J Clin Res Paramed Sci. 2020 June; 9(1):e97294. doi: 10.5812/jcrps.97294.

Published online 2020 January 19. **Research Article**

Evaluation of Antimicrobial Effect of *Ferulago angulata* on *Staphylococcus aureus* Growth Isolated From Subclinical Mastitis, in Vitro Study

Somayeh Bohlouli [1,*] and Sara Hasanvand [2]

[1]Department of Veterinary Medicine, College of Agriculture, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran
[2]Research Committee, Kermanshah University of Medical Sciences, Kermanshah, Iran

[*]*Corresponding author*: Department of Veterinary Medicine, College of Agriculture, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran. Email: sbohloli94@gmail.com

Received 2019 August 18; **Accepted** 2019 December 18.

Abstract

Background: *Staphylococcus aureus* is one of the important causative agents in mastitis.
Objectives: The aim of this study was determination antimicrobial effect of *Ferulago angulata* and different common therapeutic antibiotics on growth of *Staphylococcus aureus* isolated from subclinical mastitis cases.
Methods: In this study the antibacterial effect of *Ferulago angulata* essential oil, aqueous and hydro alcoholic extract on *Staphylococcus aureus* growth was done and compared with common therapeutic antibiotics such as tetracycline, erythromycin, kanamycin and gentamicin by diffusion disk method. Minimum inhibitory concentration and minimum bactericidal concentration were tested through tube standard method.
Results: Antimicrobial effect of essential oil and hydro alcoholic extract was increased by dose-dependently. 40 mg/mL extract and 20% essential oil are the highest dose with antimicrobial effect. Extract of *Ferulago angulata* has maximum antibacterial activity on the growth of *S. aureus*.
Conclusions: *Ferulago angulata* had remarkable anti-bacterial effect on *Staphylococcus aureus* growth isolated from subclinical mastitis cases. In comparison *Ferulago angulata* essential oil was more powerful than extract. Regarding the fact that antibiotic resistance is growing, *Ferulago angulata* as an herbal plant with anti-bacterial effect could be used in *Staphylococcus aureus* isolated from subclinical mastitis cases.

Keywords: Subclinical Mastitis, *Staphylococcus aureus*, *Ferulago angulate*, Antimicrobial Effect

1. Background

Staphylococcus aureus is a Gram-positive, round-shaped bacterium that that causes various infections in humans and animals. The prevalence of staphylococcal mastitis in the milk industry and its products has led to significant economic losses worldwide (1). The most important of these damages is the reduction in the amount of milk and the disruption of milk production. Mastitis is a response from the body to the damage to the breast tissue. Bacteria is producing mastitis from the teat enter the tubers and, after deployment in the tuber, create toxin (2).

In subclinical mastitis, the appearance of milk is normal, but milk composition changes such as increased white blood cells and breast or somatic cell cells (SC), decreased milk fat, decreased casein, lactose depletion, milk serum, increased milk chlorine, increased glycogen and reduced milk production (1). Diagnosis of subclinical mastitis can be detected by counting the number of somatic cell milk (3). Most infections caused by *Staphylococcus aureus* are subclinical and delay in diagnosis and treatment result in disease progression and form an acute form of mastitis (4). So early diagnosis of subclinical mastitis and its treatment is one of the most important the problems of the cattle industry and dairy factories (5). Drug resistance to common antibiotics in animal husbandry is important in the pharmaceutical industry and the treatment of microbial diseases common in the animal husbandry and veterinary medicine. The use of medicinal plants in various forms, such as essential oils and plant extracts, is an appropriate solution for the replacement of natural substances with chemical and synthetic drugs (6). Antimicrobial and antioxidant properties of natural herbs can be a good idea for obtaining new antibiotics (7). The Chavir, known as *Fer-*

ulago angulata, is located in the west of Iran, especially in Kermanshah Province. This herb was traditionally used as a digestive, hemorrhoid treatment. *Ferulago angulata*, as a natural and rich source of monoterpenes has an antibacterial effect (8).

Extracts and essential oils from seeds and plant aerial parts, have strong antimicrobial effect. Hydro alcoholic extract of Chavir effect in immune system of rainbow trout, which improves and strengthens the defenses of the body of the fish (9). The phenolic compounds in the extracts and essential oils of medicinal plants are considered as antioxidant and antibacterial compounds and components in these plants. Essential oil and extracts of Chavir plant have numerous antioxidant properties due to its phenolic and flavonoid combinations (10).

