HPLC profile, antioxidant, antibacterial, and anti-proliferative activity of aerial parts of *Ferula ovina* (Boiss.) Boiss.

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**A B S T R A C T**

The aerial parts of *Ferula ovina* (Boiss.) Boiss were extracted by methanol and then fractionated with solvents that were differences in their polarity. The evaluation of the bioactivity of the plant extracts including antioxidant, antimicrobial and antiproliferative activity was carried out using various assays. The phenolic and flavonoid compound profile of the extract were established using HPLC analysis. The leaves showed the highest total phenolic content and radical scavenging activity; the flowers had the maximum amount of total flavonoid content, and the stems exhibited the maximum ferrous ion chelating ability. Among ten microorganisms, the plant extract prevented the growth of three strains, namely *Pseudomonas aeruginosa, Acinetobacter baumannii*, and *Streptococcus pneumoniae*. The flower of *F. ovina* (Boiss.) Boiss shows the best anti-human cervical carcinoma property against HeLa cell lines. Gallic acid was found as the major phenolic compound in the plant extract according to the HPLC result.

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**K E Y W O R D S**

Antibacterial activity
Antioxidant activity
Ferrous ion chelating
*Ferula ovina* (Boiss.) Boiss
Radical scavenging activity

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1. Introduction

The genus *Ferula* belongs to Umbelifrerae family (Iranshahi et al., 2012) and consists of 170 species that have a widespread distribution from the west of Asia to Mediterranean region and north of Africa (Sahebkar and Iranshahi, 2011). More than 30 species of *Ferula* genus grow in Iran most of which produce resins with different phytochemical properties (Keshtkar et al., 2008). To date, more than 70 species of *Ferula* have been studied to recognize their chemical composition and bioactivity properties (Iranshahi et al., 2010b). Most plants of this genus are well-known as a good source of biologically active compounds (Arnoldi et al., 2004); such as sulfur-containing compounds derivatives and sesquiterpenes. For instance, the most abundant sesquiterpenes from the root of *Ferula flabellifolia*, are farnesiferone B, flabellilobin A, flabellilobin B (Iranshahi et al., 2010a); and mogoltacin, fesolol, badrakemin acetate, ferocaulidin, conferone, and conferol acetate from roots of *F. badrakema* (Iranshahi et al., 2010b).

Many of the *Ferula* species have been historically known as rich sources of aromatic resins. Numerous species of this genus have been used in traditional medicine for various organ disorders such as carminative, digestive, expectorant, sedative, anti-hysteric, laxative, aphrodisiac analgesic (Sahebkar and Iranshahi, 2010), antispasmodic (Sahebkar and Iranshahi, 2011) and antinociceptive (Bagheri et al., 2014). *Ferula ovina* (Boiss.) Boiss is known by the Persian name of "Koma" (Azarnivand et al., 2011). In contrast to the other *Ferula* species, the plant odor is not stinky due to the absence of sulfur-containing compounds. A previous study reported isolating stylosin, tschimgine as benzoic acid monoterpenic derivatives, and ferutinin as a benzoic acid sesquiterpene derivative, from chloroform extract of the root of *F. ovina* Boiss (Iranshahi et al., 2010a). The essential oil of the aerial parts of the...
plant was dominated by carvacrol, geranyl isovalerate, 
granyl isovalerate, and α-pinene (Ghannadi et al., 2002). 
Antihistaminic effect (Bashir et al., 2014) and smooth 
muscle (Al-Khalil et al., 1990) are among the prominent 
biological activity of this plant. Due to the lack of 
sufficient reports on antioxidant and antibacterial 
activity of extracts from *F. ovina* (Boiss.) Boiss, the 
present study focuses on measuring TPC (total phenolic 
content), TFC (total flavonoid content), and antioxidant 
activity (radical scavenging activity and ferrous ion 
chelating ability) of the extracts from aerial parts of 
the plant including flower, leaf, and stem. Antibacterial 
activity of the methanolic extract from the plant parts 
was also tested using the disc diffusion method.

**2. Experimental**

**2.1. Reagents and chemicals**

1,1-Diphenyl-2-picrylhydrazyl (DPPH), BHT (butylated 
hydroxyl toluene), α-tocopherol, and ferrozine 
[3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4 
triazine] were purchased from Sigma; ascorbic acid 
(AscA) and ethylene diamine tetraacetic acid 
(EDTA) from Merck; Na\(_2\)CO\(_3\), FeSO\(_4\).7H\(_2\)O from 
BDH; Folin-Ciocalteu’s reagent (FCR) from Flucka; 
gallic acid (GA) from Acros; and all solvents with 
analytical grade were purchased from Merck.

