Natural Products Chemistry & Research
ISSN: 2329-6836
Shahmokhtar and Armand, Nat Prod Chem Res 2017, 5:4
DOI: 10.4172/2329-6836.1000267
Research Article
OMICS International

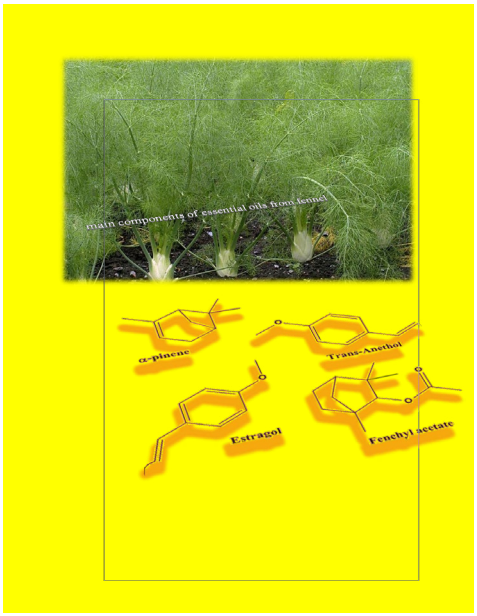
Phytochemical and Biological Studies of Fennel (*Foeniculum vulgare Mill.*) from the South West Region of Iran (Yasouj)

Mehrzad Kocheki Shahmokhtar[1*] and Samira Armand[2]

[1]*Department of Chemistry, Faculty of Science, Yasouj University, Yasouj, 75918-74831, Iran*
[2]*Department of Dentistry, Belarus State Medical University, Minsk, Belarus*

Abstract

In this study the chemical composition of essential oils of fennel (*Foeniculum vulgare* Mill.) was identified by GC-MS method. Results showed that essential oils of fennel have several organic compounds such as hydrocarbons, alcohols, ketones, aldehydes, ethers, esters and other volatile oil and so on. Essential oil of "fennel" has 32 compounds. T-Anethol, α-pinene, D-limonene, α-fenchone and fenchol were the highest percent of compounds. biological activity of Aqueous extract of fennel was evaluated by disk diffusion method and results showed that it has antibacterial properties against *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*, but have not any antifungal activity (*Aspergillus oryzae)*. The results show the extract of fennel was not a good alternative for antibiotics.



Keywords: *Foeniculum vulgare* Mill.; Essential oil; Antimicrobial; Antifungal

Introduction

The emergence of multidrug-resistant (MDR) pathogens and life-threatening infections caused by these microorganisms is a global challenge for the scientific community and some scientists believe that we are going back to the era before antibiotics [1,2]. The prevalence of bacterial infections due to opportunistic pathogens, frequentlycharacterized by high mortality rates, has increased during the past two decades [3]. Majority of antibiotics, currentlyapplied in therapy, belong to drug classes discovered prior to 1970 [4]. The current expansion of antibacterial and antifungal drugs research has occurred since there is persistent need for developing new compounds to fight life-threatening infections [5]. Besides bacterial infections, fungi are the major causes of liver, lungs, mouth, blood and skin infections [6]. Systemic mycoses are most frequently caused by Candida genus yeasts and mould particularly, the Aspergillus genus. Moreover, many of the existing drugs are toxic, ineffective and enable infection recurrence because of being bacteriostatic/fungistatic in nature. Medicinal plants are potential sources of potent antimicrobial drugs and are used in many countries to treat infectious diseases [7]. *Foeniculum vulgare* Mill. ssp. *vulgare* is a well-known medicinal plant native to the Mediterranean area [8]. 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]. Mature fennel fruit and its essential oil are used as stuffing agents in food products such as liqueurs, bread, pickles, pastries, and cheese. They are also used as a constituent in cosmetic and pharmaceutical products [10]. Widespread in middle and southern Italy, used in traditional medicine and as spice to aromatize breads, fishes, liqueurs, salads and cheeses [10]. Traditional teas were prepared from fruits [11] that are also routinely consumed for their carminative and mouth freshening effect. The main components of *F. vulgare* fruit essential oils have been reported to be trans anethole, estragol,

***Corresponding author:** Mehrzad Kocheki Shahmokhtar, Department of Chemistry, Faculty of Science, Yasouj University, Yasouj, 75918-74831, Iran, Tel: +989215410292; E-mail: mehrzad.kucheki1990@gmail.com