2. Objectives

Regarding the occurrence of drug interactions and the importance of complications of *Staphylococcus aureus* subclinical mastitis in the food industry and the production of dairy products and the importance of native herbs in each region, the aim of this study was to compare the antimicrobial effect of essential oil, aqueous and hydro alcoholic extract was used with antibiotics commonly used in the laboratory against *Staphylococcus aureus* isolated from subclinical mastitis cases.

3. Methods

3.1. Preparation of Plant

Ferulago angulata were collected from Dalahoo Mountains in Kermanshah Province in Iran. After identified by the herbarium of Islamic Azad University, Kermanshah Branch, essential oil extraction (stems, leaves and flowers) was performed by hydro distillation and clevenger-type apparatus (1928) for 3 hours (11). In order to extract, the whole plant was dried (stems, leaves and flowers) and powdered. Extracts were extracted from distilled water and ethanol 80% solvents separately. The mixture was mixed with 50 g of powder with 250 mL of ethanol 80%, and the resulting mixture was stirred and stirred for 72 hours with a magnetic stirrer. Then filtered by Watten filter and dried under reduced pressure at 37°C with rotator evaporator (9).

3.2. Collection of Milk Samples

Three hundred milk samples were collected from traditional and industrial cattle in Kermanshah Province. Sampling was carried out in a completely sanitized and sterile manner. Teats was washed with water, then dried disinfect with 70% ethanol. Ten milliliter of milk were collected in sterile tubes. Then, samples were kept in the freezer (-20°C) until the appropriate bacteriological tests were performed (12).

3.3. Detection of Subclinical Mastitis

Detection of subclinical mastitis (CMT) was used to detect subclinical mastitis (13). One hundred and eighty samples were subclinical mastitis in this study.

3.4. Isolation of Staphylococcus aureus From Milk Samples

In order to isolate *Staphylococcus aureus* from milk samples, initial culture was carried out in Baird-parker agar medium containing potassium tellurite and egg yolk emulsion (Merck Germany) and incubated at 37°C for 48 - 48 hours. Black colonies with transparent around them are suspected of *Staphylococcus aureus*. For definitive and final diagnosis, colonies in Blood Agar (Merck, Germany) contain 5% sheep blood and were incubated at 37°C for 24 hours. The formation of beta hemolysis, Gram staining, appearance of colonies under microscope, and catalase and coagulase tests were the definitive determinations of *Staphylococcus aureus*. *Staphylococcus aureus* isolated from milk samples were stored in tryptic soy broth (TSB) Brock (Merck, Germany) containing 15% glycerol in freezer (-20°C) (14).

3.5. Determine Antimicrobial Activity with Disk Diffusion Method

To determine the sensitivity of *Staphylococcus aureus* isolated from bovine mastitis to different concentrations of essential oil and extract of *Ferulago angulata*, disk diffusion test was performed using Kirby-Boer method.

A 24-hour culture of a bacterial suspension containing 0.5 McFarland dilutions was prepared as $0.5 \times 10^8 \times$ CFU/mL and uniformly grown in a medium of Mueller-Hinton agar (Merck, Germany). Antibiotic disks erythromycin, kanamycin, tetracycline and gentamicin (antibacterial medicine company) were placed on sterile foam and incubated at 37°C for 24 hours and the diameter of the inhibition zone was measured. Sterile disks were discarded at concentrations of 30, 20, and 40 mg/mL of aqueous and hydro alcoholic extracts, and then disks were transferred to the microbial contaminated area of the Mueller-Hinton agar. In order to determine the antimicrobial activity of essential oil, 10.5 and 20% essential oil was prepared by dimethyl sulfoxide (DMSO). Sterile disks were placed on the surface of the culture medium of the Mueller-Hinton agar contaminated with germs and 10 mL

of essential oil was discharged with concentrations prepared on the disks. Plates containing extracts and essential oil disks were incubated in a culture medium infected with germs at 37°C for 24 hours and the diameter of the non-growth holes with caliper was measured in millimeters. The experiments were repeated 3 times.