**2.2. Plant materials and preparation of extracts**

The aerial parts of *F. ovina* (Boiss.) Boiss. were collected 
from north of Sabzevar-Iran (May 2014). A voucher 
specimen for the plant with the number of HSUH 201 
was deposited at Hakim Sabzevari University Herbarium. 
The plant species were identified in the Iranian Research 
Institute of Forests and Rangelands. The samples were 
dried at room temperature, ground, and extracted using 
methanol by maceration method for 72 h. After filtration, 
the result was concentrated over a rotary vacuum until 
crude extracts were obtained. Next, the crude extracts 
were dissolved in methanol again and fractionated by 
using various solvents, which differ in polarity including 
* n-hexane, chloroform, ethyl acetate respectively, 
and then were concentrated over a rotary vacuum.

**2.3. HPLC analysis and identification of the main 
compounds**

The ethyl acetate extract of the flower of *F. ovina* (Boiss.) 
Boiss with 10 mg/mL concentration in methanol was 
analyzed by HPLC method (Waters 2695 (USA) (Zhou 
et al., 2020). The chromatographic assay was performed 
on a 15 cm×4.6 mm with pre-column, Eurospher 100- 
5 C18 analytical column provided by Waters (Sunfire) 
reversed-phase matrix (3.5 μm) (Waters). Elution 
was carried out in a gradient system with H\(_2\)O: HOAc 
(97:3) as the organic phase (solvent A) and methanol 
(solvent B) with the flow-rate of 1 mL.min\(^{-1}\). Peaks were 
monitored at 195-400 nm wavelength. Injection volume 
was 20 μL and the temperature was maintained at 25 
°C. The identification of the compounds was achieved 
by comparison of their retention time and UV/Vis. 
spectral reference data with those of the standard 
controls. The levels of the different compounds 
were extrapolated from calibration standard curves.

**2.4. Antioxidant activity**

The procedures for measuring radical-scavenging activity, 
ferrous ion chelating ability, and determination of total 
phenolic content, which are described below, were based 
on the methods previously used (Mahdavi et al., 2013).

**2.4.1. Determination of TPC (total phenolic content)**

A 0.5 mL of aqueous FCR reagent 10% was added to 
a mixture of 0.5 mL of each methanolic extract (1000 
µg/mL) and 1.5 mL of distilled water. The containers 
were vigorously shaken. After 5 min, 2 mL of sodium 
carbonate solution 10% was added and shaken 
again. The vials were incubated at room temperature 
in darkness for 2 h. The mixture absorbance was 
read at 760nm with the Photonix Ar 2015 UV/Vis. 
spectrophotometer. The analyses were carried out in 
three replicates. A gallic acid standard curve was used to 
calculate the total phenolic content of the extracts. The 
TPC was expressed as gallic acid equivalent (GAE) which 
means mg of gallic acid (GA) per gram of dried extract.

**2.4.2. Total flavonoid content (TFC)**

The TFC was calculated according to a previous report 
with some modifications (Farhan et al., 2012). Accordingly, 
1 mL of aluminum chloride (2% in methanol) was added 
to 1 mL of extracts in methanol (100 µg/mL). The mixture 
was shaken for 1 min and kept in darkness at room 
temperature for 30 min. The solution absorbance was 
measured at 415 nm. Methanol was used as the blank. 
A standard curve of rutin was used for comparison; the 
TFC of the extracts was obtained as rutin equivalent 
(RuE) which means mg of rutin per gram of extract.

**2.4.3. DPPH radical-scavenging activity (RSA)**

A mixture of 2 mL DPPH in methanol (0.1 mM) and 
1.5 mL of extract solution in methanol with different 
concentrations (20, 40, 80 and 120 µg/mL) were 
prepared. The vials were then shaken and put in the 
dark at room temperature for 90 min. The absorbance 
was read at 517 nm. Standards of BHT (butylated 
hydroxyl toluene) and α-tocopherol were used. The 
assay was carried out in three replicates. The following 
equation was used to calculate the relevant RSA.

\[
\text{RSA}\% = \frac{(A_c-A_e)}{A_c} \times 100
\]  

(Eqn.1)

