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Chemical composition, antibacterial and antifungal activities of seed essential oil of Ferulago angulata

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Abstract
The aim of this study was to determine the chemical composition and the in vitro antimicrobial effects of seed essential oil of Ferulago angulata. The oil analyses by GC and GC/MS resulted in the identification of 39 compounds representing 91.07% of the oil. The major constituents were (Z)-β-ocimene (19.93%), α-pinene (15.50%), p-cymene (7.67%), sabinene (7.49%), β-phellandrene (5.5%), and α-phellandrene (4.95%). The oil was also screened for its antimicrobial properties against six bacteria (Erwinia amylovora, Xanthomonas oryzae, Pseudomonas syringae, Pectobacterium carotovorum, Ralstonia solanacearum, Bacillus thuringiensis) and six fungi (Alternaria alternata, Culvularia fallax, Macrophomina phaseolina, Fusarium oxysporum, Cytospora sacchari, Colletotrichum tricbellum). According to the results of antibacterial activity, B. thuringiensis (with 8 µL mL\(^{-1}\) minimal inhibitory concentration (MIC) and 15 µL mL\(^{-1}\) minimum bactericidal concentration (MBC)) was the most sensitive bacterium; P. carotovorum and R. solanacearum (with 20 µL mL\(^{-1}\) MIC and 30< MBC) were the most resistant bacteria. Additionally, a broad differentiation against all of the tested fungi showed that the most susceptible and resistant fungi after 6 days at the highest concentration (800 µL L\(^{-1}\)) were F. oxysporum (100.0 ± 0.00%) and C. tricbellum (52.50 ± 1.67%) of growth inhibition, respectively.

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Keywords
Ferulago angulata; Biological activity; (Z)-β-Ocimene; α-Pinene; Phytopathogens

Introduction
Apiaceae (Umbelliferae) family comprises 300 genera and 2500–3000 species distributed in most parts of the world. The genus Ferulago belongs to this family consisting of 35–40 species in the world which are centred in southwestern Asia, and 7 of them are endemic in Iran, including Ferulago contracta, Ferulago angulata, Ferulago macrocarpa, Ferulago galbanifera, Ferulago trachycarpa, Ferulago phialocarpa, and Ferulago carduchrom.\(^{[1,2]}\) F. angulata (Schlecht.) Boiss. (referred to locally as ‘Chavir’ or ‘Chavil’ in Iran) is an endemic natural and native plant in western Iran.\(^{[1]}\) Traditionally, the aerial parts of the plant added to diary and edible oil by natives to prevent from the oils expiration besides adding a pleasant taste to it.\(^{[3]}\) Results of an ethnobotanical study\(^{[4]}\) indicated the aerial parts of F. angulata have been used as antiseptic, spice, and air fresher by the people of Abdanan and Dehloran districts, western Iran. Furthermore, it has been used in folk medicine as sedative, tonic, digestive, carminative, vermifuge, aphrodisiac, anti-oxidation, anti-diabetics, anti-parasitic, and for the treatment of chronic ulcers, snakebites, intestinal worms, haemorrhoids, headache, and diseases of the spleen.\(^{[5,6]}\)
Previous phytochemical studies of some species of the genus Ferulago have led to isolation of volatile oils from aerial parts of these plants which contain a variety of components with different therapeutic effects such as digestion diseases, different pains, and the subject of some earlier studies were the scant biological activity investigations. \cite{5-8} Also, the essential oils of several species in the genus Ferulago have been studied from an antimicrobial point of view. \cite{2,10}

The extraction of F. angulata essence has already been done through hydro-distillation and extraction by solvent. Its major chemical compounds have been identified and their percentages were different. \cite{2,3,7,11} The F. angulata oils predominantly included ferulagone, β-hydroxy-13-epi-manoyl oxide, α-pinene, 2,5-dimethoxy-p-cymene, p-cymene, methyl carvacrol, trans-chrysanthenyl acetate, γ-terpinene, myrcene, (Z)-β-octimene, terpinolene, 2,4,5-trimethyl-benzaldehyde, and α-phellandrene. \cite{7,12,13} The parts of the plant, its stage of development, and geographical origin can significantly influence the composition of the oils obtained from the species. \cite{14,15}