A micro dilution broth was used to determine the minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC). Dilutions of 2, 4, 8, 16, 32, and 64 mg/mL of aqueous and hydro alcoholic extracts and dilutions of 1%, 2%, 4%, 6%, 8%, and 10% of Chavir were prepared and filled into tubes test specimens were added to the Muller Hinton medium. Then to each tube, each 1 mL of the Muller Hinton medium, the concentration of 1×10^6 CFU/mL was added. All tubes were incubated at 37°C for 24 hours and then the results were examined. The minimum dilution of extract and essential oil which inhibited bacterial growth at concentrations was considered as the minimum inhibitory concentration (MIC). All of the tubes lacking turbidity (non-growth of bacteria) were cultured in a Mueller-Hinton agar and at 37°C for 24 hours at incubator, the first cultures in which no bacteria were observed as the minimum bactericidal concentration (MBC) was considered (15).

3.6. Statistical Analysis

Data analysis was done using one-way ANOVA, Tukey's test and SPSS software. P value < 0.05 was considered significant.

4. Results

Based on the results of this study, the antimicrobial effect of essential oil and extract of *Ferulago angulata* on *Staphylococcus aureus* isolated from bovine mastitis increased with increasing of concentration and dose of essential oil and extract. The diameter of the inhibition zone of the bacteria increases with increasing the concentration of essential oil and extract. Study of the *Ferulago angulata* essential oil compounds showed that cis-ocimene and alpha-pinene were the most important compounds in the essential oil. Table 1 shows the important compounds in the essential oil. The results of antimicrobial activity of various concentrations of Chavir essential oil in *Staphylococcus aureus* isolated from bovine mastitis are presented in Table 2. Using *Ferulago angulata* essential oil in all concentrations increased the diameter of the bacterial growth inhibition zone and increases in higher doses were significant ($P < 0.05$). Our findings on the antimicrobial effect of Chavir extract showed that the different concentrations of hydro alcoholic extract in *Staphylococcus aureus* isolated from bovine mastitis increased the diameter of the bacteria inhibition zone, and in doses of 30 and 40 mg/mL have a significant increase ($P < 0.05$). However, different concentrations of aqueous extract in *Staphylococcus aureus* isolated from bovine mastitis increased the diameter of the bacterial growth but this increase was not significant except 40 mg/mL ($P > 0.05$) and only 40 mg/mL of aqueous extract had antimicrobial effect ($P < 0.05$) (Table 3). The results of the study of the susceptibility pattern of *Staphylococcus aureus* isolated from bovine mastitis compared with the commonly used antibiotics in laboratory and the diameter of the inhibition zone are visible in Table 4. Gentamicin with the highest susceptibility and erythromycin with the least sensitivity to *Staphylococcus aureus* isolated from bovine mastitis were identified. The results of the minimum inhibitory concentration and minimum bactericidal concentration of essential oil and extract showed that in Table 5.

5. Discussion

Staphylococcus aureus is one of the most important factors in subclinical mastitis, which can be transmitted through environmental factors such as milking equipment and infected hands (16). *Staphylococcus aureus*, with its survival and survival capability in the bovine breast, can cause subclinical mastitis and in the absence of treatment of acute and chronic mastitis and can be considered as the source of the disease by disposing of milk. Studies have shown that a high percentage of subclinical mastitis caused by *Staphylococcus aureus* (17). In this study, the antimicrobial effect of essential oil and aqueous and hydro alcoholic extracts of *Ferulago angulata* were compared to common antibiotics in *Staphylococcus aureus* isolated from subclinical mastitis. Based on the results of this study, essential oil and hydro alcoholic extract of *Ferulago angulata* has significant antibacterial effects on *Staphylococcus aureus* isolated from bovine mastitis. Comparing the antibacterial effect of the essential oil and extract of the plant, essential oil has a stronger antibacterial effect than hydroalcoholic and aqueous extracts. The phenolic compounds in the extract and essential oil of *Ferulago angulata* have been identified (18). The phenolic compounds in the extracts and essential oil of medicinal plants are considered as antioxidant and antibacterial compounds and components in these plants (19, 20). In other study identified the compounds in the essential oil by GC/MS and water distillation methods. Cis-osimen and alpha-pineneare are The major compounds in *Ferulago angulata* essential oil (21).