Where \(A_c\) is the control absorbance (control was 
DPPH solution without extract) and \(A_e\) is the 
extract absorbance (extract within DPPH solution).
2.4.4. Ferrous ion chelating ability (FIC)

A mixture of 50 µL FeSO₄ (2 mM), 1 mL of each extract in methanol with a concentration of 400, 600, 800, and 1000 µg/mL, and 2 mL of distilled water was prepared. Then, 100 µL of ferrozine (5 mM) was added to every vial. The container of the reaction mixture was shaken well and allowed to stand at room temperature for 10 min. The mixture absorbance was then measured at 562 nm. All measurements were carried out in triplicate. EDTA and ascorbic acid (AscA) were used as standards. Using the following equation, the inhibition percentage of ferrozine-Fe²⁺ complex formation was obtained:

\[
\text{Inhibition}\% = \left(\frac{A_C - A_S}{A_C}\right) \times 100
\]

(Eqn. 2)

Where \( A_c \) is the control absorbance (50 µL of the FeSO₄, 1 mL of methanol, and 100 µL of the ferrozine) and \( A_s \) is the sample absorbance consist of the extract, FeSO₄ and ferrozine.

2.5. Antibacterial activity: Disc diffusion assay

The antibacterial activity of the plant methanolic extracts was investigated using the disc diffusion method. The assay was run according to CLSI and (Mahdavi et al., 2012). Briefly, first, a sterile cotton swab was impregnated with a suspension containing 10⁸ CFU/mL of microorganisms. Then the plates of Mueller-Hinton agar medium were vaccinated using the swabs. Next, the discs (6 mm in diameter), which were impregnated with methanolic extract in the concentration of 100 mg/mL were placed on the inoculated agar. The plates were put in an incubator at 37.5 °C for 24 h. The antimicrobial activity of the extracts was determined by measuring of inhibition zone diameter against the microorganisms. The test was run in triplicate for each bacterium. Chloramphenicol (30 µg) and vancomycin (30 µg) were used as the positive control. The test bacterial strain was accomplished on nine Gram-negative including Pseudomonas aeruginosa, Echerishi acoli, Staphylococcus coagulase, Citrobacter frurdii, Enterobacter aerogenes, Agrobacterium tumefaciens, Acinetobacter baumannii, Serratia marcescens, Klebsiella pneumonia, and one Gram-positive of Streptococcus pnemoniae was tested. All the strains were obtained from the Microbiology Laboratory, Department of Biology, Faculty of Science, Hakim Sabzevari University and also Microbiology Laboratory of Sabzevar Medical Science University.

2.6. Antiproliferative assay

The cytotoxicity of ethyl acetate extract of different parts of F. ovina (Boiss.) Boiss. was evaluated on Human cervical carcinoma cell line (HeLa cells) using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay according to a previously reported study (Li et al., 2020). In this regard, the cells were evenly distributed in 96-well plates and incubated at 37 °C with 5% CO₂ overnight. Next, the cells were treated with extracts (202-120 µg/mL) and incubated for 24 h. 

Then, the medium was replaced with 20 µL MTT (5 mg/mL in PBS) and incubated at 37 °C for 4 h. The formazan crystals were dissolved in 100 µL Dimethyl sulfoxide (DMSO) and the absorbance was measured at 510 and 630 nm using a plate reader (Thermo Lab systems, Franklin, MA USA). Finally, IC₅₀ (concentration of oil that achieved a 50% of mortality) of the extract was calculated.

3. Results and Discussion

3.1. HPLC analysis

Fig. 1 presents the HPLC chromatogram of the ethyl acetate extract of the flowers of F. ovina (Boiss.) Boiss.. The HPLC profile of the extract is presented in the Table 1. Among the four selected standards including phenolic compounds of gallic acid (GA) and caffeic acid (CA) and flavonoids of rutin (Ru) and quercetin (Qu). The analysis approved the presence of all the compounds with differences in their concentration. According to the result, gallic acid with concentration of 7.59 µg/mg was the major compound in the plant extract followed by caffeic acid (2.64 µg/mg), quercetin (1.53 µg/mg), and rutin (0.67 µg/mg). The results revealed the presences of phenolic compounds predominates over flavonoids.

