Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2016; 8(11): 1505-1509

ISSN- 0975 1556

Research Article

Antibacterial Activity and Phytochemical Screening of Essential Oil of *Foeniculum vulgare*

Azadeh Foroughi[1], Pouya Pournaghi[2], Mohsen Zhaleh[3], Akram Zangeneh[1,3], Mohammad Mahdi Zangeneh[1,3*], Rohallah Moradi[4]

[1]*Microbiology section, Pathobiology & Basic sciences department, Veterinary faculty, Razi University, Kermanshah, Iran.*
[2]*Department of Biology, Kermanshah Center of Payame Noor University, Kermanshah, Iran.*
[3]*Department of Laboratory Science, School of Paramedicine, Kermanshah University of Medical Science, Kermanshah, Iran.*
[4]*Department of Chemistry, Kermanshah Center of Payame Noor University, Kermanshah, Iran.*

Available Online: 15th November, 2016

ABSTRACT
Certainly, using herbal plants is the oldest way of mankind to treat the diseases. Considering the drug resistance and the side effects of chemical antibacterial drugs, the research approach is increasingly going toward using natural resources. The aim of the study was to evaluate the chemical composition and antibacterial activity of essential oil of *Foeniculum vulgare* against *Escherichia coli O157:H7* and *Staphylococcus aureus*, Gas chromatography mass spectrometry was done to specify chemical composion. As a screen test to detect antibacterial properties of the essential oil, agar disk and agar well diffusion methods were employed. Macrobroth tube test was performed to determinate MIC. The results indicated that the most substance found in *F. vulgare* essential oil was Trans-anethole (47.41 %), also the essential oil of *F. vulgare* with 0.007 g/ml concentration has prevented *E. coli* and with 0.003 g/ml concentration has prevented *S. aureus*, from the growth. Thus, the research represents the antibacterial effects of the medical herb on *E. coli* and *S. aureus*. We believe that the article provide support to the antibacterial properties of the essential oil. The results indicate the fact that the essential oil of *F. vulgare* can be useful as medicinal or preservatives composition. Fractionation and characterization of active molecules will be the future work to investigate.

Keywords: *Foeniculum vulgare*, Essential oil, Chemical composition, Antibacterial activity.

INTRODUCTION

Bacterial infections are responsible for many deaths each year. Antibiotics are undeniably one of the most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infections. However, only one third of the infectious diseases known have been treated from these synthetic products. Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multi-resistant[1]. One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Plants as a source of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times[2]. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant[3]. Phytochemicals such as vitamins (A, C, E and K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes and minerals that have antimicrobial and antioxidant activity[4]. The specific function of many phytochemicals is still unclear; however, a considerable number of studies have shown that they are involved in the interaction of plants/pests/diseases. Phytochemical studies have attracted the attention of plant scientists due to the development of new and sophisticated techniques. These techniques played a significant role in the search for additional resources of raw material for pharmaceutical industry[5]. Essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants. The main constituents of essential oils –mono- and sesquiterpenes including carbohydrates, phenols, alcohols, ethers, aldehydes and ketones are responsible for the biological activity of aromatic and medicinal plants as well as for their fragrance. Due to these properties, spices and herbs have been added to food since ancient time, not only as flavouring agents but also as preservatives[6]. Plants and

**Author for Correspondence: m.mehdizangeneh@yahoo.com*

their essential oils are potentially useful sources of antimicrobial compounds. Antimicrobial screening of plant essential oils and phytochemicals, then, represents a starting point for antimicrobial drug discovery. Essential oils are effective on a wide range of Gram-negative and positive bacteria such as *Staphylococcus aureus, Bacillus subtilis,* and *Escherichia coli* O157:H7[7]. *Foeniculum vulgare*, commonly known as *Fennel*, is a flowering plant species in the carrot family. It is a hardy, perennial herb with yellow flowers and feathery leaves. It is a highly aromatic and flavorful herb and, along with the similar-tasting anise, is one of the primary ingredients of absinthe. It is widely cultivated throughout the temperate and tropical regions of the world for its aromatic fruits, which are used as culinary spices[8]. The *F. vulgare* fruit has a long history of use as both a food and medicine. Mature fruit of the plant and its essential oil are used as flavoring agents in food products such as liqueurs, bread, pickles, pastries, and cheese. Traditionally, it is said to act as a carminative (assists with flatulence control) and increase breast milk production. They are also used as a constituent in cosmetic and pharmaceutical products[9,10]. *F. vulgare* is used in traditional medicine for its antiseptic, palliative and anti-inflammatory effects. Traditionally, the plant is utilized for treating female infertility[11]. The most substance found in *F. vulgare* essential oil is Trans-anethole. The aim of this study was to screen the in vitro antibacterial activity of the plant essential oil against *E. coli O157:H7* and *S. aureus.*

MATERIAL AND METHODS

Plant sample collection

In the empirical-experimental study, medicine plant collected from Kermanshah. The sample was cleaned from any strange, plants, dust, or any other contaminants.

