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Research Article

Chemical Composition and *in Vitro* Antibacterial Activity of *Ferulago angulata* (Schlecht.) Boiss Essential Oil

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ABSTRACT

Background: Essential oils usually exhibit different characteristics such as antimicrobial and flavoring effects. The aim of the present study was to investigate chemical composition and *in vitro* antibacterial activity of the essential oil of *Ferulago angulata* (Schlecht.) Boiss aerial parts against bacterial food-borne pathogens (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella typhimurium* and *Escherichia coli* O157:H7). ***Methods:*** The chemical composition of the essential oil was analysed by gas chromatograph coupled with mass spectrometer detector (GC-MS). The antibacterial activity of the essential oil was assessed using broth micro-dilution and agar disk diffusion methods. ***Results:*** According to results of GC-MS analysis, 33 constituents were identified. The dominant components were α-pinene (28.43%), (Z)-beta-ocimene (20.12%), bornyl acetate (7.92%), ɣ-terpinene (5.72%), germacrene D (5.63%), myrcene (4.67%) and *p*-cymene (2.17%). The results obtained in this study showed that *L. monocytogenes* and *B. cereus* had the most sensitivity to the essential oil (MIC and MBC= 40µg/mL; Inhibition zone: 9mm). ***Conclusions:*** Our results indicated that *F. angulata* essential oil might be a potential rich source of compounds with antibacterial properties against food-borne bacteria.

Introduction

In the last decade, a great proportion of food-borne illness outbreaks are attributed to *Staphylococcus aureus*, *Bacillus* spp., *Listeria monocytogenes*, *Salmonella* spp. *Escherichia coli*, *Yersinia* spp. and *Clostridium* spp.[1-3] The outbreaks associated with foods usually are characterized by self-limiting symptoms such as nausea, vomiting, mild fever, abdominal pain and diarrhea.[4] But some bacteria such as *L. monocytogenes* seems to be a major concern for consumers due to severe disease caused by the bacterium, listeriosis, that may lead to abortion, meningitis and perinatal septicemia.[4,5] *S. aureus* and *E. coli* are mainly responsible for toxic shock syndrome and hemorrhagic cholecystitis, respectively.[6,7] On the other hand, it is noteworthy that some bacteria such as *Pseudomonas aeruginosa* and *Enterobacter aerogenes* can cause significant economic loss due to spoilage of foods.[8] In recent years, besides the use of chemicals, food preservation by natural products and preservatives has been considered as a new and safe approach for inhibiting the growth of food-borne pathogens and spoilage bacteria.[9]

Essential oils usually exhibit different characteristics such as antimicrobial and flavoring effects.[10] The *Ferulago* genus plants (known as "Chavir" in Persian), belonging to the Apiaceae family, are perennial or annual plants with small flowers and yellow fruits which are widely distributed in Iraq, Turkey and west of Iran (from flora of Iran especially in Ilam, Kurdistan, Kermanshah and Lorestan Provinces).[11-13] Many species of *Ferulago* have been served as rich sources of bioactive compounds such as monoterpenes and sesquiterpenes.[11-13] Phytochemical investigations of different *Ferulago* species have indicated the presence of *α*- pinene, *α*-phellandrene, *β*-hydroxy-13-epi-manoyl oxide, (*Z*)-*β*-ocimene, *p*-cymene, methyl carvacrol, myrcene, terpinolene, trans chrysanthenyl acetate, and trimethyl-benzaldehyde.[14-18] Several *in vitro* studies have demonstrated that these natural compounds possess potential activities as antibacterial, anthelmintic, antifungal and antiviral agents.[15,16,18] Generally, the genus of *Ferulago* extensively has been employed to treat of intestinal worms, wound skin infections, snake bites, headache and diseases of the spleen and gastrointestinal tract (digestive system) problems.[12,13,17] It also has been applied as preservative agent of traditional oils and different foods such as cheese and meat products.[12,16]

To demonstrate the usefulness of *F. angulata* essential

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oil as potential antimicrobial agent in pharmaceutical and food industries, the antibacterial efficacy of the essential oil must be investigated in a laboratory medium and food models. Hence, the objective of the current study was to investigate the chemical composition and antimicrobial effect of essential oil obtained from the aerial parts of *F. angulata* against selected food-borne bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Escherichia coli* O157:H7) with broth micro-dilution and agar disk diffusion methods.

Materials and Methods

Collection of plant material

The fresh aerial parts of *F. angulata* were gathered from Dalahoo Mountain (Kermanshah province, west of Iran) at the full flowering stage in July 2014. The plant was carefully dried one week at room temperature (25±2°C) in dark space. Authentication of the plant and voucher specimen (No. 4175) was conducted by Dr. Seyed Mohammad Masoumi, Faculty of Agriculture, Razi University, Kermanshah, Iran.