![Fig. 1. HPLC chromatogram of the ethyl acetate extract of flowers of Ferula ovina (Boiss.) Boiss.](image)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>UV ( \lambda_{max} ) (nm)</th>
<th>RT ( ^a ) (min)</th>
<th>Concentration µg/mg extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>272</td>
<td>3.6</td>
<td>7.59</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>324</td>
<td>2.8</td>
<td>2.64</td>
</tr>
<tr>
<td>Rutin</td>
<td>256</td>
<td>26.6</td>
<td>0.67</td>
</tr>
<tr>
<td>Quercetin</td>
<td>370</td>
<td>34.2</td>
<td>1.53</td>
</tr>
</tbody>
</table>

\(^a\) RT: Retention time

Table 1

High-performance liquid chromatography analysis of phenolic compounds content of the ethyl acetate extract of the flowers of Ferula ovina (Boiss.) Boiss.
3.2. Antioxidant activity

As shown in Table 2, among the methanolic (M), n-hexane (H), chloroform (C) and ethyl acetate (Et) extracts of the aerial parts of the plant, the Et extracts exhibited the highest TPC with an amount of 91.33 ± 3.2; 63.96 ± 2.1; and 40.33 ± 2.47 mgGAE/g for leaves, flower, and stem, respectively. Similar to TPC results ethyl acetate extracts showed a higher TFC than the others. The flower, leaf, and stem ethyl acetate extract with 491.7 ± 4.61; 378.42 ± 19.94; and 159.16 ± 6.46 mgRuE/g showed the highest TFC, respectively.

Table 2

<table>
<thead>
<tr>
<th>Plant Part</th>
<th>Extract</th>
<th>TPC</th>
<th>TFC</th>
<th>RSA</th>
<th>FIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg GAE/g</td>
<td>mgRuE/g</td>
<td>IC₅₀ µg/mL</td>
<td>IC₅₀ µg/mL</td>
</tr>
<tr>
<td>Flower</td>
<td>Hexane</td>
<td>NT</td>
<td>NT</td>
<td>71.11 ± 4.53*</td>
<td>475.56 ± 6.15</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>26.61 ± 5.15</td>
<td>3.55 ± 0.89</td>
<td>71.99 ± 2.27</td>
<td>306.98 ± 5.21</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>63.96 ± 2.08</td>
<td>480.01 ± 4.6</td>
<td>28.63 ± 1.12</td>
<td>357.65 ± 6.12</td>
</tr>
<tr>
<td>Leaf</td>
<td>Hexane</td>
<td>NT</td>
<td>NT</td>
<td>46.4 ± 2.71</td>
<td>605.15 ± 4.74</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>13.97 ± 2.66</td>
<td>8.2 ± 1.2</td>
<td>64.24 ± 3.44</td>
<td>481.94 ± 7.37</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>91.33 ± 3.2</td>
<td>366.74 ± 19.94</td>
<td>4.15 ± 0.71</td>
<td>521.34 ± 4.47</td>
</tr>
<tr>
<td>Stem</td>
<td>Hexane</td>
<td>NT</td>
<td>NT</td>
<td>563.43 ± 6.39</td>
<td>643.36 ± 6.19</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>31.8 ± 1.26</td>
<td>9.45 ± 7.04</td>
<td>388.17 ± 4.17</td>
<td>287.67 ± 4.66</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>40.33 ± 2.47</td>
<td>147.49 ± 6.46</td>
<td>57.38 ± 1.85</td>
<td>325.71 ± 3.18</td>
</tr>
<tr>
<td>Standards</td>
<td>α-Tocopherol</td>
<td>-</td>
<td>-</td>
<td>14.29 ± 3.54</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>BHT</td>
<td>-</td>
<td>-</td>
<td>16.21 ± 1.46</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>EDTA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>72.44 ± 6.31</td>
</tr>
<tr>
<td></td>
<td>AscA</td>
<td>-</td>
<td>-</td>
<td>40.28 ± 1.06</td>
<td>1475.47 ± 70.12</td>
</tr>
</tbody>
</table>

*NT (Not – Tested), the measuring was not carried out on the fraction, due to the polarity of phenolic and flavonoid compounds that are insoluble in n-hexane. **Values are presented as means ± SD (n = 3).