The control of plant disease is a considerable problem in agriculture practice. Plant pathogens include fungi and bacteria and can cause diseases or damages in plants, also being responsible for significant losses of crop production. \cite{16,17} For many years, a variety of different synthetic chemicals such as benzimidazoles, aromatic hydrocarbons, and sterol biosynthesis inhibitors have been used as antifungal agents to inhibit the growth of plant pathogenic fungi. \cite{18} Moreover, many phytopathogenic bacteria have acquired resistance to synthetic pesticides. \cite{19} Therefore, there is an increasing interest in the antimicrobial activities of some natural composition, especially those found in medicinal plants, which may play a role in preventing various diseases. Despite the high degree of activity shown by these plants against phytopathogens, antimicrobial investigation of aromatic plants has been limited. \cite{20} Among the plant products, essential oils have been widely investigated for their toxicities against various fungi, bacteria, and insects. \cite{21} Essential oils are natural mixtures of complex compounds formed by several volatile compounds, mainly monoterpenoids and sesquiterpenoids \cite{22}, which are characterized by a strong odour and play an important role in the protection of the plants against bacteria, virus, fungi, insects, herbivores, and as attractant to pollinators. Moreover, they are used in agriculture and food industries as food preservers, additives, and natural remedies, owing to their notable antimicrobial and antioxidant properties. \cite{23} Owing to the increasing incidence of adverse side effects associated with conventional synthetic antimicrobial poisons, essential oils may therefore constitute effective organic alternatives to synthetic compounds of chemical composition in agro-industries. \cite{24,25}

Considering the capability of plant essential oils for substituting synthetic antimicrobial products, the study of potential of F. angulata can be useful for industrial application of this plant as a natural product. Therefore, the objective of the present work was to characterize the chemical composition of the F. angulata seed oil, and investigate and compare the antimicrobial effects of the oil against some phytopathogenic bacteria and fungi for the first time.

**Materials and methods**

**Plant material**

F. angulata seeds were harvested from the plants grown in Kohgiluyeh va Boyer Ahmad province, southwestern of Iran. One herbarium sample of plant was collected and a voucher specimen of plant was deposited at the Herbarium of the Faculty of Sciences, Isfahan University, Isfahan, Iran.

**Isolation of the essential oil**

Essential oil was isolated from the dried seeds of the plants that were subjected to hydrodistillation for 4 h using a Clevenger-type apparatus. The essential oil was collected over water, separated, and dried over anhydrous sodium sulphate and stored in sealed vials at 4°C until chemical analysis and antimicrobial studies.
**Analysis of essential oil**

**GC and GC-MS analysis**
Composition of the essential oils was determined by GC and GC-MS. GC analysis was done on an Agilent Technologies 7890 GC equipped with FID and a HP-5MS 5% capillary column (30.00 m × 0.25 mm, 0.25 µm film thicknesses). The carrier gas was helium at a flow of 0.8 mL min⁻¹. Initial column temperature was 60°C and programmed to increase at 4°C min⁻¹ to 280°C. The split ratio was 40:1. The injector temperature was set at 300°C. The purity of helium gas was 99.999% and 0.1 µL samples were injected manually in the split mode. GC-MS analysis was done on the mentioned Agilent Technologies 5975 Mass system. Mass spectra were recorded at 70 eV. Mass range was from m/z 50–550.

**Compounds identification**
Constituents were identified by comparison of their Kovats index (KI) relative to C₅–C₂₄ n-alkanes obtained on a nonpolar HP-5 MS column by comparison of the KI, provided in the literature, by comparison of the mass spectra with those recorded by the NIST 08 (National Institute of Standards and Technology) and Willey (ChemStation data system). The individual components were identified by retention indices and compared with constituents known from the literature.[26,27] The percentage composition was computed from the GC peak areas without using any correction factors.

**Antifungal activity**

**Fungal species**
Six phytopathogenic fungi, including *Alternaria alternata* strain C1445, *Culvularia fallax* strain I21, *Macrophomina phaseolina* strain M21, *Fusarium oxysporum* strain 104, *Cytospora sacchari* strain 125, and *Colletotrichum tricbellum* strain 108, were obtained from the collection of Department of Plant Diseases, Tehran University, Iran.

**Antifungal activity assays**
Activities of oil were tested against six fungi pathogens above using a range of different concentration (100, 200, 400, and 800 µL L⁻¹) with two different methods in four replicates.