Table 1. The Most Important Compounds of the Essential Oil of *Ferulago angulata*

Compound	Compound	Compound	Compound	Compound
Cis-Ocimene	Noe-Allo-Ocimene	Spathulenol	Alpha-Copaene	Borneol
Alpha-Pinene	Beta-Phellandrene	Alpha-Terpinene	Geranyl	Epi-alpha-Cadinol
Trans-Beta-Ocimene	Alpha-Terpinolene	alpha-Phellandrene	Isovalerate	Cis-Jasmone
Gamma-Terpinene	Bicyclogermacrene	Beta-Bourbonene	Methyl Eugenol	Gamma-Elemene
Germacrene-D	Delta-Cadinene	Linallo	Trans-Caryophllen	Cuparene
Limonene	Delta-3Carene	Beta-Cubebene	Beta-Elemen	Alpha-Cadinene
Bornyl Acetate	Germacrene-B	Alpha-amorphene	Alpha-Cadinol	Ledene
Camphene	Para-Cymene	Terpinene-4-01	Beta-Bisabolene	Alpha-Terpineol

Table 2. The Zone of Inhibition of *Staphylococcus aureus* Growth by Different Concentrations of Essential Oil[a]

	Different Concentrations *Ferulago angulata* Essential Oil, %		
Essential oil, %	10	15	20
Zone of inhibition, mm	14.8 ± 0.15^{A}	19.2 ± 0.33^{B}	24.4 ± 0.35^{C}

[a]Values are expressed as mean ± SD. Non-identical letters in a row indicate a significant difference between the antimicrobial effects of various concentrations of the essential oil ($P \leq 0.05$).

Table 3. The Zone of Inhibition of *Staphylococcus aureus* Growth (mm) by Different Concentrations of Aqueous and Hydro Alcoholic Extract (mg/mL)[a]

	Concentration of Extract, mg/mL		
	20	30	40
Hydro alcoholic extract	14.1 ± 0.25^{A}	18.6 ± 0.15^{B}	23.8 ± 0.33^{C}
Aqueous extract	12.9 ± 0.33^{A}	13.9 ± 0.18^{A}	15.7 ± 0.2^{B}

[a]Values are expressed as mean ± SD. Non-identical letters in a row indicate a significant difference between the antimicrobial effects of different concentrations. The same letters in a row indicate no significant difference ($P \leq 0.05$).

Chavir essential oil with antioxidant compound have antimicrobial activity, it can be used to control staphylococcal poisoning, especially in food products (22). In survey of antimicrobial activity of several plants on Gram-positive and negative bacteria was announced, *Ferulago angulata* have the highest antimicrobial activity (23). In another study, it has been suggested that *Ferulago angulata* essential oil has antimicrobial effects on *Staphylococcus aureus* and has inhibitory effects on Gram-positive bacteria more than Gram-negative bacteria (20). It seems that natural herbal compounds such as essential oil and herbal extracts against Gram-positive bacteria have more antimicrobial effects than Gram-negative bacteria (23). In other study the essential oil of *Ferulago angulata* showed stronger antimicrobial activity than other species (24). The antimicrobial effects of *Ferulago angulata* that collected from Chaharmahal and Bakhtiari Province in Iran, was survey on different bacteria and antibiotics in the laboratory. Researchers found that the hydro alcoholic extract of *Ferulago angulata* extract compared to conventional antibiotics has a greater inhibitory effect on some bacteria such as *Staphylococcus epidermidis*. *Ferulago angulata* extracts on Gram-positive bacteria also have a more inhibitory effect on Gram-positive bacteria than the Gram-negative bacteria. By increasing the dose, the antimicrobial activity of the extract increases. The results of these studies are similar with our results research (25). Findings of other study indicated that the essential oil of *Ferulago angulata* seed have antibacterial and antifungal activities, it could be used as a natural antimicrobial agent (26).

5.1. Conclusions

The results of this study showed that essential oil and extract of *Ferulago angulata* have significant antibacterial effect on *Staphylococcus aureus* isolated subclinical mastitis.

Regarding the healthy appearance of milk in the subclinical mastitis, it can be stated that the disease is one of the important health problems in the milk industry and its products and significant economic and health consequences. It entails innumerable economic losses to the dairy industry of the country. On the other hand, increasing the resistance of antibiotics in human and animal made serious dangers in humans. Use of natural herbs such as *Ferulago angulata* with high and impressive antimicrobial effect can be a good alternative to synthetic antibiotics in the pharmaceutical industry.

Table 4. Mean Diameter of *Staphylococcus aureus* Non-Growth Caused by Different Antibiotics[a]

	Erythromycin	Kanamycin	Tetracycline	Gentamicin
Antibiotics dosage, μg/disk	15	30	30	10
Zone of inhibition, mm	16.1 ± 0.35	19.47 ± 0.23	20.03 ± 0.51	21.2 ± 0.37

[a]Values are expressed as mean ± SD.