Received April 20, 2017; **Accepted** May 03, 2017; **Published** May 08, 2017

Citation: Shahmokhtar MK, Armand S (2017) Phytochemical and Biological Studies of Fennel (*Foeniculum vulgare Mill.*) from the South West Region of Iran (Yasouj). Nat Prod Chem Res 5: 267. doi: 10.4172/2329-6836.1000267

fenchone, and α-phellandrene and the relative concentration of these compounds varies considerably depending on the phenological state and origin of the fennel [12]. There have been several reports on fennel oils, including extraction methods, biological activities of the essential oil, antioxidant activity, anti-inflammatory activity, anti diabetic activity. In addition, the essential oil of fennel seeds had significant antifungal activity [13,14]. Although the chemical compositions and antimicrobial properties of essential oil of fennel have been studied, these informations are still limited the essential oil composition proved to exhibit considerable chemo diversity depending upon the method of extraction and geographical origin [15]. Our knowledge of the constituents of fennel's oleoresin is mostly related to the fruit, while knowledge relating to the chemical Compounds of the oils from the leaves is scarce. Fennel leaves are traditionally used to treat diarrhoea, bronchitis and to treat impotency [16]. Recently methyl chavicol, fenchone, limonene and exo-fenchyl acetate were established as the main components of the essential oil from leaves of *Foeniculum vulgare* ssp. piperitum growing wild in south Anatolia (Turkey) in different years [17]. In fact, agricultural practices influenced the yield and quality of the essential oils whose composition depends upon environmental and agricultural practices in addition to other factors affecting the plant such as genetics and ecological conditions [18]. The aim of the present study was conducted to investigate the chemical composition of essential oil and antibacterial activity of extract of the fennel from the south west region of Iran (yasouj).

Materials and Methods

Plant material and essential oil

In July with the use of plant distribution maps for fennel, region for sampling was selected. The different geographical characteristics of the region were measured. Latitude, longitude and altitude to the global coordinate system (GPS) were measured Table 1. Then samples (leaf ad twigs) were dried away from the sun for 10 days, dried whole fennel seeds were stored at -10°C until analysis, dried leaves transferred to the Phytochemistry laboratory and extraction essential oils by Clevenger for 3.5 hours (according to the method outlined by the British Pharmacopeia) [19]. Finally, essential oils after dehydration by Na_2SO_4, kept in dark glasses bottle in the refrigerator (in 4°C).

Preparation of the extract

The aqueous extract was obtained as follows: 80 mL of water added in 10 g of plant material in round-bottomed flask, then put it on the electric mixer for 24 h. Then it was smooth operation and filtered by Whatman paper No. 42.

Preparation of the culture medium

Based on the directions on the container culture media, culture media powder is dissolved in distilled water and heater stirred until the boil, Then the flask door closed with cotton for 20 min at 121°C inside the autoclave to be sterilized. After leaving autoclave poured about 15 mL of culture medium in each plate after cooling to keep in the refrigerator. Broth as the liquid culture medium is not necessary to pour plates can be directly stored in the fridge after sterilization and cooling.

Analysis of the essential oil

For determine the composition of the essential oils of the leaves was used gas chromatography and gas chromatography (Agilent, Model N 6890) connected mass spectrometry (GC-MS). HPS column (30 meter), its diameter was 250 micrometers and thickness of stationary phase 0.25 μm. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. Diluted samples (1/100 in n-hexan, v/v) of 1 μL were injected manually in split mode (split ratio 1/30). Oven temperature was programmed from 50 to 220°C at a rate of 5°C/min and from 220°C to 280°C at a rate 20°C/min and held at for 2 min Oil constituents were identified by MS data obtained from Wiley and NIST libraries (Table 2).

