbituric acid reactive species (TBARS) and β-carotene bleaching (BCB) spectrophotometric methods.

#### *2.7.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH)*

50 µg/ml of plant extracts were added to 4 ml of DPPH solution (25 mg DPPH/l ethanol) in the test tube and incubated

at room temperature for 30 min. After incubation, the absorbance at 517 nm was measured against an ethanol blank. 4 ml of DPPH solution was used as a control (Shimada et al., 1992). The decreasing absorbance gives the remaining amount of DPPH in the solution and the free radical scavenging activity. The DPPH scavenging activity was calculated using the following equation:

DPPH scavending activity % = $[(A_0-A_1)/A_0]x100$ (1)

### *2.7.2. 2,2'-azino-bis(3-ethylbenzothiazoline-6 sulphonic acid (ABTS) radical scavenging activity*

ABTS (Sigma-Aldrich, Canada) radical cation solution was prepared by reacting 7.0 mM ABTS stock solution with 2.45 mM (final concentration) potassium persulfate ($K_2S_2O_8$) was mixed in a 1:1 ratio and shaking continuously for 16 h at room temperature in the dark until a stable oxidation state was reached. The $ABTS^+$ radical solution was then diluted with ethanol to give an absorbance of 1.850±0.05 at 734 nm. This absorbance was used as the control absorbance. For the spectrophotometric assay, $ABTS^+$ (4 ml) and 50 µg of plant extract were thoroughly mixed and incubated at room temperature for 2 hours. After this time, the absorbance of the samples was recorded at 734 nm against phosphate buffer (PBS, pH = 7.4) as a blank (Wu et al., 2009). The decreasing absorbance gives the amount of $ABTS^+$. $ABTS^+$ was calculated using the following formula:

% ABTS radical scavenging activity= $[(A_0-A_1)/A_0]x100$ (2)

### *2.7.3. Thiobarbituric acid reactive species (TBARS) assay*

A modified thiobarbituric acid reactive species (TBARS) assay was used to measure the potential antioxidant capacity of the essential oil as a lipid-rich compound (Ruberto & Baratta, 2000). Briefly, 500 µl of 10% (w/v) essential oil and 0.1 ml of sample solutions (4.0, 20.0, and 40.0 g/L respectively), prepared immediately before use, to be tested in methanol, were made up to the 1.0 ml with distilled water. 0.05 ml of 2,20-azobis (2-amidinopropane) dihydrochloride (ABAP) solution (0.07 mol/L) in water was added to induce lipid peroxidation. 1.5 ml of 20% acetic acid (pH 3.5) and 0.8% (w/v) thiobarbituric acid were added in 1.1% (w/v) sodium dodecyl sulphate solution were added and the resulting mixture was vortexed and incubated at 95 °C for 60 min. The mixture was cooled, and 5 ml of butane-1-ol was added to each vial and centrifuged at 1200 g for 10 minutes. The supernatants were measured with a spectrophotometer at 532 nm.

The antioxidant index (AI%) was calculated from the following formula:

AI%=1-T/Cx100 (3)

(*C* is the absorbance value of oxidized control, and *T* is the absorbance of the sample)

### *2.7.4. β-carotene bleaching (BCB) assay*

The antioxidant capacity of *F. orientalis* essential oil was determined using the β-carotene bleaching method with some modifications (Kulisic et al., 2004). β-Carotene (0.1 mg) was added to a boiling flask together with linoleic acid (20 mg) and Tween 40 (100 mg) was dissolved in chloroform. The solvent was evaporated, under vacuum at 50 °C in a rotary evaporator, 50 ml of oxygenated distilled water was added and the emulsion was allowed to stand for 1 minute in sonificator to form emulsion A. The emulsion was then mixed with 200 µl of the ethanolic stock solutions of each antioxidant (0.1; 0.5; 1.0; 2.0; 3.0 and 4.0 g/L respectively) in 5 ml of open-tope cuvettes. A control without antioxidants was prepared, consisting of 200 µl ethanol and 5 ml of emulsion A. In addition, a second emulsion (B) was prepared, consisting of 25 ml of oxygenated water, 10 mg of linoleic acid, and 50 mg of Tween 40. 5 ml of emulsion B and 200 µl of ethanol were used to zero the spectrophotometer. The absorbances of the samples were read immediately (t=0) and every 15 min interval for 120 min at 50 °C using a microplate reader at 470 nm. The percentage of bleaching of β-carotene was calculated according to by Mallet et al. (1994) using the following formula.