**Agar dilution method**
The oil was dissolved in ethyl alcohol and 5% Tween 80, and added to the culture medium at a temperature of 40–45°C, then poured into Petri dishes (90 mm). The fungi were inoculated as soon as the medium had solidified. Discs (5 mm) of mycelia material, taken from the edge of 7-day-old fungal cultures, were placed at the centre of each Petri dish. The controls set were prepared similarly by inoculating fresh medium with ethyl alcohol + 5% Tween 20 and aqueous solutions were used as second controls. The Petri dishes with the inoculum were placed in incubator under controlled temperature condition of 25 ± 2°C. The efficacy of treatments was evaluated after 3 and 7 days. The percentage of inhibition of mycelia growth was calculated from the mean values of colony diameter of treated and control (ethyl alcohol + 5% Tween 80).

**Disc diffusion method**
Sterile filter paper discs (7 cm diameter) soaked in 100, 200, 400, or 800 µL L⁻¹ pure essential oil were placed on the inner surface of the Petri dish lid. The dishes were sealed with parafilm and incubated upside-down at 25°C. Colony radius (in millimetre) was measured at third and sixth days. Control consists of sterile distilled water-soaked filter paper in the lid of Petri dish. The percentage of inhibition of mycelia growth was calculated from the mean values of colony diameter of treated and control.
Antibacterial activity assays

Microbial strains and growth conditions

Five Gram-negative bacteria (including *Erwinia amylovora* strain BPD, *Xanthomonas oryzae* strain IR42, *Pseudomonas syringae* strain 32, *Pectobacterium carotovorum* strain 863, *Ralstonia solanacearum* strain 145) and a Gram-positive bacterium (*Bacillus thuringiensis* strain KD2) were obtained from the collection of Department of Plant Diseases, Ferdowsi University, Iran. All strains were maintained as stock strains in 25% glycerol in Eppendorf microtubes and kept at −70°C until use. Bacteria strains were grown in nutrient broth at 28°C for 24 h and adjusted to approximately 1 × 10^6 CFU mL⁻¹.

Disc diffusion assay

The antibacterial activity of *F. angulata* seed essential oil was tested against six phytopathogens by disc diffusion method. Whatman No.1 sterile filter paper discs (6 mm diameter) were impregnated with defined concentrations of essential oil (40 µL/disc), prepared in 1% DMSO. The discs were allowed to dry for 15 min and placed on the inoculated agar. The standard reference drug, ampicillin (40 µL/disc), was used as a positive control. Negative controls were prepared using the same solvents employed to dissolve the samples. The plates were incubated at 28°C for 24 h. The degree of the essential oil activity is revealed by the size of inhibition zone that is expressed by the diameter of the inhibition zone (in millimetre) and usually the diameter of the disc is included. Each assay in this experiment was replicated three times. [28]

MIC and MBC assay

In order to determine the minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) by microtitre plates, bacteria were cultured overnight at 28°C. The oils were dissolved in 1% DMSO. Dilutions were prepared in a 96-well microtitre plates to get final concentrations from 0 to 30 µL mL⁻¹. Finally, 40 µL of inoculums (10^6–10^7 CFU mL⁻¹) was inoculated onto the microplates and the tests were performed in a volume of 200 µL. Plates were incubated at 28°C for 24 h. The antibiotic, gentamicin/ampicillin, was used as a positive control for the tested plant pathogenic bacteria. The lowest concentrations of tested samples, which did not show any visual growth after macroscopic evaluation, were determined as MICs, which were expressed in µL mL⁻¹. Using the results of the MIC assay, the concentrations showing complete absence of visual growth of bacteria were identified and 50 µL of each culture broth was transferred onto the agar plates and incubated for the specified time and temperature as mentioned above. The complete absence of growth on the agar surface in the lowest concentration of sample was defined as the MBC. [29] For more accurate determination of MIC, all the bacterial cultures were co-cultivated with various concentrations of oil in 5 mL of nutrient broth medium (in higher volume for less error). After the specified incubation period (28°C, for 24 h), 0.1 mL of cultures from all the test tubes were placed on nutrient agar medium to find out the MIC. [29] All the experiments were done in triplicate.

Statistical analysis

In the disc sensitivity test, zones of inhibition were measured millimetres with a centimetre rule. Data on inhibitory effect of essential oils on mycelia growth were subjected to analysis of variance using SPSS 21. The means comparisons were made using Duncan’s multiple-range tests at p ≤ 0.05. All data are presented as mean values ± standard deviation (SD).