Essential oil extraction

Essential oil from fresh, clean, weighed aerial part *F. vulgare* fruits extracted by hydro-steam distillation using the Clevenger apparatus were collected and stored in sterile vials. Briefly, 100 to 150 g of plant was introduced in the distillation flask (1L), which was connected to a steam generator via a glass tube and to a condenser to retrieve the oil. This was recovered in a funnel tube. Aromatic molecules of the essential oil were released from the plant material and evaporated into hot steam. The hot steam forced the plant material to release the essential oil without burning the plant material itself. Then, steam containing the essential oil was passed through a cooling system in order to condense the steam. The steam was applied for 3h. After settling the recovered mixture, essential oil was withdrawn. The supernatant essential oil was filtered through anhydrous Na_2SO_4 to dry the yielded essential oil. Afterward, the essential oil was collected in tightened vials and stored in a refrigerator. For the antimicrobial activity test, several dilutions of the essential oil were done using dimethyl sulfoxide (DMSO).

Gas chromatography mass spectrometry (GC/MS)

F. vulgare essential oil was analysed using GC/MS (Shimadzu capillary GC-quadrupole MS system QP 5000) with two fused silica capillary column DB-5 (30 µm, 0.25 mm i.d, film thickness 0.25 µm) and a flame ionization detector (FID) which was operated in EI mode at 70 eV. Injector and detector temperatures were set at 220°C and 250°C, respectively. One microliter of each solution in hexane was injected and analyzed with the column held initially at 60°C for 2 min and then increased by 3°C/min up to 300°C. Helium was employed as carrier gas (1 ml/min). The relative amount of individual components of the total essential oil is expressed as percentage peak area relative to total peak area. Qualitative identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds and mass spectra.

Source of microorganisms

Tow bacterial species namely *E. coli O157:H7* (ATCC No. 25922) and *S. aureus* (ATCC No. 25923) were procured from Veterinary school of Tehran University as lyophilized. Each bacterial strain was activated on Tryptic Soy broth, constant at 37°C for 18 h. Then 60 µl of the broth was transferred to Nutrient agar and incubated at 37°C for another 24 h; cell concentration was then adjusted to obtain final concentration of 10^8 cfu/ml using Muller Hinton broth.

Culture media

Mueller-Hinton Agar (Müller-Hinton agar is a microbiological growth medium that is commonly used for antibiotic susceptibility testing) was prepared according to the manufacturer's instruction (Oxoid, UK), autoclaved and dispensed at 20 ml per plate in 12 x 12cm Petri dishes. Set plates were incubated overnight to ensure sterility before use.

Evaluation of antimicrobial activities

Agar disk diffusion and agar well diffusion were used as screen tests to evaluate antibacterial property of essential oil of *F. vulgare* based on standard protocol. The solution of the essential oil was yielded in 1g/ml from which six fold serial dilutions (v/v) were prepared. 60 µl of each dilution was poured on each disk in order. After a period of 24 hours incubation, the diameters of growth inhibition zones around the disks were measured. DMSO was used as negative control whereas kanamycin and cephalexin were used as positive controls in case of *E. coli* and *S. aureus*, respectively. Minimum inhibitory concentration (MIC) means the lowest concentration of the probable antimicrobial agent which prevents growing of bacteria (regardless of killing the bacteria or stopping the growth of them). The lowest dilution which no gross microbial growth has been seen indicates MIC. Minimum bactericidal concentration (MBC) means the lowest concentration of the agent which causes death to test bacteria. The last can be revealed by pouring 60 µl of MIC tube and six dilutions before contents on agar plate. In the case, after incubation period, the lowest concentration which makes no growth indicates MBC. For determination of MIC value, macrobroth dilution method was applied. Interpretation of the results was done due to national accepted letter[12].