Isolation of essential oil

The extraction of the essential oil was performed using Clevenger-type apparatus according to standard technique.[17] For this purpose, 100g of the fine powdered-aerial parts of the *F. angulata* were subjected to hydro-distillation for until full recovery essential oil (3h). The extraction process of *F. angulata* was performed in three times. Finally, the collected volatile oil was dehydrated with anhydrous sodium sulfate (0.5g) (Merck, Darmstadt, Germany) and then filtered through a 0.22µm filter (Millipore™, Bedford MA, USA) and stored at 4˚C for further analysis (chemical analysis and antibacterial tests).

Gas chromatography–mass spectrometry (GC–MS) analysis of essential oil

The analysis of *F. angulata* essential oil was performed using an analytical gas chromatograph (Thermo Quest 2000, UK). The GC apparatus was HP-5MS (5% phenyl methyl silicone and 95% dimethylpolysiloxane), that fitted with DB5 capillary column (30m×0.25mm ID, 0.25µm film thickness). The column temperature was initially 50°C, and then gradually increased to 120˚C at a 2˚C/min rate, held for 3 min, and finally increased to 265˚C and held for 6°C. The temperature of the injector was 250°C. The carrier gas was helium with purity 99.99%, constant flow rate 1.2ml per min, and a split ratio equal to 1:20. Also, the chemical analysis of the essential oil was carried by analytical gas chromatograph coupled with mass spectrometer detector (Thermo Quest Finningan, UK) (GC-MS). The capillary column and temperature condition of mass spectrometer detector was similar with gas chromatograph that described above. The procedure was operated at 70 eV. The GC-MS analysis was done in triplicate.

Identification of the major constituents of the essential oil was accompanied based on comparison between their retention indices (RIs), retention indices of published data,[12-17] Standard Mass Spectral fragmentation pattern (Wiley/NBS) and the NIST (National Institute of Standards and Technology). The percentage of each essential oil compositions was calculated from GC peak areas.

Preparation of test microorganisms

The strains used in this study were *Staphylococcus aureus* (ATCC6538), *Bacillus subtilis* (ATCC6633), *Bacillus cereus* (ATCC11774), *Listeria monocytogenes* (ATCC19118), *Salmonella typhimurium* (ATCC14028) and *Escherichia coli* O157:H7 (ATCC10536). Lyophilized cultures of the organisms were obtained from the Pasteur Research Institute, Tehran, Iran. Before the antibacterial tests, all bacteria were sub-cultured in Brain Heart Infusion broth (BHI; Merck, Darmstadt, Germany) medium in twice and incubated at 37°C for 18h. The density of bacterial cultures needed for the antibacterial tests examined by using a spectrophotometer at 600nm. The determination of inoculum dose (10^6CFU/mL) also was assessed using plate count on Brain Heart Infusion agar (BHI; Merck, Darmstadt, Germany) medium.

Antibacterial tests

Determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC).

The MIC and MBC values were assessed using serial micro-dilution test according to Azizkhani *et al*., method.[7] For this purpose, the different concentrations of the essential oil (5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100µg/mL) were incorporated with BHI broth containing 5% (v/v) dimethyl sulfoxide (DMSO) (Merck, Darmstadt, Germany) as an emulsifier and 0.05% (w/v) agar-agar (Merck, Darmstadt, Germany) as a stabilizer of essential oil. The 96-well sterile micro-dilution plates with U-bottom wells (Extragene, USA) were used. Briefly, for every experiment, 180µl/mL BHI broth containing different concentrations of the essential oil and 20µl/mL of inoculum diluted in BHI broth with 10^6CFU/mL of each bacterium were located into each well. The last well containing 180µL BHI broth and 20µL of inoculum without the essential oil was designed as the positive control sample. For negative control, un-inoculated BHI broth was used in order to determine sterility. Contents of each well were mixed using a plate shaker at 300rpm for 30s and afterwards, microplates were incubated at 37°C for 48h. The microbial growth was assessed by using ELx 800universal micro-plate reader (Biotek Instrument Inc., Winooski, VT, USA) by reading the absorbance of each well at 600nm. To determine MBC value, 20µl/mL of each well without any invisible growth was

cultured on BHI agar and incubated at 37°C for 24h.[7,19,20]

Agar disk diffusion assay

To investigation of antibacterial activity of *F. angulata* essential oil, agar disk diffusion method also was used according to Bajalan *et al.*, with some modification.[21] Firstly, the initial inoculum (10^8CFU/mL) was cultured on BHI agar by surface method and sterile cotton swabs. Then, the filter paper disc (6mm Ø; Whatman#1) impregnated with 20µl of the essential oil was placed on the surface of BHI agar. Positive (ampicillin, amoxicillin, trimethoprim and oxytetracycline) and negative (only DMSO) controls were considered in the present test. Afterwards, the plates were incubated 37°C for 24h. In order to evaluate of antibacterial activity, the radius of the inhibition zone was measured.[21,22] All Experiments were repeated in triplicate.

