Original Article

J Babol Univ Med Sci
Vol 20, Issu 2; Feb 2018. P:49-55

A Comparision of Aqueous and Hydroalcoholic Extracts of Foeniculum Vulgare and Carum Copticum with Gentamicin on Escherichia Coli Strains: in Vitro Study

F. Abedi (MD)[1], R. Rajabnia (PhD)[2], H. Sorkhi (MD)[3], Z. Memariani (PhD)[1], H. Shirafkan (PhD)[4], S.A. Mozaffarpur (MD,PhD)[5*]

1.Department of Persian Medicine, Faculty of Persian Medicine, Babol university of Medical Sciences, Babol, I.R.Iran.
2.Infection Diseases Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, I.R.Iran.
3.Non-Communicable Pediatric Disease Research Center, Health Research Institute, Babol Medical University, Babol, I.R.Iran.
4.Department of Epidemiology and Biostatistics, Faculty of Public Health, Tehran university of Medical Sciences, Tehran, I.R.Iran.
5.Traditional Medicine and History of Medical Sciences Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, I.R.Iran.

J Babol Univ Med Sci; 20(2); Feb 2018; PP: 49-55
Received: Nov 20th 2017, Revised: Jan 30th 2018, Accepted: Feb 12th 2018.

ABSTRACT

BACKGROUND AND OBJECTIVE: Due to bacterial resistance to antibiotics, new antibacterial agents is essential. In Persian medicine Fennel (*Fuenoculum vulgare* Mill.) and Ajwain (*Carum copticum* (L.) Benth. & Hook.f.) are recommended for the treatment of some infections. In this research, bacteriostatic and bactericidal effects of aqueous and hydroalcoholic extracts of fennel and Ajwain on *E. coli* were investigated.

METHODS: In an in-vitro study 30 clinical isolates of urine culture of children with urinary tract infection from Amirkola Pediatric Hospital in Babol and a standard sample were used. Antibacterial effects of 4 grouos including aqueous and hydroalcoholic extracts of fennel and Ajwain by measuring the diameter of the inhibition zone using disc diffusion (concentrations 16, 32, 64, 128, 256 and 512 mg/disc) and determination of Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC) with Microdilution method was compared with Gentamicin (30mg/disc) as a positive control

FINDINGS: There was no significant difference in inhibition zone with Gentamicin at concentrations of 64, 128, 256, and 512 mg/disc in standard and clinical samples. At concentrations of 16 and 32, Gentamicin was significantly better. The extract of 512 mg/disc (12.93±2.66) of hydroalcoholic extract of *Carum copticum* was significantly better than 256 mg/disc (9.53±1) (p=0.002). The MIC and MBC for standard samples were 4 and 8, respectively, and for clinical samples 3.83±2.36 and 5.8 mg/ml, respectively. Other extracts were not able to inhibit the growth of *Escherichia coli*.

CONCLUSION: The results showed that the Hydroalcoholic extract of *Carum copticum* has bacteriostatic and bactericidal effects on standard and clinical isolates of *Escherichia coli*.

KEY WORDS: *Anti-Bacterial Agents, Ajwain, Fennel, Persian Medicine*.

Please cite this article as follows:

Abedi F, Rajabnia R, Sorkhi H, Memariani Z, Shirafkan H, Mozaffarpur SA. Comparision of Aqueous and Hydroalcoholic Extracts of Foeniculum Vulgare and Carum Copticum with Gentamicin on Escherichia Coli Strains: in Vitro Study. J Babol Univ Med Sci. 2018;20(2):49-55.

* **Corresponding Author; S.A. Mozaffarpur.**
Address: Traditional Medicine and History of Medical Sciences Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, I.R.Iran.
Tel: +98 11 32194728.
E-mail: seyyedali1357@gmail.com

Introduction

Urinary tract infection is one of the most common bacterial infections in the population. The prevalence of urinary tract infection varies according to age and sex, and is more common in women due to anatomical causes (1). According to available statistics, 1% of boys and 3% of girls suffer from urinary tract infection in the first decade of life (2). In girls, 75 – 90% of cases of infection are caused by *E. coli*, and in the next stage by *Klebsiella* and *Proteus*. In some reports, boys above one year have an equal prevalence of E. coli (3). Microorganisms can adapt to the environment in a variety of ways. One of these adaptations is drug resistance (4, 5).