RESULTS

Chemical composition

The most substance found in *F. vulgare* essential oil was Trans-anethole. In contrast, *β-Pinene* was the least constituents discovered in *F. vulgare* essential oil. Composition of the plant using Gas chromatography mass spectrometry method can be perceived in table 1.

Agar disk diffusion test

In case of *F. vulgare*, the widest zone was formed due to 0.031 g/ml and 0.015 g/ml of the essential oil in *E.coli* culture (equivalent to positive control: kanamycin) and it was no halo in 0.002 g/ml and less for two bacteria. No inhibition zone was observed due to DMSO. Growth inhibition zones due to different dilutions are listed in table 2.

Agar well diffusion test

In regard to *F. vulgare* essential oil, the widest zone was seen in 0.031 g/ml, due to *S. aureus* (10 mm). It was no growth inhibition in 0.001 g/ml and less for two bacteria. The data are discoverable in table 3.

MIC and *MBC ascertaining*

The values of MIC and MBC are same for *E. coli* and it is 0.007 g/ml. in case of *S. aureus*, the values of MIC and MBC are same and it is 0.003 g/ml (Table 4). As the table showed, *F. vulgare* essential oil have prevented the growth of *E. coli* and *S.aureus*. Also, by increasing the concentration of *F. vulgare* essential oil, the inhibition zone increased ($p \leq 0.001$). The results determined that in tested bacteria, there was a significant difference ($p \leq 0.001$) in terms of sensitivity to the essential oil.

DISCUSSION

The development of resistance in bacteria is one of the mechanisms of natural adaptation to the presence of an antimicrobial agent that inhibits susceptible organisms and selects the resistant ones. The problem of antibiotic resistance, which has limited the use of cheap and old antibiotics, has necessitated the need for a continued search for new antimicrobial compounds. Plants as a source of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. According to the World Health Organization plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. Over 50% of all modern clinical drugs are of natural product origin[2]. Medicinal plants may have the ability to treat bacterial resistance to many types of antibiotics. The search for such compounds which can be combined with antibiotics in the treatment of drug resistant infections may be an alternative to overcoming the problem of resistance in bacteria. Essential oils of medicinal plants stand out as veritable sources of potential resistance modifying agents. Essential oils are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties[13,14]. *F. vulgare* is a small genus of annual, biennial or perennial herbs distributed in central Europe and Mediterranean region. It is widely cultivated throughout the temperate and tropical regions of the world for its aromatic fruits, which are used as a culinary spice[9,15]. Mature *F. vulgare* fruit are used as flavoring agent in food products such as liqueurs, bread, pickles, pastries, and cheese. They are also used as a constituent in cosmetic and pharmaceutical products[9,10]. *F. vulgare* is one of the medicinal plants which have been used for different purposes in traditional medicine of Iran, such as antimicrobial, antifungal, and antibacterial. Concerning the method of essential oil and preventing from using high temperature to decrease the rate of destruction of effective herbal compound. 16 compounds such as Trans-anethole, Limonene, β-Ocimene Z, Cis-anethole, Fenchone, α-Fenchyl acetate, α-Phellandrene, β-Fenchyl acetate, β-Myrcene, α-Pinene, Germacrene-D, β-Ocimene E, β-Farnesene, α-Copaene, Camphene, β-Pinene representing 93/21% of the total essential oil composition of *F. vulgare* were identified using mass gas-chromatograph. The most substance found in *F. vulgare* essential oil was Trans-anethole with 47.41 %. Trans-anethole is an alkyl alkyl-phenolether. Both the cis and trans isomers of trans-anethole occur in nature with the trans isomer always being the more abundant. Natural anethole occurs in *F. vulgare* essential oil. It has been shown to block grow of bacteria, inflammation and carcinogenesis. In contrast, *Camphene* (0.12) was the least constituents discovered in *F. vulgare* essential oil. There is a partial difference between these results and the similar studies. The composition of medicinal plant can highly be affected by their secretary tissue condition and developmental stage[16]. The previous findings showed that terpenes, phenols, aldehydes and ketones are the major components of essential oils[17], and it is generally believed that essential oils principally performed against the cell cytoplasmic membrane of microorganisms. The hydrophobicity is an important characteristic of essential oils and their components which enables them to accumulate in cell membranes, disturbing the structures and causing an increase of permeability[18]. Some chemical constituents from *F. vulgare* have been identified as active antimicrobial principles such as a phenyl propanoid derivative – Dillapional was found to be the active antimicrobial principle of the *F. vulgare* stem. Another molecule – Scopoletin which is a coumarin derivative has been isolated from *F. vulgare* and reported to possess marginal antimicrobial effect[19]. Findings from the current study revealed that *F. vulgare* essential oil has potential inhibitory effects on both of tested bacteria. The *F. vulgare* essential oil have maximum activity against *E. coli* (10 mm), which is comparable with a zone of inhibition exhibited by kanamycin (22 mm). Also the results indicated that essential oil of *F. vulgare* with 0.007 g/ml concentration has prevented *E. coli* bacteria and with 0.003 g/ml concentration has prevented *S. aureus*, from the growth. In the study, the levels of MBC were observed ranges from 0.003 and 0.007 g/ml for *F. vulgare*. Thus, the research represents the antibacterial effects of the medical herb on *E. coli* and *S. aureus*. There have been several reports on *F. vulgare* essential oils, including reports on the relative concentration of *F. vulgare* antibacterial activity[20-22]. A number of authors have mentioned the antimicrobial activity of essential oils of the plant, however, the mechanism of action has not been studied in great detail[23]. The essential oil extracted from the fruits of