Table 5. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Essential Oil and Extract of *Ferulago angulata*

	Essential Oil, %	Hydro Alcoholic Extract, mg/mL	Aqueous Extract, mg/mL
MIC	4	4	8
MBC	6	8	16

Footnotes

Authors' Contribution: Somayeh Bohloli designed the experimental, set up the study and wrote the manuscript. Sara Hasanvand performed the experiments, and analyzed the data.

Conflict of Interests: The authors declare no conflict of interest regarding the compilation or publication of this article.

Ethical Approval: All experimental were reviewed and approved by Ethics Committee of Islamic Azad university.

Funding/Support: This study has not received any funds or financial support.

References

1. Barlow J. Mastitis therapy and antimicrobial susceptibility: A multispecies review with a focus on antibiotic treatment of mastitis in dairy cattle. *J Mammary Gland Biol Neoplasia*. 2011;**16**(4):383–407. doi: 10.1007/s10911-011-9235-z. [PubMed: 21984469].
2. Mork T, Waage S, Tollersrud T, Kvitle B, Sviland S. Clinical mastitis in ewes; bacteriology, epidemiology and clinical features. *Acta Vet Scand*. 2007;**49**:23. doi: 10.1186/1751-0147-49-23. [PubMed: 17892567]. [PubMed Central: PMC2048968].
3. Kivaria FM, Noordhuizen JP, Nielen M. Interpretation of California mastitis test scores using Staphylococcus aureus culture results for screening of subclinical mastitis in low yielding smallholder dairy cows in the Dar es Salaam region of Tanzania. *Prev Vet Med*. 2007;**78**(3-4):274–85. doi: 10.1016/j.prevetmed.2006.10.011. [PubMed: 17137660].
4. Atyabi N, Vodjgani M, Gharagozloo F, Bahonar A. Prevalence of bacterial mastitis in cattle fromthe farms around Tehran. *Iran J Vet Res*. 2006;**7**(3):76–9. doi: 10.22099/ijvr.2006.2654.
5. Francoz D, Wellemans V, Dupre JP, Roy JP, Labelle F, Lacasse P, et al. Invited review: A systematic review and qualitative analysis of treatments other than conventional antimicrobials for clinical mastitis in dairy cows. *J Dairy Sci*. 2017;**100**(10):7751–70. doi: 10.3168/jds.2016-12512. [PubMed: 28755947].
6. Berahou A, Auhmani A, Fdil N, Benharref A, Jana M, Gadhi CA. Antibacterial activity of Quercus ilex bark's extracts. *J Ethnopharmacol*. 2007;**112**(3):426–9. doi: 10.1016/j.jep.2007.03.032. [PubMed: 17513077].
7. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: A molecular, biological, and epidemiological study. *Lancet Infect Dis*. 2010;**10**(9):597–602. doi: 10.1016/s1473-3099(10)70143-2.
8. Razavi SM, Ravansalar A, Mirinejad S. The investigation on phytochemicals from Ferulago angulata (Schlecht) Boiss, indigenous to central parts of Iran. *Nat Prod Res*. 2015;**29**(21):2037–40. doi: 10.1080/14786419.2015.1017725. [PubMed: 25743037].
9. Bohlouli S, Sadeghi E. Growth performance and haematological and immunological indices of rainbow trout (Oncorhynchus mykiss) fingerlings supplemented with dietary Ferulago angulata (Schlecht) Boiss. *Acta Veterinaria Brno*. 2016;**85**(3):231–8. doi: 10.2754/avb201685030231.
10. Sadeghi E, Mahtabani A, Etminan A, Karami F. Stabilization of soybean oil during accelerated storage by essential oil of ferulago angulata boiss. *J Food Sci Technol*. 2016;**53**(2):1199–204. doi: 10.1007/s13197-015-2078-7. [PubMed: 27162399]. [PubMed Central: PMC4837741].
11. Sadeghi E, Karami F, Etminan A. The effect of Ferulago angulata(Schlecht) boiss essential oil on stabilization of sunflower oil during accelerated storage. *J Food Process Preserv*. 2017;**41**(1). e12745. doi: 10.1111/jfpp.12745.
12. Anueyiagu KN, Isiyaku AW. Isolation, identification of Staphylococcus aureus from bovine milk and its antibiotics susceptibility. *Int J Livest Prod*. 2015;**6**(6):74–7. doi: 10.5897/ijlp2015.0248.
13. Duarte CM, Freitas PP, Bexiga R. Technological advances in bovine mastitis diagnosis: An overview. *J Vet Diagn Invest*. 2015;**27**(6):665–72. doi: 10.1177/1040638715603087. [PubMed: 26450837].
14. Patel RK, Kumar R, Savalia CV, Patel NG. Isolation of Staphylococcus aureus from raw cattle milk and their drug resistance pattern. *Int J Curr Microbiol Appl Sci*. 2018;**7**(2):836–40. doi: 10.20546/ijcmas.2018.702.105.
15. Piotr S, Magdalena Z, Joanna P, Barbara K, Sławomir M. Essential oils as potential anti-staphylococcal agents. *Acta Veterinaria*. 2018;**68**(1):95–107. doi: 10.2478/acve-2018-0008.
16. Saglam AG, Sahin M, Celik E, Celebi O, Akca D, Otlu S. The role of staphylococci in subclinical mastitis of cows and lytic phage isolation against to Staphylococcus aureus. *Vet World*. 2017;**10**(12):1481–5. doi: 10.14202/vetworld.2017.1481-1485. [PubMed: 29391690]. [PubMed Central: PMC5771174].
17. Pumipuntu N, Tunyong W, Chantratita N, Diraphat P, Pumirat P, Sookrung N, et al. Staphylococcus spp. associated with subclinical bovine mastitis in central and northeast provinces of Thailand. *PeerJ*. 2019;**7**. e6587. doi: 10.7717/peerj.6587. [PubMed: 30886776]. [PubMed Central: PMC6421060].
18. Alami-Rostami S, Rafieirad M. The effect of hydro-alcoholic Ferulago angulata extract on locomotor activity and anxiety caused by hypoperfusion ischemia in adult male rats. *J Herbmed Pharmacol*. 2017;**7**(1):51–5. doi: 10.15171/jhp.2018.09.
19. Akhlaghi H. The essential oils from flowers, stems and leaves of Ferulago angulata from Iran. *Chem Nat Compd*. 2008;**44**(3):396–7. doi: 10.1007/s10600-008-9076-1.