Due to drug resistance and side effects of chemical drugs, the approach of scientific researches to natural and herbal sources has increased in recent decades (6 – 12). One of the medicinal herbs used in the treatment of urinary tract infections in Iranian medicine is fennel and ajwain (13). Thymol is a major chemical compound in ajwain and is known as an antimicrobial, antispasmodic and antifungal agent (14). In addition, the active ingredients of essential oils of the fennel include carvacrol and trans-anethole (15).

Various studies have been carried out on the antimicrobial effects of the fennel and ajwain extract. Fennel seeds essential oil has antimicrobial activity against foodborne pathogens such as *E. coli* (16, 17). Limited studies have been conducted on the antimicrobial effects of fennel and ajwain extracts (12 – 18). In a study in Pakistan, the methanolic and ethanolic extracts of ajwain at concentrations of 30 mg/ml did not have antimicrobial effects on the standard strain of *E. coli* (19).

In another study, aqueous and ethanolic extracts of fennel and ajwain (200 mg/ml) showed significant antimicrobial effects on standard strains of *E. coli* compared to gentamicin (10 mg/ml) (20, 21). In another study, the antimicrobial effect of aqueous extract of fennel and ajwain (10% concentration) on *E. coli* was found to be significant compared to gentamicin (10 mg/ml) and cefixime (5 mg/ml) (22). Considering the fact that most studies have been conducted regarding the effect of fennel extract on standard strains of *E. coli* strain, this study was conducted to determine the effects of various concentrations of aqueous and hydroalcoholic extracts of fennel and ajwain on the strains of *E. coli* isolated from urine culture samples of children with urinary tract infection.

Methods

This experimental laboratory study was conducted after being approved by the Ethics Committee of Babol University of Medical Sciences (code MUBABOL.REC.1396.47). Sample size was determined to be 30 using PASS software (Power Analysis & Sample Size) with a confidence level of 5% and a power of 80%, assuming a correlation of 0.45 between the two groups.

Preparation of extracts: Soaking or maceration method was used to prepare the hydroalcoholic extract. After weighing and powdering, 50 g of fennel and ajwain seeds was added to 300 ml of 80% ethanol and kept for three days at laboratory temperature. The solution was then filtered by Whatman 42 Filter Paper and placed at room temperature to evaporate the solvent. To prepare the aqueous extracts, the seeds of fennel and ajwain were first weighed to be 100 g and powdered and then, distilled water was added six times its weight. The resulting mixture was placed in a hot bath at 60 °C for 15 minutes and then passed through a Whatman 42 Filter Paper. Eventually, each was separately exposed at ambient temperature to evaporate the solvent.

Studied microbial strains: In this study, the standard strain of *E. coli* (ATCC25922) was used. In addition, 30 clinical samples of *E. coli* from the urine culture of children with urinary tract infection, which were feverish, had pyuria and (from the middle urine samples in children with the ability to use a toilet and a catheter sample in the rest) had positive urine culture with *E. coli* were collected from Amirkola Children's Hospital in Babol. Clinical strains were identified again by biochemical tests.

Measurement of antimicrobial activity using disc diffusion method: In this culture method, the bacteria were prepared at a concentration equal to the standard opacity of 0.5 McFarland. The surface of the plate, containing the Mueller Hinton Agar, was inoculated uniformly using sterile cotton swab. Then, according to the six studied concentrations study (16, 32, 64, 128, 256 and 512 mg/disc), six blank discs were placed in culture media and the concentrations of hydroalcoholic extract of each plant were separately added (23). Plates were kept in incubator at 37 °C for 18 – 24 h. After the intended time, the inhibition zone diameter was measured using a ruler and the results were recorded. Gentamycin (30 mg/disc) was also used to test positive control. All of the above steps were performed for standard and clinical samples with two replications.

Measurement of antimicrobial activity by microdilution method: Determination of the minimum inhibitory concentration of the extracts was done by microdilution method and based on the standard protocol of the Clinical and Laboratory Standards Institute (CLSI) (24). In this method, 96–well microplate was used. First, 100 µl of concentrations of 1, 2, 4, 8, 16, 32, 64, 128, 256, 512 mg/ml of the extracts were added to the first 10 wells of each row of microplate by serial dilution method (25). Then, 100 µl of microbial suspension, equal to 0.5 McFarland, was added to each well. Wells 11 and 12 were considered as negative control (culture medium containing no bacteria) and positive (culture medium containing bacteria), respectively. Microplates were then incubated at 37 °C for 18-24 hours and the first well without opacity was considered as the minimum inhibitor concentration (MIC). Minimum bactericidal concentration (MBC) of the extract was determined according to the results of minimum inhibitor concentration.