Results and discussion

Oil analysis

The essential oil from *F. angulata* seed was obtained in the yield of 0.7% (v/w). The chemical composition of the oil was examined by GC and GC/MS. The major components of the oil are
presented in Table 1. In total, 39 compounds representing 91.07% of the oil have been identified. The oil consisted mainly of monoterpene hydrocarbons (77.04%), oxygenated monoterpenes (10.86%), sesquiterpene hydrocarbons (1.05%), oxygenated sesquiterpenes (0.71%), and other components (0.22%), respectively. The major constituents were (Z)-β-ocimene (19.93%), α-pinene (15.50%), p-cymene (7.67%), sabinene (7.49%), β-phellandrene (5.50%), and α-phellandrene (4.95%).

Based on the literature surveys, the species of Ferulago genus are rich in essential oil. Earlier studies have been shown that aerial parts of F. angulata plant including stems, leaves, flowers, and

| No | Component                  | RT<sup>a</sup> | RI<sup>b</sup>     | Percentage |
|----|----------------------------|-----------------|--------------------|------------|
| 1  | Hexane                     | 1.45            | 603.23             | 1.19       |
|    | **Monoterpene hydrocarbons** |                |                    | **77.04**  |
| 2  | α-Thujene                  | 3.96            | 929.20             | 0.58       |
| 3  | α-Pinene                   | 4.20            | 941.35             | 15.50      |
| 4  | Camphene                   | 4.37            | 949.92             | 0.56       |
| 5  | Verbenene                  | 4.47            | 955.67             | 0.22       |
| 6  | Sabinene                   | 4.84            | 974.17             | 7.49       |
| 7  | β-Pinene                   | 4.92            | 978.25             | 1.18       |
| 8  | Myrcene                    | 5.12            | 988.36             | 1.96       |
| 9  | α-Phellandrene             | 5.51            | 1006.09            | 4.95       |
| 10 | Δ-3-Carene                 | 5.65            | 1011.39            | 1.02       |
| 11 | α-Terpine                  | 5.78            | 1016.31            | 1.12       |
| 12 | p-Cymene                   | 6.10            | 1028.46            | 7.67       |
| 13 | β-Phellandrene             | 6.18            | 1031.53            | 5.30       |
| 14 | Limonene                   | 6.20            | 1032.34            | 2.76       |
| 15 | (Z)-β-Ocimene              | 6.31            | 1036.26            | 19.93      |
| 16 | (E)-β-Ocimene              | 6.56            | 1045.80            | 0.89       |
| 17 | γ-Terpine                  | 6.84            | 1056.43            | 3.33       |
| 18 | α-Terpinolene              | 7.51            | 1081.68            | 2.38       |
|    | **Oxygenated monoterpenes** |                |                    | **10.86**  |
| 19 | Linalool                   | 7.98            | 1099.51            | 1.12       |
| 20 | Terpin-4-ol                | 10.23           | 1172.70            | 4.34       |
| 21 | α-Terpineol                | 10.65           | 1186.25            | 1.18       |
| 22 | Citronellol                | 11.88           | 1225.13            | 1.17       |
|    | **Esters**                 |                |                    |            |
| 23 | Borneol acetate            | 13.69           | 1281.51            | 2.10       |
| 24 | Thymol                     | 13.89           | 1287.81            | 0.11       |
| 25 | Carvacrol                  | 14.19           | 1297.04            | 0.40       |
| 26 | Methyl eugenol             | 17.43           | 1401.52            | 0.44       |
|    | **Sesquiterpene hydrocarbons** |            |                    | **1.05**   |
| 27 | α-Cubebene                 | 16.41           | 1368.83            | 0.04       |
| 28 | β-Elemene                  | 16.94           | 1385.82            | 0.07       |
| 29 | Germacrene B               | 21.81           | 1548.05            | 0.12       |
| 30 | β-Caryophyllene            | 17.76           | 1412.24            | 0.27       |
| 31 | γ-Elemene                  | 18.20           | 1426.68            | 0.04       |
| 32 | Germacrene D               | 19.63           | 1474.23            | 0.11       |
| 33 | β-Selinene                 | 19.77           | 1478.79            | 0.10       |
| 34 | Bicyclogermacrene          | 20.08           | 1489.18            | 0.22       |
| 35 | Δ-Cadinene                 | 20.92           | 1516.73            | 0.08       |
|    | **Oxygenated sesquiterpenes** |            |                    | **0.71**   |
| 36 | Elemol                     | 21.69           | 1542.40            | 0.24       |
| 37 | Spathulenol                | 22.45           | 1569.77            | 0.39       |
| 38 | Caryophyllene oxide        | 22.60           | 1574.86            | 0.08       |
|    | **Others**                 |                |                    | **0.22**   |
| 39 | cis-Jasmonic acid          | 17.19           | 1393.77            | 0.22       |
|    | **Total**                  |                |                    | **91.07**  |