Results and Discussion

Chemical composition of F. angulata (Schlecht.) Boiss essential oil

Major chemical compositions of essential oil isolated from *F. angulata* (Schlecht.) Boiss was presented in Table 1. According to GC-MS analysis, 33 constituents were identified, representing 93.40% of the total oil, that belonged to monoterpenes (73.85% hydrocarbon and 12.26% oxygenated monoterpenes) and sesquiterpenes (6.29% hydrocarbon and 0.7% oxygenated sesquiterpenes) groups of constituents.

Table 1. Major chemical composition of essential oil isolated from *Ferulago angulata* (Schlecht.) Boiss.

No.	Compound name	Composition%	Retention time (min.)	KI[a]
1	Tricyclene	0.05	11.17	924
2	*α*-Thujene	0.25	11.32	927
3	***α*-Pinene**	**28.43**	**11.75**	**935**
4	Camphene	2.13	12.6	952
5	Verbenene	0.17	12.83	956
6	Sabinene	0.84	13.79	975
7	*β*-Pinene	1.42	14.05	980
8	**Myrcene**	**4.67**	**14.63**	**992**
9	δ-2-Carene	0.43	15.62	1011
10	*α*-Terpinene	0.07	16.11	1021
11	***p*-Cymene**	**2.17**	**16.61**	**1030**
12	Limonene	2.74	16.77	1033
13	*β*-Phellanderene	0.16	16.89	1036
14	**(*Z*)-*β*-Ocimene**	**20.12**	**17.14**	**1040**
15	(*E*)-*β*-Ocimene	2.13	17.65	1050
16	***γ*-Terpinene**	**5.72**	**18.32**	**1063**
17	Terpinolene	0.28	19.69	1089
18	Linalool	0.4	20.5	1105
19	allo-Ocimene	2.07	21.88	1133
20	*cis*-Verbenol	0.5	22.77	1151
21	*trans*-Verbenol	2.45	22.98	1155
22	Borneol	0.7	24.35	1183
23	Terpinene-4-ol	0.09	24.69	1189
24	α-Terpineol	0.2	25.47	1205
25	**Bornyl acetate**	**7.92**	**29.52**	**1291**
26	*α*-Copaene	0.25	33.49	1380
27	*β*-Bourbonene	0.14	33.87	1389
28	*β*-Cubebene	0.2	34.02	1392
29	Methyl eugenol	0.3	34.9	1413
30	**Germacrene D**	**5.63**	**38.1**	**1489**
31	δ-Cadinene	0.07	39.55	1526
32	Spathulenol	0.6	41.49	1575
33	Neryl isovalerate	0.1	42.1	1590
	Monoterpene hydrocarbons	73.85	Oxygenated monoterpenes	12.26
	Sesquiterpene hydrocarbons	6.29	Oxygenated sesquiterpenes	0.7
	Other	0.3		
	Total	**93.40**		

[a] Koat´s index

The main components were α-pinene (28.43%), followed by (*Z*)-*β*-ocimene (20.12%), bornyl acetate (7.92%), ɣ-terpinene (5.72%), germacrene D (5.63%), myrcene (4.67%) and *p*-cymene (2.17%). Various studies have been conducted on the chemical composition and antimicrobial effect of essential oil of plants belonging to Apiaceae family,[23-26] *F. angulata* has been the subject of numerous studies through the food industry.[11-18,27,28] Azarbani *et al.*, indicated *F. angulata* essential oil as a potent antimicrobial agent which inhibited growth of bacterial food-borne pathogens.[12] Also, some studies intended to extend the shelf-life of foods (such as cheese) by preventing microbial contaminations.[16] The composition of our essential oil was generally in agreement with other investigations.[13-15,17,18] Rezazadeh *et al.*, reported *α*-pinene (17.3%), bornyl acetate (14.45%) and cis-ocimene (14.41) as the main compounds of the oil.[27] According to another study, cis-ocimene (30.17%) and *α*-pinene (15.41%) were determined as the major components.[13] Also, Akhlaghi investigated chemical composition of aerial parts of *F. angulata* obtained from northeast of Iran, and reported that α-pinene (10.5%), limonene (9.6%), β-myrcene (5.5%) and α-fenchyl acetate (4.2%) were the major components.[15] In mentioned former study, the aerial parts oil of the plant has been reported to consist monoterpenes (75.1%), sesquiterpenes (11.9%) and nonterpene hydrocarbons (6.2%). Taran *et al.*, who investigated antibacterial and chemical composition of *F. angulata* obtained from Shahoo Mountain (Kermanshah Province, west of Iran), reported cis-ocimene (27.9%) in its essential oil. Other major components were *α*-pinene (25.7%), bornyl acetate (3.9%), *α*-terpinene (0.1%), germacrene D (22.3), and trans-ocimene (3.3%).[14] As well as, Sefidkon *et al.*, Javidnia *et al.*, and Rustaiyan *et al.*, reported *α*-terpinene and cis-ocimene as the major components of *F. angulata* essential oil.[17,18,28] The variability and diversity of the reports regarding the chemical composition content of *F. angulata* Boiss. essential oil can be attributed to different method used for extraction of the essential oil, geographical conditions, climate and seasonal variations and the stage of the plant growth.[24,26,29] The antibacterial activity of *F. angulata* essential oil could be attributed to the presence of monoterpene hydrocarbon (*α*-pinene, myrcene, *α*-terpinene, *β*-pinene and ocimene), oxygenated monoterpene (bornyl acetate) and sesquiterpene (germacrene D) compounds.[12,16]