Five microliters of wells, in which bacterial growth was completely stopped, were transferred to the plates containing the medium and kept in incubator at 37 °C for 18-24 hours. Concentrations that did not have bacterial growth were reported as minimum bactericidal concentration (MBC). All the steps were performed for standard samples and clinical samples with two replications. In this study, gentamicin disks (10 mg/disc) were used as a positive control. After collecting the data, they were analyzed by correlation coefficient analysis and $p<0.05$ was considered significant.

Results

Patients in the two study groups did not have a statistically significant difference in demographic characteristics (Table 1). The highest incidence of pruritus was from 30 to 60 minutes after injection. Of the 45 patients, 10 (22.2%) patients in the ondansetron group and 12 (26.7%) patients in the propofol group had pruritus during the procedure. Considering the sample size and type 1 error with alpha 0.05, and according to the ratio of pruritus in the study group (22.2% to 26.7%), the power of the statistical test in this study was 66%. Three (6.7%) patients in the ondansetron group, and four (8.9%) patients in propofol groups had pruritus in recovery (p=0.41) (p=0.5) (Table 2).

Table 1. Demographic characteristics of patients in the two groups

Group Variable	Propofol Mean±SD	Ondansetron	P-value
Age (year)	32.42±6.21	32.78±5.67	0.23
Height(centimeters)	159.61±3.83	160.02±3.25	0.12
Weight (kg)	83.73±7.69	84.02±8.07	0.36
BMI (Kg/m^2)	33.9±3.23	32.85±3.44	0.9
Operation duration (min)	89.9±11.93	88.07±8.28	0.29

Table 2. Distribution of absolute and relative frequency of pruritus during surgery and recovery in the two groups

Group Variable	Ondansetron N(%)	Propofol N(%)	P-value
Pruritus during surgery	10(22.2)	12(26.7)	0.4
Pruritus in recovery	3(6.7)	4(8.9)	0.5

Comparing the severity of pruritus during the operation between the two groups, the mean severity of pruritus in the ondansetron group was 1.85±0.69 and in the propofol group was 1.66±0.81 (p = 0.65). The mean severity of pruritus in the ondansetron group was 1.33±0.57 and in the propofol group was 1.25±0.5 in recovery (p=0.84). 12 (26.7%) patients in the ondansetron group, and 22 (48.9%) patients propofol group had nausea during the operation (p=0.02). Nausea in the recovery was reported in three (6.7%) patients in the ondansetron group and two (4.4%) patients in the propofol group (p=0.26). Four (8.9%) patients in the ondansetron group and six (13.3%) patients in the propofol group had vomiting during the operation (p=0.37). Two (4.4%) patients in the ondansetron group and one (2.2%) patient in the propofol group had vomiting in the recovery (Table 3).

Table 3. Distribution of absolute and relative frequency of nausea and vomiting during operation and recovery in two groups

Group Variable	Ondansetron N(%)	Propofol N(%)	P-value
Nausea during surgery	12(26.7)	22(48.9)	0.02
Nausea in recovery	3(6.7)	2(4.4)	0.26
Vomiting during surgery	4(8.9)	6(13.3)	0.37
Vomiting in recovery	2(4.4)	1(2.2)	0.5

Discussion

Based on the results of this study, hydroalcoholic extract (80%) of fennel and ajwain were found to have antimicrobial effects on standard samples and clinical samples of *E. coli* isolated from children with urinary tract infections. In some studies, the antibacterial effects of hydroalcoholic extract on the standard sample of *E. coli* have been shown.

In the study of Shafaghat et al., hydroalcoholic extract (70%) of ajwain with a minimum inhibitor concentration of 5 mg/ml was able to inhibit the growth of standard strains of *E. coli* resistant to cefixime, erythromycin and tetracycline (26). Amiri et al. reported the antimicrobial effect of hydroalcoholic extract (50%) of ajwain on a standard strain of *E. coli* with a minimum inhibitory concentration of 50 mg/ml. In this study, there was no statistical comparison with antibiotics (27).