<sup>a</sup>Retention time (min).
<sup>b</sup>Retention indices (RI) relative to C<sub>5</sub>–C<sub>24</sub> n-alkanes on HP-5 MS capillary column.
seeds contain essential oil. Chemical compounds and biological activity of some species from the genus *Ferulago* have been the subject of preceding studies. The reports on the chemical compositions of the oils isolated from the aerial parts *F. angulata* are abundant as most of these investigations showed patterns of oil compositions similar to our present study. Although differences can be observed in the percentage distribution, in conformity with our findings (Z)-β-ocimene and α-pinene were dominant oil compounds in an earlier investigation. Moreover, regarding the previously reported contents of the aerial parts, oil of the plant consists of monoterpenes as a major constituent which is in conformity with our results. On the other hand, our findings differ from those reported in some investigations, in which other components such as α-phellandrene and β-phellandrene, suberosin, spathulenol, trans-β-caryophyllene, arcurcumene, and bicyclogermacrene, α-pinene, limonene, β-myrcene, and fenchyl acetate were the major compounds. A qualitative comparison of the oil constituents of *F. angulata* from different studies indicated significant differences in varying compositions, while some components that were not detected in our sample have been reported as the main compounds in others. This variation could have aroused from this fact that the plants used in each study were collected from different regions. Also, another important factor was the harvesting time. Previous investigations showed that the oil yield and its chemical composition were under various factors including species and genotype, ecological conditions, growth stage, and extraction methods.

**Antifungal assay**

The antifungal effect of *F. angulata* oil against six tested plant pathogenic fungi at different concentrations with two methods is shown in Table 2. Our findings indicated that all of the tested fungi could significantly inhibit fungal growth on all concentrations compared to the control and exhibited a moderate to high antifungal activity. According to the per cent of mycelia growth inhibition for each fungus, a difference between the resistance against the tested fungi in relation to the oil concentrations and times can be observed.

**Agar dilution method**

The inhibition of mycelia growth percentage by agar dilution method is shown in Table 2. As mentioned in our results, various concentrations had different effects on each fungus. In this method, all fungi were controlled by four concentrations of the oil. The oil exhibited antifungal activity at 100, 200, 400, and 800 µL L\(^{-1}\) concentrations against the tested fungi with various mycelia growth due to the concentrations and times from 17.6 ± 2.50 to 100 mean values. Our data indicated that *F. angulata* seed essential oil on all concentrations except at 100 µL L\(^{-1}\) could completely (100%) inhibit the *F. oxysporum* growth. Moreover, the weakest effect of the oil was observed against *C. tricbellum*. So, the most susceptible and the most resistant fungi at the highest concentration (800 µL L\(^{-1}\)) after 6 days were *F. oxysporum* and *C. tricbellum*, respectively (Table 2).

**Disc diffusion method**

The per cent inhibition of mycelia growth by disc diffusion method is indicated in Table 2. A broad differentiation in the antifungal properties of essential oil against various fungi was observed. The oil exhibited antifungal activity at 100, 200, 400, and 800 (µL L\(^{-1}\)) concentrations against the tested fungi with inhibition of mycelia growth percentage from 23.60 ± 2.10 to 100 in different concentrations and times. At the highest oil concentration (800 µL L\(^{-1}\)), *C. tricbellum* showed a greater tolerance and *F. oxysporum* indicated the major susceptibility within the selected concentration range. So, in this concentration the most susceptible fungus was *F. oxysporum* and the most resistant fungus was *C. tricbellum* with 100.0 ± 0.00 and 52.5 ± 1.67% inhibition of mycelia growth, respectively. Furthermore, after 3 days the essential oil at 400 and 800 µL L\(^{-1}\) concentrations completely inhibited from *C. sacchari* growth. In this method completely fungistatic activities of the oil in all concentrations were observed only on *F. oxysporum*. According to the results, the various concentrations had different effects on each
Table 2. Antifungal activity of the essential oil from seeds of *Ferulago angulata* by different methods.