% Bleaching of β-Carotene= $(100-A_{A(t1)}-A_{C(t1)})/A_{C(t0)}-A_{C(t1)}x100$ (4)

(A: absorbance $_A$=antioxidant, $_C$=control, t1:120 min)

## 3. Results and Discussion

### *3.1.* Composition of the Essential Oil

The constituents of the studied *F. orientalis* essential oil are shown in Table 2 and 14 bioactive compounds were identified by the GC-MS analysis. The highest percentage of compounds of *F. orientalis* essential oil was δ-3-Carene (40.38%), followed by γ-Terpinene (17.24%) and (E)-β-Ocimene (10.51%), respectively.

Several essential oils from *Ferula* species contain monoterpene hydrocarbons (α-pinene, β-pinene, myrcene, and limonene), oxygenated monoterpenes (linalool, α-terpineol, and neryl acetate), sesquiterpene hydrocarbons (α-caryophyllene, germacrene B, germacrene D, and δ-cadinene), oxygenated sesquiterpenes (caryophyllene oxide, α-cadinol, and guaiol), and sec-butyl disulphide derivatives (Sahebkar & Iranshahi, 2013).

Kartal et al. (2007) showed that *F. orientalis* EO from aerial parts contained high levels of β-phellandrene (23.6%), β-o-cymene (13.8%), α-pinene (12.5%), and α-phellandrene (11.5%). Ozkan et al. (2014) were determined, α-cadinol (10.45%), δ-cadinene (8.1%), germacrene D-4-ol (6.8%), and epi-α-muurolol (5.9%) as the major compounds of *F. orientalis* leaf and flower EOs. Topdas et al. (2020) found α-pinene (20.6%) to be the most abundant compound. These differences

may be due to various factors such as geographical origin, soil composition, climate, harvest time, and plant parts (Celiktas et al., 2007; Topdas et al., 2020).

**Table 2.** The main components of essential oil of *Ferula orientalis*.

| RI[a*] | Components | *Ferula orientalis* (%) | Identification methods |
|---|---|---|---|
| **932** | α Pinene | 2.44 | GC, MS, RI |
| **969** | Sabinene | 0.76 | GC, MS, RI |
| **988** | Myrcene | 1.10 | GC, MS, RI |
| **1008** | δ-3-Carene | 40.38 | GC, MS, RI |
| **1020** | p-Cymene | 3.66 | GC, MS, RI |
| **1025** | β-Phellandrene | 8.49 | GC, MS, RI |
| **1032** | (Z)-β-Ocimene | 1.00 | GC, MS, RI |
| **1044** | (E)-β-Ocimene | 10.51 | GC, MS, RI |
| **1054** | γ-Terpinene | 17.24 | GC, MS, RI |
| **1086** | Terpilone | 0.79 | GC, MS, RI |
| **1471** | 7-epi-1,2-dehydrosesquicineole | 6.28 | GC, MS, RI |
| **1481** | Widdra-2,4(14)-diene | 2.42 | GC, MS, RI |
| **1559** | Germacrene-β | 1.13 | GC, MS, RI |
| **1666** | 14-hydroxy-(Z)-caryophyllene | 2.45 | GC, MS, RI |

[*]**RI[a]:** retention indices in elution order from an HP-5 column.

### *3.2.* Antibacterial Activity Assays

The antibacterial activities of *F. orientalis* essential oil and extracts against eight plant pathogenic bacteria were evaluated by recording IZ and MIC. The results are presented in Table 3.