In another study, Khosravipour et al. showed the antibacterial and dose-dependent effects of ajwain essential oil on bacterial agents such as *E. coli* (28). In other studies, the antimicrobial effect of ajwain essential oil has been considered (29, 30). In all of the mentioned studies, the standard samples of *E. coli* has been used. However, the study of clinical samples isolated from urinary tract infections is important due to their pathogenicity in accordance with geographical area (31). In this study, one standard sample and 30 clinical samples collected from urinary tract infection at the Medical Education Center were used and the effect of hydroalcoholic and aqueous extracts of fennel and ajwain on this pathogen was studied.

Considering the fact that, other than hydroalcoholic extract of ajwain, other extracts did not show antimicrobial effects neither on standard strain nor on the clinical samples and given the similar condition of extraction, the effect of geographic area of the plant on its components can be mentioned.

In the study of Zakeri et al. that examined the effects of ecosystem on the antimicrobial activity of the extract of medicinal plants, there was a significant difference between the minimum inhibitory concentrations of plants in different regions on bacterial agents (32).

In another study in Pakistan, the methanolic and ethanolic extracts of fennel at concentrations of 30 mg/ml did not have antimicrobial effects on the strains of the *E. coli* (33). Moreover, Manonmani et al. showed that ethanolic extract of fennel had no antibacterial effect on strains of *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* (34). In addition to the impact of the geographic area on the antibacterial agents of plants, gram-negative bacteria are generally more resistant to the antimicrobial effects of the plants due to the presence of the cell wall lipopolysaccharide (35). Gulfraz et al. reported that the effect antimicrobial activity of ethanolic extract of fennel on gram-positive bacteria is higher than gram-negative bacteria (36).

In another study in Turkey, fennel did not show any developmental inhibitory effect on microorganisms, including *E. coli* (37). But in another study, the aqueous and ethanolic extracts of fennel and ajwain with relatively high concentrations (200 mg/ml) showed antimicrobial effects on standard strains of *E. coli* (38, 39). In this study, two methods of disc diffusion and microdilution have been used to investigate the antibacterial effect of fennel and ajwain plants, which were also used in other studies.

Regarding the extraction of aqueous and hydroalcoholic extracts, contrary to other studies, in order to maintain the maximum effective amount of the plant, heating for concentration of the extract was avoided in this study and the extracts were concentrated at ambient temperature. In a study, Kaur et al. showed that with increasing temperature in the process of preparation of aqueous extract from ajwain and fennel, the antimicrobial effects of ajwain were significantly decreased and the aqueous extract of fennel did not have an antimicrobial effect on *E. coli* (38). In addition to the differences in the method of extraction, according to the different results of studies, the variation in the amount of chemical compounds present in plants is considered.

The most important active ingredients of essential oils are Thymol, gamma-terpinene, beta-Pinene, saimin and Sabinene, whose antimicrobial effects have been reported in various studies (40). Other compounds in the extract of ajwain include flavonoid compounds with antimicrobial effect. The presence of various amounts of different compounds in the plant extract increases their biological effect and increases the effect against microbial resistance (41). In this study, gentamicin was used as a standard antibiotic. One of the limitations of this study is the lack of use of other antibiotics to evaluate the probable microbial resistance of clinical strains.

By examining the data obtained from the study on the standard sample and clinical sample of *E. coli*, it can be concluded that the hydroalcoholic extract of

ajwain has bacteriostatic and bactericidal effects on the standard and clinical samples of *E. coli*. Further studies are required to investigate the antimicrobial activity of ajwain extract on antibiotic-resistant strains and also to investigate the synergistic effects of ajwain extract with common antibiotics.

Acknowledgments

Hereby, we express our deepest sense of gratitude and indebtedness to Deputy of Research and Technology of Babol University of Medical Sciences for their support and Experts of the Microbiology Department of this university.

References

1.Colgan R, Nicolle LE, McGlone A, Hooton TM. Asymptomatic bacteriuria in adults. Am Fam Physician. 2006;74(6):985-90.

2.Jonathan HC. Investigation of urinary tract infection in children. current paediatric. 2006,16(4):248-253.

3.Shokrollahei MD. Epidemiology of urinary tract infections in hospitolized children in fatemi-sahamieh hospital (2005-2006). Qom Univ Med Sci. 2008;2(1):35-41. [in Persian]

4.Sharma R, Sharma Ch, Kapoor B. Antibacterial resistance :current problems and possible solution. Indian J Med Sci. 2005;59(3):120-295.