| Fungi               | Concentration (µL L\(^{-1}\)) | 3rd Day   | 6th Day   | 3rd Day   | 6th Day   |
|---------------------|-------------------------------|-----------|-----------|-----------|-----------|
|                     |                               | Agar dilution | Disc diffusion | |
| *Alternaria alternate* | 100                           | 51.90 ± 2.72 | 39.40 ± 1.43 | 63.50 ± 3.80 | 51.50 ± 4.38 |
|                     | 200                           | 57.80 ± 8.46 | 37.10 ± 4.13 | 72.20 ± 1.57 | 59.20 ± 2.46 |
|                     | 400                           | 59.10 ± 7.68 | 35.40 ± 3.07 | 72.30 ± 2.75 | 66.95 ± 1.06 |
|                     | 800                           | 75.50 ± 4.05 | 55.80 ± 4.74 | 90.50 ± 1.92 | 75.83 ± 1.90 |
| *Macrophoma phaseolina* | 100                           | 41.93 ± 5.03 | 30.64 ± 6.65 | 75.21 ± 3.36 | 67.22 ± 1.43 |
|                     | 200                           | 49.31 ± 5.85 | 39.17 ± 4.38 | 75.69 ± 4.31 | 70.83 ± 4.66 |
|                     | 400                           | 73.04 ± 4.99 | 47.42 ± 5.63 | 88.26 ± 1.47 | 72.78 ± 4.67 |
|                     | 800                           | 76.94 ± 4.75 | 63.33 ± 3.51 | 88.68 ± 2.98 | 75.28 ± 2.47 |
| *Culvularia fallax*  | 100                           | 54.17 ± 8.25 | 39.44 ± 3.79 | 74.65 ± 2.33 | 66.11 ± 2.31 |
|                     | 200                           | 57.18 ± 5.61 | 22.73 ± 5.10 | 77.64 ± 1.82 | 71.39 ± 1.06 |
|                     | 400                           | 74.44 ± 7.53 | 46.29 ± 3.98 | 87.15 ± 2.30 | 72.22 ± 5.52 |
|                     | 800                           | 75.83 ± 4.29 | 63.05 ± 2.46 | 88.75 ± 2.66 | 76.39 ± 1.40 |
| *Cytospora sacchari* | 100                           | 31.50 ± 8.30 | 23.90 ± 4.15 | 79.40 ± 3.52 | 64.70 ± 4.29 |
|                     | 200                           | 62.90 ± 3.46 | 51.20 ± 5.66 | 89.90 ± 1.04 | 73.30 ± 3.27 |
|                     | 400                           | 88.60 ± 2.60 | 61.20 ± 0.93 | 100.0 ± 0.00 | 81.40 ± 2.46 |
|                     | 800                           | 100.0 ± 0.00 | 72.50 ± 1.89 | 100.0 ± 0.00 | 85.00 ± 3.80 |
| *Fusarium oxysporum* | 100                           | 64.40 ± 4.05 | 61.80 ± 4.60 | 100.0 ± 0.00 | 100.0 ± 0.00 |
|                     | 200                           | 100.0 ± 0.00 | 100.0 ± 0.00 | 100.0 ± 0.00 | 100.0 ± 0.00 |
|                     | 400                           | 100.0 ± 0.00 | 100.0 ± 0.00 | 100.0 ± 0.00 | 100.0 ± 0.00 |
|                     | 800                           | 100.0 ± 0.00 | 100.0 ± 0.00 | 100.0 ± 0.00 | 100.0 ± 0.00 |
| *Colletotrichum tricbellum* | 100                           | 35.40 ± 2.41 | 17.60 ± 2.50 | 37.08 ± 4.99 | 23.60 ± 2.10 |
|                     | 200                           | 35.40 ± 3.16 | 22.90 ± 3.88 | 46.04 ± 5.91 | 33.90 ± 3.21 |
|                     | 400                           | 44.80 ± 2.17 | 31.11 ± 3.95 | 54.03 ± 4.03 | 42.30 ± 4.73 |
|                     | 800                           | 58.80 ± 3.10 | 47.80 ± 2.40 | 64.60 ± 3.36 | 52.50 ± 1.67 |

*a* Data are mean ± SD.
fungus. In contrast, in all cases with rise in the concentration of the oil, the zones of growth inhibition increased. Also about all fungi with passing the time the resistances were increased. Overall, the antifungal activity of the essential oil is related to the respective composition of the herbal essential oil, the structural configuration of the constituent components, and their functional groups and possible synergistic interactions between components.\textsuperscript{[34]}

The comparison between two methods at the highest concentration (800 µL L\textsuperscript{-1}) of the oil indicated that disc diffusion was more effective to assay the percentage of growth inhibition of all of the tested fungi after 3 and 6 days (Figs. 1 and 2). Therefore, all of the tested fungi were shown to be more resistant in agar dilution method. Although after 3 days there were no significant differences between two methods about \textit{C. sacchari} and \textit{F. oxysporum}, after 6 days \textit{C. sacchari} showed lower percentage of growth inhibition by agar dilution method. So, \textit{C. sacchari} was more resistant in this method. In total, according to the applied methods, the susceptibility and resistance of the tested fungi were different (Figs. 1 and 2).