DPPH is a stable nitrogen-centered radical, that has been used for the estimate of the radical scavenging capacity of the natural compound such as plant extract (Tirzitis and Bartosz, 2010). In the presence of the antioxidant compound, DPPH radical is scavenged by donating of hydrogen to form a stable structure and the purple color of the compound change to yellow that have strong absorption at 517 nm (Prakash et al., 2001). Table 1 shows the RSA results of different fractions of F. ovina (Boiss.) Boiss. Among the fractions of each part, the ethyl acetate displayed the highest potential for radical scavenging. The leaf ethyl acetate fraction with IC₅₀ of 4.15 ± 0.71 µg/mL showed more activity than the other parts and standards. The RSA is followed with flower and stem fraction (IC₅₀ of 28.63 ± 1.12 and 57.38 ± 1.85 µg/mL, respectively). The poor activity belonged to the stem part with IC₅₀ of 388.17 ± 4.17 and 563.43 ± 6.39 µg/mL for chloroform and hexane fractions respectively. Ferrozine reacts with Fe²⁺ to form a complex in purple color, presence of the other chelating agents lead to fading out complex and so measuring of the color allows estimating the chelating activity of the agents. The active plant extracts challenge ferrozin to interact with ferrous ions to the formation of the complex (Hassan et al., 2013). The FIC results are shown in Table 1, on the contrary to RSA results of F. ovina (Boiss.) Boiss, the highest activity was measured for the chloroform fraction of each part. The chloroform fraction of stem with IC₅₀ of 287.67 ± 4.66 µg/mL and the hexane fraction of the stem part with IC₅₀ of 643.36 ± 6.19 µg/mL showed the most and lowest activity respectively. The flower fractions were more active than the leaves one. The ethyl acetate fractions of the plant parts showed FIC ability more than hexane and less than chloroform extracts. The FIC ability of EDTA, as a positive control, was more than those of the plant fractions; however, the ability of ascorbic acid (AscA) to chelate ferrous ion was less than plant fractions. A previous study reported 94.8 ± 5.9 mgGAE/g for TPC of methanolic extract from aerial parts of F. assafoetida (Dehpour et al., 2009). The ethanol/water extract of flower, leaf and stem of F.gummosa exhibited TPC of
20.8 ± 0.91; 18.5 ± 0.58; and 12.9 ± 0.39 mgGAE/g respectively (Nabavi et al., 2010). According to our result, F. ovina (Boiss.) Boiss. extracts exhibited higher TPC compared to those of F. gummosa. reported the TFC of F. assafoetida with 90.9 ± 6.3 mgRuE/g (Dehpour et al., 2009). The TFC of F. gummosa was measured with an amount of 9.2 ± 0.46; 8.2 ± 0.23; and 6.9 ± 0.31 mgRuE/g for flower, leaf, and stem parts (Nabavi et al., 2010). The results of the present study are comparable with the previous one, in which the flower of F. ovina and F. gummosa exhibited the highest TFC, however, all F. ovina (Boiss.) Boiss. extracts showed higher TFC than the same part of F. gummosa. Previous studies on RSA of other Ferula species reported the IC_{50} of 380 ± 12 µg/mL for F. assafoetida and 798, 906, and 1130 µg/mL for leaf, flower, and stem of F. gummosa, respectively (Dehpour et al., 2009; Nabavi et al., 2010), which are less than those of F. ovina (Boiss.) Boiss. The FIC of F. assafoetida with IC_{50} of 0.57 ± 0.02 µg/mL was reported previously (Dehpour et al., 2009). The result illustrated a very high FIC ability of the plant. However, the ability of F. gummosa to chelate of ferrous ions was lower than F. assafoetida. The IC_{50} of flower, stem, and leaf from F. gummosa were reported 726 ± 28.4, 634 ± 19.8, and 534 ± 21.5 µg/mL, respectively (Dehpour et al., 2009).

3.3. Antibacterial activity

The result of the antibacterial assay for the different parts of F. ovina (Boiss.) Boiss. is summarized in Table 3. Among 10 microorganisms, which are used to screen the antibacterial activity, the F. ovina (Boiss.) Boiss. extracts inhibited the growth of three test bacteria namely P. aeruginosa, A. baumannii, and S. pneumonia. According to the results, the plant extracts weakly prevented the growth of microorganisms. The maximum inhibition zone was found for stem extract against Gram-positive bacterium of S. pneumoniae with 11 mm diameter, which is less than control positive of Chloramphenicol and vancomycin with 21.6 and 14.2 mm inhibition zone diameter. Most previous studies on the antibacterial activity of Ferula species were carried out using the essential oils from different species of the genus. According to these reports, the plants are more sensitive to Gram-positive bacteria than Gram-negative. Essential oil of F. gummosa prevented the growth of Listeria monocytogenes and Staphylococcus aureus with MIC of 1.56 µL/mL; and inhibited the growth of Salmonella enteritidis, E. coli, and P. aeruginosa with MIC of 6.25, 12.50, and 50.00 µL/mL respectively (Abedi et al., 2009). The antibacterial activity of F. lycia essential oil was tested against Enterococcus faecalis and S. aureus as Gram-positive bacteria; and E. coli, K. pneumonia, Enterobacter cloacae, S. marcescens, Proteus vulgaris, Salmonella typhimurium, Pseudomonas aeruginosa, Haemophilus influenzae as Gram-negative bacteria. The essential oil exhibited weak activity against S. aureus and E. faecalis with inhibition zone diameters of 10 and 8 mm respectively. Among the Gram-negative bacteria, the essential oil only prevented the strain of H. influenza with 14 mm for inhibition zone diameter for disc diffusion test (Kose and Sarikuum, 2010). As mentioned before, the extracts of F. ovina (Boiss.) Boiss. were not potent to prevent the growth of the tested bacteria strains. The extracts just inhibition P. aeruginosa, A. baumannii, and S. pneumonia.