5.Moghadam Nia A, Ghadimi R, Fatemi A. Sensitivity of bacteria involving pediatric urinary tract infection to some available antibiotics. J Babol Univ Med Sci. 1999;1(2):47-53. [In Persian].

6.Mahmoudpour Z, Shirafkan H, Mojahedi M, Gorji N, Mozaffarpur SA. Digesters in traditional persian medicine. Caspian J Intern Med. 2018;9(1):1-6.

7.Esmaeilidooki MR, Mozaffarpur SA, Mirzapour M, Shirafkan H, Kamalinejad M, Bijani A. Comparison between the cassia fistulas emulsion with polyethylene glycol (peg4000) in the pediatric functional constipation: a randomized clinical trial. Iran Red Crescent Med J. 2016;18(7):33998.

8.Mozaffarpur SA, Khodadust M, Shirafkan H, Yousefi M, Mirzapor M. Introducing a model for prioritization of drugs, based on iranian traditional medicine references. Med History. 2014;6(19):11-28.[In Persian].

9.Mozaffarpur SA, Naseri M, Esmaeili Dooki, MR, Bijani A, Kamalinejad M, Yousefi M, Mojahedi M, Khodadust M. Introduction of natural medicinal materia effective in treatment of constipation in Persian traditional medicine. History Med J. 2012;3(9):79-95. [In Persian].

10.Mozaffarpur SA, Naseri M, Esmaeili Dooki MR, Kamalinejad M, Bijani A. The effect of cassia fistula emulsion on pediatric functional constipation in comparison with mineral oil: a randomized, clinical trial. Daru. 2012; 20(1): 83.

11.Bazzaz BS, Memariani Z, Khashiarmanesh Z, Iranshahi M, Naderinasab M. Effect of galbanic acid, a sesquiterpene coumarin from Ferula szowits. Brazil J Microbiol. 2010;41:574-80.

12.Saeedi M, Ebrahimzadeh MA, Morteza-Semnani K, Akha A, Rabiei K. Evaluation of antibacterial effect of ethanolic extract of foeniculum vulgare mill. J Mazand Univ Med Sci. 2010;20(77):88-91. [In Persian]

13.Aghili Shirazi MH. Tehran: Institute of Meical History, Islamic Medicine and Complementary Medicine; 2008. Moalejat-e Aghili (Persian) PP. 133-223.

14.Haghiroalsadat BF, Vahidi AR, Azimzadeh M, Kalantar SM, Bernard F, Hokmollahi F. Chemical assessment of active ingredients and anti-oxidant effects of trachyspermum copticum's seeds harvested in yazd province. J Rafsanjan Univ Med Sci. 2012;11(3):197-206. [In Persian]

15.Diao WR, Hu QP, Zhang H, Xu JG. Chemical composition, antibacterial activity and mechanism of action of essential oil from seeds of fennel (Foeniculum vulgare Mill.). Food Control. 2014;35(1):109-16.

16.Rather MA, Dar BA, Sofi SN, Bhat BA, Qurishi MA. Foeniculum vulgare: A comprehensive review of its traditional use, phytochemistry, pharmacology, and safety. Arabian J Chem. 2016;1(9):1574-83.

17.Mohsenzade M. Evaluation of antibacterial activity of selected Iranian essential oil against Staphylococcus areus and E coli in nutrient broth medium. Pak J Book Sci. 2007;10(20):3693-7.

18.Mahdavi S, Alizad M, Sajjadi P, Baleghi M. A Study of the Antioxidant and Antimicrobial Effects of Ethanolic Extract of Fennel (Foeniculum vulgare Mill) Seeds. J Babol Univ Med Sci. 2017;19(5):32-8. [In Persian]

19.Anwar F, Ali M, Hussain AI, Shahid M. Antioxidant and antimicrobial activities of essential oil and extracts of fennel (Foeniculum vulgare Mill.) seeds from Pakistan. Flav Fragranc J. 2009;24(4):170-6.

20.Kaur GJ, Arora DS. Antibacterial and phytochemical screening of Anethum graveolens, Foeniculum vulgare and Trachyspermum ammi. BMC Complement Alternat Med. 2009;9(1):30.

21.Mahmoudi H, Arabestani MR, Molavi M, Karamolah KS, Fahim NZ. The study effects antimicrobial of Foeniculum vulgare mill and Achilles mille folium plant on bacterial pathogens causing urinary tract infections and nosocomial infection. Int J Pharmacogn Phytochem Res. 2016;8(9):1549-54.