Previous studies on the analysis and antifungal activity of essential oil of some species of different plants genera have shown that they have various degrees of growth inhibition effects against some phytopathogenic fungal species.\textsuperscript{[35]} Expression of different levels of antifungal activity may be due to the differences in the content of known antimicrobial compounds in each essential oil. In recent years, several researchers have reported that monoterpenes and sesquiterpene hydrocarbons and their oxygenated derivatives are the major components of essential oils of plant origin, which have enormous potential to strongly inhibit microbial pathogens.\textsuperscript{[2]} In the preceding literature, the antifungal activity of \textit{p}-cymene, \textit{α}-pinene, linalool, terpinen-4-ol, 4-terpineol, \textit{α}-terpineol, \textit{γ}-terpinene, myrcene, \textit{β}-caryophyllene, and \textit{β}-pinene has been reported.\textsuperscript{[36]} This inhibition effect of the essential oil against the fungal species may be due to the presence of a relatively high proportion of oxygenated monoterpenes in the oil. It is also possible that the minor components might be involved in some type of synergism with the other active compounds.\textsuperscript{[34]}

In addition, some earlier papers on the analysis and antifungal properties of the essential oils of some species of various genera have shown that they have varying degrees of growth inhibition effects against some \textit{Fusarium}, \textit{Botrytis}, and \textit{Rhizoctonia} species due to their different chemical compositions.\textsuperscript{[35–37]} The strong antifungal activity of \textit{Cuminum cyminum} oil against \textit{F. solani} and \textit{F. oxysporum} was reported earlier which was related to main components, such as \textit{α}-pinene, 1,8-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Effect of antifungal activity methods on per cent of growth inhibition different fungi after 3 days at the highest concentration (800 µL L\textsuperscript{-1}).}
\end{figure}
cineole, and linalool. It is necessary to mention that there is a direct relationship between inhibitory effects of essential oil on fungal growth and fusariotoxins production.

**Antibacterial assay**

In present work, it was aimed to examine the *in vitro* antibacterial activity of the *F. angulata* seed oil against six different phytopathogenic bacteria. It was evaluated against a Gram-positive (*B. thuringiensis*) and five Gram-negative (*E. amylovora, X. oryzae, P. syringae, P. carotovorum*, and *R. solanacearum*) phytopathogenic bacteria which were morphologically and physiologically different and their potency were assessed qualitatively and quantitatively by the presence inhibition zones, MIC, and MBC values (Fig. 3 and Table 3).

The oil exhibited antibacterial activity against all of the tested strains, but in variable degrees (Fig. 3). The inhibition zones for bacterial strains sensitive to the oil were in range of 2–5 mm. The data indicated that the highest activity of the oil was observed against *B. thuringiensis* with the strongest inhibition zones (5 mm). *E. amylovora* among the Gram-negative bacteria was the most sensitive strain tested. Also, *P. carotovorum* and *R. solanacearum* were the most resistant bacteria to the oil with a weak inhibition zones (Fig. 3). The resistance of the bacteria might be derived from the presence of hydrophilic properties of their impermeable outer membrane to lipophilic compounds such as oils. Essential oils are phytochemical complex blends of different aromatic components with different lipophilic and hydrophilic properties influencing the antimicrobial property in respect of its dilution in the tested medium.

Furthermore, in this investigation the bacteriostatic and bactericidal effectiveness of the oil estimated by MIC and MBC, respectively, are shown in Table 3. Although the oil showed activity on all of the tested bacteria, the results indicated a high variation of MICs and MBCs. The MIC and MBC values for bacterial strains sensitive to the plant products were in the range of 8–20 and 15 to >30 µL mL⁻¹, respectively. The seed essential oil had the most bactericidal and bacteriostatic properties against the Gram-positive bacterium, *B. thuringiensis*. Moreover, the seed essential oil had the lowest MIC and MBC against *E. amylovora* and *X. oryzae* strains, among the Gram-negative bacteria. According to the results, *B. thuringiensis* was the most sensitive bacterium to the oil (8 µL mL⁻¹ MIC). In addition, *P. carotovorum* and *R. solanacearum* were the most resistant bacteria (Table 3).
The antibacterial activities of several medicinal plants and their oils have been investigated in several studies and could be attributed to the major components. All of the Apiaceae family plants are aromatic that are known to possess antimicrobial properties, particularly due to their essential oils and can produce monoterpene, sesquiterpene, and phenyl components and related resins in their secretory ducts, roots, stem, leaf, flowers, and seeds and fruits.[2] The antimicrobial efficacy of essential oils is the consequence of interaction between the minor and major components and conditioned by the activity of their components.[34]