**Table 3.** The antibacterial activities of essential oil, extracts of *Ferula orientalis* and standard antibiotics and MIC values of essential oil.

| Strains | IZ * (mm) | MIC | M | A | C | H | P | K | DMSO |
|---|---|---|---|---|---|---|---|---|---|
| F-408 | 20 | 125 | - | - | - | - | - | - | - |
| F-491 | 21 | 62.5 | - | - | - | - | - | - | - |
| F-492 | 12 | 250 | - | - | - | - | - | - | - |
| F-502 | 15 | 250 | - | - | - | - | - | - | - |
| F-544 | 15 | 250 | - | - | - | - | - | - | - |
| F-709 | 19 | 125 | - | - | - | - | - | - | - |
| F-713 | 9 | 250 | - | - | - | - | - | - | - |
| F-723 | 20 | 125 | - | - | - | - | - | - | - |

*IZ: Inhibition zones of essential oil (12.5 µl/ml), MIC: Minimal inhibition concentration, M: Methanol extract, A: Aceton extract, C: Chloroform extract, H: Hexan extract, P: Penicillin, K: Kanamycin, DMSO: Dimethilsulfoxoide.

Essential oil of *F. orientalis* showed antibacterial activity against all *C. indologenes* isolates with different ratios (9-21 mm) of inhibition zones compared to the positive standard antibiotics (penicillin and kanamycin). The highest inhibition zone was observed against strain F-491 (21 mm), followed by F-408 and F-723 (20 mm), respectively. The lowest inhibition zone was observed against isolate F-713 (9 mm). The degree of efficacy of essential oils with antimicrobial properties may vary between species belonging to the same genus. The four different extracts (n-hexane, chloroform, methanol, and acetone) of *F. orientalis* do not have any antibacterial activity against *C. indologenes* isolates. Similarly, the standard antibiotics penicillin and kanamycin, used as positive controls, and 10% DMSO, used as a negative control, were found to have no antibacterial activity.

Recent studies on the essential oils of species of the *Ferula* genus have shown that most of these plants have a wide range of biological, especially antimicrobial activities, which are generally related to the chemical composition of the oil (Pavlovic et al., 2012; Al-Ja'fari et al., 2013; Maggi et al., 2016;

Nguir et al., 2016; Topdas et al., 2020). It has been demonstrated that the essential oil of *F. orientalis* can be used in the control of *C. indologenes* (soft rot disease pathogen) by this study.

### *3.3.* Determination of Minimal Inhibition Concentration (MIC)

The MIC values of the essential oil and extracts obtained from the *F. orientalis* plant in Petri dishes against *C. indologenes* isolates are given in Table 3. The MIC values were determined to be 62.5-250 µl/ml.

Copper compounds and antibiotics have been used to control phytopathogenic bacteria, but these applications have many disadvantages. Many studies have reported that phytopathogenic bacteria become resistant to many antibiotics over time. As a result, the use of antibiotics is banned in many countries. For example; there are a number of studies indicating that there are resistant strains of *Xanthomonas campestris* pathovars to kanamycin, ampicillin, penicillin, and streptomycin (Sahin & Miller, 1997; Schlesier et al., 2002; White et al., 2002). In this study, medium or high resistance to tested antibiotic was observed in pathogenic bacteria.

### *3.4.* Determination of Antioxidant Activity

The DPPH method is applied widely used to measure the antioxidant activities of plant extracts. In the DPPH test, the antioxidants reduce the stable DPPH radical to the yellow coloured diphenylpricryhydrazine. In this study, acetone, methanol, water, chloroform, hexane extracts and essential oil obtained from *F. orientalis* were tested for their ability to scavenge free radicals (DPPH). The results are presented in Table 4. It can be seen that the acetone extract has the highest free radical scavenging capacity (DPPH) with 24.2 ($IC_{50}$ mg/ml). The free radical-scavenging capacity of methanol, chloroform, hexane and water extracts and essential oil were found to be 23.3, 19.6, 17.2, 18.3, and 0.88 (ABTS $IC_{50}$ (g/l)), respectively. The results indicated that the essential oil and extracts obtained from *F. orientalis* exhibited potential DPPH radical scavenging activity. The radical scavenging activity of the extracts and essential oil obtained from *F. orientalis* was determined by radical cation (ABTS) according to the reported procedure (Wu et al., 2009). In our study, the scavenging ability of the essential oil and extracts on the ABTS free radical is shown in Table 4. In this reaction system, the ABTS scavenging activities of methanol, acetone, chloroform, hexane, water extracts and essential oil were determineted to be 8.1, 12.4, 9.7, 14.2, 6.2 and 0.67 (ABTS $IC_{50}$ (g/l)), respectively. The results showed that the acetone extract had the highest ABTS free radical scavenging activity with 12.4 (ABTS $IC_{50}$ (g/l)).