Antibacterial activity

Antibacterial effect of *F. angulata* (Schlecht.) Boiss essential oil against six common food-borne bacteria was presented in Table 2 and 3.

Table 2. Antibacterial activity of *Ferulago angulata* (Schlecht.) *Boiss* essential oil indicated as Minimum Inhibitory/Bactericidal Concentrations-MIC/MBC (µg/mL).

Bacteria	MIC (µg/mL)	MBC (µg/mL)
Gram-positive		
S. aureus	50	50
B. subtilis	50	50
B. cereus	40	40
L. monocytogenes	40	40
Gram-negative		
S. typhimurium	50	50
E. coli O157:H7	50	50

Table 3. Antibacterial effect of *Ferulago angulata* (Schlecht.) Boiss essential oil by agar disk diffusion assay.

Bacteria	Inhibition zone (mm)				
	EO	Ampicillin	Amoxicillin	Trimethoprim	Oxytetracycline
Gram-positive					
S. aureus	7	0.5	0.5	NT	NT
B. subtilis	8	9	10	NT	NT
B. cereus	9	9	11	NT	NT
L.monocytogenes	9	13	15	NT	NT
Gram-negative					
S. typhimurium	7	NT	NT	10	8
E. coli O157:H7	7	NT	NT	13	7

NT: Not tested

Based on our results, the MIC and MBC values of the essential oil did not differ between gram negative and positive bacteria. As it was shown in Table 2 and 3, *S. aureus*, *B. subtilis*, *S. typhimurium* and *E.coli* O157:H7 had similar sensitivity to the essential oil, and *L. monocytogenes* and *B. cereus* showed more sensitivity (MIC and MBC= 40µg/mL; Inhibition zone: 9mm). The results obtained in this study showed that *F. angulata* essential oil exhibited strong antibacterial activity against all test bacteria. Results presented in this study were in agreement with previous studies.[12,14] Taran *et al.*, reported that the essential oil of aerial parts of *F. angulata* had the higher antibacterial activity against *S. aureus* (15µg/ml), but had no significant activity against *Shigella boidii*, *Pseudomonas aeruginosa*, *E. coli* and *Enterococcus faecalis*.[14] The results of Azarbani *et al.*, exhibited that aqueuos and methanolic extracts from flower, leaf and stem of *F. angulata* showed remarkable antibacterial activity against *B. cereus*, *S. aureus*, *E. coli* and *K.*

pneumonia.[12] Different reported MICs and inhibition zones of *F. angulata* essential oil among various studies can be attributed to the use of different bacterial species, media, volume of essential oil placed on the paper discs and thickness of the agar layer.[10,30,31] The mechanism of action of essential oils is related to the destabilization of the phospholipid bilayer structure, disruption of the cellular membrane, interaction with membrane enzymes and proteins and their action as a proton exchanger reducing the pH gradient across the membrane.[32-35] These antimicrobial effects have been reported for monoterpenes and sesquiterpenes.[36]

The present study indicated that *F. angulata* essential oil showed remarkable antibacterial activity against common food-borne bacteria associated with outbreaks (*S. aureus*, *B. subtilis*, *B. cereus*, *L. monocytogenes*, *S. typhimurium*, and *E. coli* O157:H7). Further research is required to evaluate the combination of this essential oil with other antibacterial constituents such as nisin, lysozyme, monolaurin and other essential oils. In conclusion, *F. angulata* essential oil could be considered as potential strong antimicrobials that can be used for the growth inhibition of various bacteria in food products.

Conflict of interest

The authors report no conflicts of interest.

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