3.4. Antiproliferative activity

Due to the results of antioxidant activity, we run the cytotoxicity assay on the ethyl acetate extracts of the different plant parts. Fig. 2 shows the results of the antiproliferative activity of ethyl acetate extract of the plant parts. According to the results flowers showed the highest activity with value of 76.07 ± 3.75 µg/mL followed by leaves (63.76 ± 3.71 µg/mL) and stems (61.47 ± 1.07 µg/mL). The finding shows a dose depending anticancer activity for the all extracts. The anticancer activity of the plant extract against Hela cell line can be contributed to the presence of phenolic and flavonoids compounds in the extract, which was approved by HPLC analysis. According to many previous studies, gallic acid (Hsu et al., 2011); rutin (Iriti et al., 2017); caffeic acid (Rocha et al., 2012); and quercetin (Wu et al., 2018) have been potent agents in induced apoptosis or MTT assay against the different cancer cell lines.
Fig. 2. Antiproliferative activity of the ethyl acetate extract of the flowers, leaves, and stems of Ferula ovina (Boiss.) on the HeLa cell line. The values are presented as means ± SD (n = 3).

### Table 3
Antibacterial activity of the aerial parts of Ferula ovina (Boiss.) Boiss from Iran using disc diffusion method.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>DD* of the plant part</th>
<th>DD of antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flower</td>
<td>leaf</td>
</tr>
<tr>
<td><strong>Gram-Negative</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>8.2 ± 0.3**</td>
<td>9.1 ± 0.1</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>NA***</td>
<td>NA</td>
</tr>
<tr>
<td>Staphylococcus coagulase</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Citrobacter frudii</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Agrobacterium tumefaciens</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>9.3 ± 1.1</td>
<td>8.8 ± 0.3</td>
</tr>
<tr>
<td>Staphylococcus coagulase</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Gram-Positive</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>9.3 ± 1.5</td>
<td>9.1 ± 0.9</td>
</tr>
</tbody>
</table>

*DD: Disc diffusion method as recommended by NCCLS. **Diameter of inhibition zone (mm) including disc diameter of 6 mm *** NA: Not Active Values are presented as means ± SD (n = 3).

4. Concluding remarks

According to the findings of this study, ethyl acetate fraction of the leaf from F. ovina (Boiss.) Boiss. showed the highest total phenolic content and radical scavenging activity (91.33 ± 3.2 mg GAE/g extract and IC₅₀ = 4.15 ± 0.71 µg/mL, respectively); the ethyl acetate of flower represented the highest flavonoid content (480.01 ± 4.6 mgRuE/g extract), and the chloroform fraction of the stem part with IC₅₀ of 287.67 ± 4.66 µg/mL was the most active fraction. The HPLC analysis revealed that gallic acid is as the major phenolic compound in the plant extract. In MTT assay the plant showed cytotoxicity effect against Hela cell line. Besides, the plant extracts were susceptible to a small range of bacteria. Among 10 microorganisms, the methanolic extracts of flower, stem, and leaf from F. ovina (Boiss.) Boiss. prevented the growth of P. aeruginosa, A. baumannii, and S. pneumonia. Hence F. ovina (Boiss.) Boiss. can be proposed as a rich source of antioxidant agents that can be dependent on the TPC and TFC of the plant. However, determination of the total bioactivity of the plant such as antiviral, anticancer, and further antimicrobial activity should be tested in future studies.

Conflict of interest

The authors declare that there is no conflict of interest.

References

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