### *3.5.* Determining the Amount of Total Phenolic Compounds

The amounts of total phenolic compounds in the extracts were determined according to the Folin-Ciocalteu procedure (Singleton et al., 1999). The results (Table 4) show that the total phenolic compounds in *F. orientalis* extracts ranged from 4.21 (mg GAE/g) to 15.13 and the highest amount of phenolic compounds was found in the water extract. In our study, we determined the antioxidant capacity and total phenolic content of extracts obtained from *F. orientalis* extracts, which have not been studied before. The results obtained are similar when the antioxidant capacities of the extracts are determined by different tests. The results of the investigation show that the higher the concentration of total phenolic compounds the lower the amount of residual DPPH and $ABTS^{+}$ radical cation, the higher the free radical scavenging activity.

**Table 4.** The antioxidant capacity and total phenolic compounds of *Ferula orientalis*.

| Antioxidants | ABTS $IC_{50}$ (g/l) | DPPH $IC_{50}$ (g/l) | Total Phenolic Compounds (mg GAE/g) |
|---|---|---|---|
| *F. orientalis* water | 6.2 | 18.3 | 15.13±3.82 |
| *F. orientalis* Acetone | 12.4 | 24.2 | 6.37±0.98 |
| *F. orientalis* methanol | 8.1 | 23.3 | 9.62±1.23 |
| *F. orientalis* hexane | 14.2 | 17.2 | 4.21±0.88 |
| *F. orientalis* chloroform | 9.7 | 19.6 | 6.12±0.83 |
| *F. orientalis* essantial oil | 0.67 | 0.88 | - |
| Bütilhidroksitoluen (BHT) | $6x10^{-2}$ | $9x10^{-2}$ | - |
| α-tocopherol | $2.8x10^{-3}$ | $4.7x10^{-3}$ | - |
| Ascorbic acid | $1.6x10^{-3}$ | $1.4x10^{-3}$ | - |

### *3.6.* Thiobarbituric Acid Reactive Species (TBARS) Assay

The ability of *F. orientalis* essential oil to act as a radical scavenger was investigated in conjunction with α-tocopherol and BHT. As shown in Table 5, the antioxidant capacity of *F. orientalis* essential oil, BHT and α-tocopherol increased respectively.

**Table 5.** Antioxidant activity of *Ferula orientalis* essential oil, α-Tocopherol and BHT measured by TBARS method.

| Antioxidants | Antioxidant Index (AI%) | | |
|---|---|---|---|
| | 100 ppm | 500 ppm | 1000 ppm |
| *F. orientalis* essential oil | 12.3± 3.2 | 18.4±4.4 | 27.8±6.2 |
| BHT | 26.1±2.2 | 30.4±3.3 | 38.3±7.6 |
| α-tocopherol | 62.8±5.7 | 68.9±9.8 | 73.1±7.8 |

### *3.7.* Determination of Antioxidant Activity with the β-Carotene Bleaching (BCB) Test

BCB method based on the measurement of β-carotenoids yellow colour due to the reaction with radicals was formed by oxidation of linoleic acid in an emulsion. The bleaching rate of β-carotene decreases in the presence of antioxidants. Figure 1 shows the decrease in absorbance of β-carotene in the presence of the *F. orientalis* essential oil. The antioxidant effects of BHT and *F. orientalis* essential oil were compared in Figure 2. It can be seen that the bleaching of β-carotene is prevented in the presence of essential oil and BHT. It was observed that the essential oil is effective even when compared to a very strong antioxidant BHT (Figure 2).