20. Ghasemi Pirbalouti A, Izadi A, Malek Poor F, Hamedi B. Chemical composition, antioxidant and antibacterial activities of essential oils from Ferulago angulata. *Pharm Biol*. 2016;**54**(11):2515–20. doi: 10.3109/13880209.2016.1162816. [PubMed: 27102982].
21. Shahbazi Y. Variation in chemical composition of essential oil of Ferulago angulata collected from West Parts of Iran. *Pharm Sci*. 2016;**22**(1):16–21. doi: 10.15171/ps.2016.04.
22. Sadeghi E, Ebadi S, Karami F, Bashiry M, Sharafi H. Evaluation of antimicrobial effect of Ferulago angulata essential oil and nisin on Staphylococcus aureus growth in Iranian white cheese production and preservation trend. *Int J Pharm Technol*. 2016;**8**(3):17927–41.
23. Zareii B, Seyfi T, Movahedi R, Cheraghi J, Ebrahimi S. Antibacterial effects of plant extracts of Alcea digitata L., Satureja bachtiarica L. and Ferulago angulata L. *J Babol Univ Med Sci*. 2014;**16**(1):31–7.
24. Ozkan AMG, Demirci B, Demirci F, Baser KHC. Composition and antimicrobial activity of essential oil of Ferulago longistylis Boiss. fruits. *J Essent Oil Res*. 2008;**20**(6):569–73. doi: 10.1080/10412905.2008.9700090.
25. Tabatabaei Yazdi F, Alizade Behbahani B, Heidari Sureshjani M. The comparison of antimicrobial effects of Chevil (Ferulago angulata) extract with a variety of common therapeutic antibiotics in vitro. *J Arak Univ Med Sci*. 2014;**17**(3):35–46.
26. Moghaddam M, Mehdizadeh L, Mirzaei Najafgholi H, Ghasemi Pirbalouti A. Chemical composition, antibacterial and antifungal activities of seed essential oil of Ferulago angulata. *Int J Food Prop*. 2018;**21**(1):158–70. doi: 10.1080/10942912.2018.1437626.