The existence of some antimicrobial constituents such as linalool,[23], α-terpinene, p-cymene,[14,41], and terpinen-4-ol[42] combined with other minor constituents might be involved in improving the overall antimicrobial activity of essential oils. According to our results, the *Ferulago angulata* seed oil contains more monoterpene hydrocarbons including (Z)-β-ocimene, α-pinene, p-cymene, and sabi- nene. In the previous literature, the antibacterial and antifungal effects of α-pinene, β-pinene, 4-terpineol, α-terpineol, and Caryophyllene oxide have been reported.[14] Referring to the literature, the antimicrobial activity of essential oil may be due to monoterpene hydrocarbons.[2] The stronger antimicrobial activity of the essential oil is likely due to the presence of these mentioned components. Results of our study indicated that p-cymene might be related to a synergistic phenomenon. Our findings as well as literature data allow us to suggest that constituents such as linalool, terpinen-4-ol, and 1,8-cineole, alongside oxygenated sesquiterpenes, might contribute to antimicrobial activity agree with earlier reports.[23,42,43-44] Although these compounds are not abundant in essential oil,
their activity is important. It is necessary to signal that other components can contribute to improve this activity.

A number of *Ferulago* species have previously been studied for their essential oil compositions, biological activity, and the antimicrobial activity.\(^{[12]}\) Some of these compounds have both antibacterial and antifungal activities.\(^{[3,13]}\) Moreover, the antibacterial and antifungal activities have previously been investigated for some *Ferulago* species as *Ferulago asparagifolia*, *F. galbanifera*, *Ferulago humilis*, *F. trachycarpa*, *Ferulago thrysiflora*, *Ferulago sylvatica*, *Ferulago bernardii*, *Ferulago nodosa*, *Ferulago longistylis*, and *Ferulago angulata* subsp. *carduchorum* and inhibitory effects for microorganisms have been observed.\(^{[2,10]}\) The essential oil of *F. angulata* showed stronger antimicrobial activity than other species.\(^{[45]}\) In a study, the essential oil of *F. bernardii* exhibited a low level of the antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* and no activity against *Pseudomonas aeruginosa*.\(^{[2]}\) Although in earlier research antimicrobial effects of essential oils obtained from aerial parts and seeds of *F. angulata* subsp. *Carduchorum* against tested bacteria and fungi have been reported\(^{[10]}\), in other study essential oils of *F. angulata* ssp. *angulata* exhibited no considerable biological activities.\(^{[46,47]}\)

The major components or their derivatives may also contribute to antimicrobial activity, thus it is still worthwhile to test the individual major components also against other pathogens in future works. As far as the mechanism of the antimicrobial activity is concerned, the complex and variable chemical composition of essential oils, which can include many different molecules, prevents the understanding of the mechanism of action. This study indicated a potent antimicrobial activity of *F. angulata* seed essential oil against different bacteria and fungi is noteworthy, but needs further survey to evaluate the suitability of this remarkable antimicrobial property.

**Conclusion**

In recent years, interest has been generated in the development of safer antifungal agents such as plant-based essential oils to control phytopathogens in agricultural systems. Essential oils are promising natural antimicrobial agents with potential applications in agro-industries to control plant pathogenic microorganisms causing severe destruction in crops. Due to the interest in antimicrobial substances from plant sources, we performed a study on *F. angulata* oil which revealed significant antimicrobial activity against different plant pathogenic microorganisms causing severe diseases in plants. Our findings indicated that the oil of this plant is effective for inhibition or to control the tested bacteria and fungi. In this way, the oil has a good capacity as an alternative to synthetic products in various industries. The essential oil of *F. angulata* seed could be used as a natural antimicrobial agent. Finally, it would also be interesting to study the effects of essential oils of *F. angulata* against other important bacteria and fungi for developing new natural antibacterial and antifungal agents to control serious fungal diseases in plants. Further work is necessary to explore the efficacy and potential of suitable concentrations of this essential oil in foods and bearing industries to consider the possible interactions of the oil with food ingredients.

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