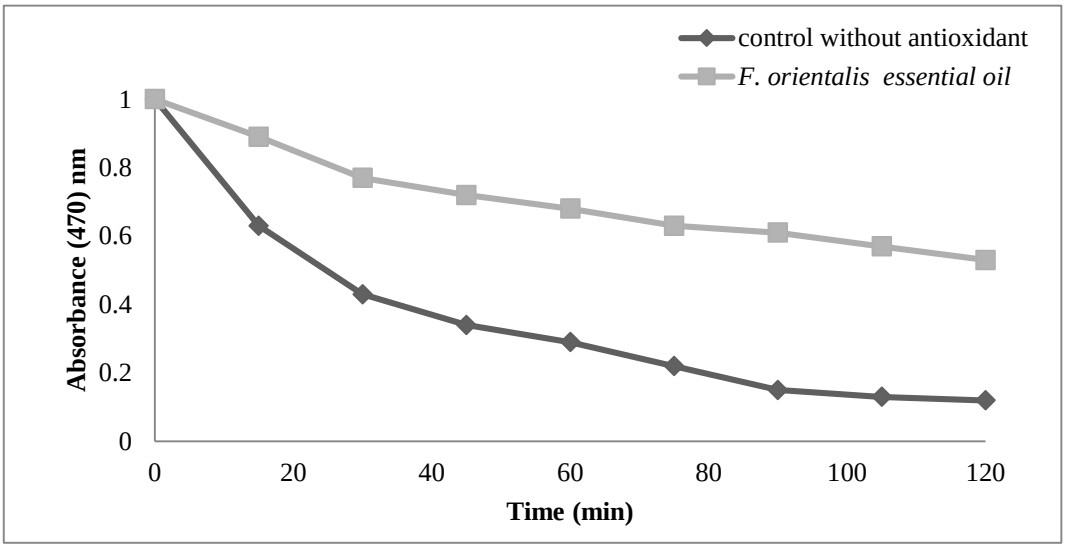


**Figure 1.** β-Carotene bleaching activity of *Ferula orientalis* essential oil.

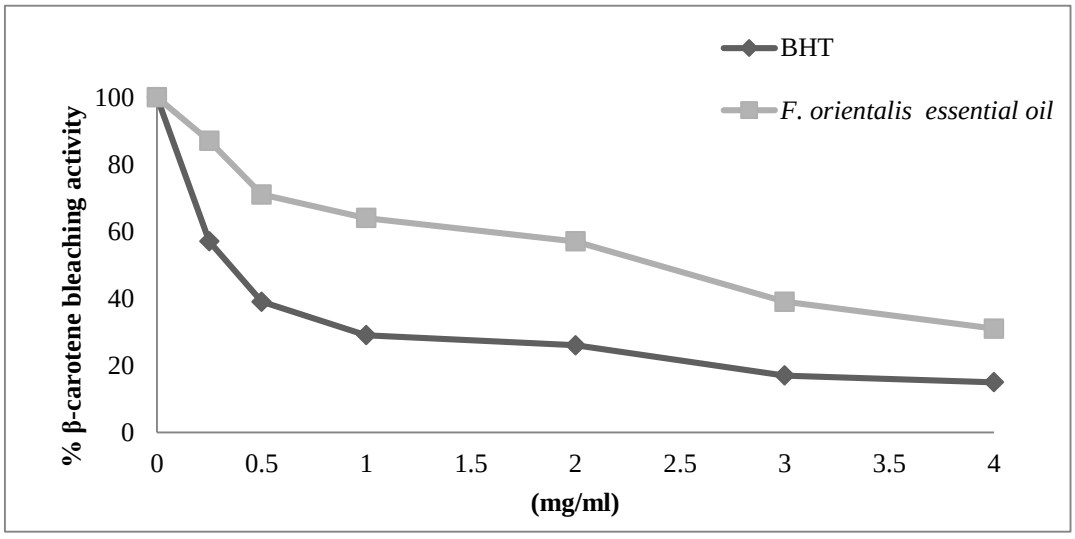


**Figure 2.** Antioxidant activity of the *Ferula orientalis* essential oil with the β-Carotene bleaching (BCB) test.

As a result, the most important feature of this study is that it is the first study in which the essential oil and extracts obtained from the *F. orientalis* plant, naturally grown in Türkiye and other regions of the world, have been used against the eight soft

rot *C. indologenes* isolates. The successful results obtained from the essential oil of the plant used in the study are also the first results obtained for this group of pathogens. The fact that the antibiotics used in the study had no effect and that the essential oil was lethal to all strains has increased the importance of the work. This is evidence that the pathogens have become more resistant to antibiotics over time.

## 5. Conclusion and Recommendations

The results obtained from this study include conclusions and recommendations to organic farming, and sustainable agricultural systems that are increasingly important in recent years. In addition, the concentrations that are effective from the results obtained will be tested in practical applications, and if the expected results are obtained, the targeting and marketing of products intended for use in the control of these pathogens will be targeted.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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