Table 1: Identified main composition of the essential oil of *F. vulgare* using Gas chromatography mass spectrometry method.

Compounds	RI	MS%
α-Pinene	937	0.71
Camphene	951	0.12
β-Pinene	979	0.09
β-Myrcene	993	0.73
α-Phellandrene	1007	1.22
Limonene	1032	32.21
β-Ocimene Z	1038	2.41
β-Ocimene E	1046	0.23
Fenchone	1089	2.37
α-Fenchyl acetate	1216	1.65
β-Fenchyl acetate	1230	1.12
Cis-anethole	1251	2.22
Trans-anethole	1285	47.41
α-Copaene	1369	0.14
β-Farnesene	1451	0.21
Germacrene-D	1474	0.37
Total	-	93.21

Table 2: The diameters of growth inhibition zones in agar disk diffusion test in different dilutions of *F. vulgare* essential oil.

Dilution(g/ml)	Inhibition zone in disk diffusion (mm)	
Microorganism	*E. coli*	*S. aureus*
Positive control	22	16
1/32 (0.031)	10	9
1/64 (0.015)	10	8
1/128 (0.007)	8	8
1/256 (0.003)	8	8
1/512 (0.002)	0	0
1/1024 (0.001)	0	0
Negative control	0	0

Table 3: The diameters of growth inhibition zones in agar well diffusion test in different dilutions of *F. vulgare* essential oil.

Dilution(g/ml)	Inhibition zone in well diffusion (mm)	
Microorganism	*E. coli*	*S. aureus*
1/32 (0.031)	9	10
1/64 (0.015)	9	9
1/128 (0.007)	8	9
1/256 (0.003)	8	8
1/512 (0.002)	0	8
1/1024 (0.001)	0	0
Negative control	0	0

Table 4: MIC and MBC of essential oil of *F. vulgare*.

Microorganism	*E. coli*	*S. aureus*
MIC (*F. vulgare*)	1/128 (0.007)	1/256 (0.003)
MBC (*F. vulgare*)	1/128 (0.007)	1/256 (0.003)

F. vulgare exhibited antibacterial effect against foodborne pathogens such as *Bacillus megaterium* and *Listeria monocytogenes*[20,21,24]. The seed essential oil of *F. vulgare* has also been reported to possess antibacterial activity against some human pathogenic bacteria. Ethanol and water extracts of *F. vulgare* have shown activity against Campylobacter jejuni and Helicobacter pylori[25].

CONCLUSION

F. vulgare is an aromatic medicinal plant with antibacterial activity toward *E. coli (ATCC No. 25922)* and *S. aureus (ATCC No. 25923)*. The growth of both *E. coli* and *S. aureus* were inhibited by the essential oil tested, these results indicate that essential oil of *F. vulgare* has its own chemical composition, which may be correlated with its antibacterial activity. The essential oil of the plant displayed antibacterial properties, and its activities could be attributed to qualitative and quantitative differences in the chemical constituents of the individual essential oil. It can be used as antibacterial supplement in the developing countries towards the development of new therapeutic agent. Additional *in vivo* studies and clinical trials would be needed to justify. Also, further evaluation is necessary on potential of it as an antibacterial agent in topical or oral applications.

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