22.Arora DS, Kaur GJ. Antibacterial activity of some Indian medicinal plants. J Nat Med. 2007;61(3):313-7.

23.Tehrani FA, Azizian S, Modaresifar KH, Peirovi H, Niknejad H. The antibacterial effect of low temperature stored amnion on growth of escherichia coli, staphylococcus aureus and pseudomonas aeruginosa. J Babol Univ Med Sci. 2018;20(1):19-3. [In Persian].
24.Clinical and Laboratory Standards Institute (CLSI), 2009a. Methods for Diluition Antimicrobial Susceptibility Tests; Approved Standard Eighth Edition. CLSI document M07–A8 (ISBN1-56238- 689-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania19087-1898,USA.
25.Zeighami H, Haghi F, Hajiahmadi F, Kashefiyeh M, Memariani M. Multi-drug-resistant enterotoxigenic and enterohemorrhagic Escherichia coli isolated from children with diarrhea. J Chemother. 2015;27(3):152-5.
26.Shafeghat M, Sharifi-Mood B, Metanat M, Saeidi S, Sepehri- Rad N. The Antibacterial Activity of the Ajowan Extract. Iran J Infec Dis Trop Med. 2015;19(67): 37-40. [In Persian]
27.Amiri A, Jomehpour N. Evaluation the Effect of Anti bacterial of Ferula assa-foetida L, Carum copticum, Mentha piperita L Hydroalcoholic Extract on Standard Sensitive and MethicillinResistant Staphylococcus aureus, Escherichia coli O157H7 and Salmonella typhimurium. J Ilam Univ. 2016;24(2):72-9. [In Persian].
28.Khosravipour R, Rezaeian-Doloei, R. Antibacterial activity Carum copticum essential oil against Pectinobactrrium carotovorum subsp. Carotovorum and Escherichia coli in nutrient broth medium. Curr Microbiol. 2016;73(2):265-72.
29.Mahboubi M, Kazempour N. Chemical composition and antimicrobial activity of Satureja hortensis and Trachyspermum copticum essential oil. Iran J Microbiol. 2011;3(4):194-200.
30.Kavoosi G, Tafsiry A, Ebdam AA, Rowshan V. Evaluation of antioxidant and antimicrobial activities of essential oils from Carum copticum seed and Ferula assafoetida latex. J Food Sci. 2013;78(2):356-61.
31.Zeidabadi Nejad M, Amini, K. Detection of pap, fim, sfa and afa genes in eschreshia coli strains isolated from patients with urinary tract infection by multiplex-pcr in kerman, iran. Jundishapur J Microbiol. 2017;16(4):393-400. [In Persian]
32.Zakerin AR, Ahmadi E, Fasihi-Ramandi M, Abdollahi S, Molazadeh AR, Jafari S, Allahverdi Gh. The Effects of Ecologic Condition on Antimicrobial Activity of Endemic Herbal Extracts in Fars Province. Fasa Univ Med Sci. 2015;5(1):111-9. [In Persian].
33.Manonmani R, Khadir VMA. Antibacterial screening on foeniculum vulgare Mill. Int J Pharma Bio Sci. 2011;2(4):390-4.
34.Lo Cantore P, Iacobellis NS, De Marco A, Capasso F, Senatore F. Antibacterial Activity of coriandrum sativum L and foeniculum vulgare miller var vulgare (Miller) essential Oils. J Agric Food Chem. 2004;52(26):7862-6.
35.Gulfraz M, Mehmood S, Minhas N, Jabeen N, Kausar R, Jabeen K, Arshad G. Composition and antimicrobial properties of essential oil of Foeniculum vulgare. Afri J Biotech. 2008;7(24):4364-8.
36.Sağ dıç O, Özcan M. Antibacterial activity of Turkish spice hydrosols. Food Cont 2003;14(3):141-3.
37.Ashrafi Tamai I, Zahraei Salehi T, Khosravi A, Sharifzadeh A, Balal A. Chemical composition and anti-candida activity of trachyspermum ammi essential oil on azoles resistant candida albicans isolates from oral cavity of hiv+ patients. J Med Plant. 2013;2(46):137-49. [In Persian].
38.Hassan W, Gul S, Rehman S, Noreen H, Shah Z. Chemical composition, essential oil characterization and antimicrobial activity of carum copticum. Vitam Miner. 2016;5:139.