Biological activity

In vitro biological activity of the extract against two Gram negative bacteria such as *Escherichia coli* (ATCC 25922) and *Pseudomonas aeroginosa* (ATCC 9027) and a Gram positive bacteria including *S. Bacillus subtilis* (ATCC: 6633) and also a fungal strain such as *A. oryzae*, were carried out using disk diffusion method [20,21]. For this mean, the petridishes were prepared as follows: *i*) a layer of culture medium (Muller Hinton Agar (Merck, Germany) for bacterial strains and Sabouraud dextrose agar (SDA) for fungal strains was poured on the surface of plates, *ii*) 100 μL of spore suspension of bacteria and/or fungi including nearly 0.5×10^6 colony-forming units (CFU/mL) was inoculated on the surface of culture medium, *iii*) The prepared discs (that had been soaked in the various concentrations of extract; 12.5, 25 and 50 mg/mL in physiological saline) were placed at different positions on a surface of plates. Then, the plates were incubated at 37°C for 24 h for bacterial strains and at 27°C for 3 days for *Aspergillus oryzae* [22]. Antimicrobial activities of extract were evaluated based on diameter of zone of inhibition (mm) and listed in Table 3. Amoxicillin, Erythromycin, Fluoxamine and Cephalexin were used as reference bactericidal drugs (positive controls) Table 4. A 0.5 McFarland standard is prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate ($BaCl_2 \bullet 2H_2O$), with 9.95 mL of 1% sulfuric acid (H_2SO_4) [23].

Results and Discussion

The geographical characteristics of the study region (latitude, longitude and altitude) were shown in Table 1. Essential oil extraction by Clevenger and dehydration by Na_2SO_4 then compounds in essential oils were identified by chromatography connected to mass spectrometry (GC-MS). Chemical compounds were identified and was shown in Table 2. The essential oil of fennel was identified 32 compounds that t-Anethol, α-pinene, D-limonene, α-fenchone and fenchol were the highest percent of compounds and others were found to be the minor components in the essential oil of fennel seeds. The profile obtained in the present study was very similar to the previous results reported by Viuda-Martos et al. who identified 20 components and also found that trans-anethole (65.59%), estragole (13.11%), limonene (8.54%) and fenchone (7.76%) were the major compounds in the essential oil from Egyptian fennel [23]. These differences in components and its content of essential oil fromfennelmay be concerned in the geographical origins. The antibacterial activity of the extract was listed in Table 3 as diameter of inhibition zone from the growth (in mm) [24]. Recently, some researchers have reported that monoterpene or sesquiterpene hydrocarbons and their oxygenated derivatives, which are the major components of essential oils, exhibit potential antimicrobial activities [25]. The results show that extract have the good antibacterial activity against all studied bacteria strains but The Aqueous extract of fennel has not any antifungal activity against *Aspergillus oryzae* (Figure 1).

Location	Altitude (m)	Latitude	Longitude
Yasouj	1759	30°40′55″	51°31′67″

Table 1: Geographical characteristics of study regions.

Row	Compounds name	RI	Percentage
1	3,5-dimethyl-1,6-heptadien-4-ol	920	1.2
2	α-thujene	925	0.9
3	α-pinene	936	11.4
4	D-limonene	1024	5.6
5	Sylvestrene	1027	1.7
6	δ-3-carene	1030	2.2
7	α-fenchone	1076	4.4
8	Epi camphor	1114	1.8
9	endo fenchol	1115	1.9
10	exo fenchol	1118	2.4
11	Bornan-3-one	1138	1.9
12	Estragol	1186	4.5
13	Fenchyl acetate	1233	4.7
14	Cis-Anethol	1253	7.5
15	Trans-Anethol	1258	15.1
16	β-cubebene	1317	0.8
17	β-caryophellene	1386	3.3
18	10s-11s-Himachala-3(12),4-diene	1399	1.1
19	β-farnesene	1460	1.6
20	Germacrene D	1480	1.8
21	Bisabolene	1506	0.7
22	Bronyl isovalerate	1514	0.9
23	Eugenol acetate	1526	2.4
24	δ-cadinene	1541	3.3
25	Spathulenol	1570	1.5
26	Caryophellene oxide	1580	1.3
27	Unknown	1601	0.8
28	ţ-cadinol	1618	0.7
29	α-cadinol	1627	1.5
30	ţ-muurolol	1641	0.4
31	Phytol	2002	0.6
32	Dipenthyl phthalate	2130	0.5

Table 2: Compounds in essential oils of fennel.

Name	Gram negative						Gram positive		
	Escherichia coli			*Pseudomons aeruginosa*			*Bacillus subtilis*		
	50 (mg/mL)	25 (mg/mL)	12.5 (mg/mL)	50 (mg/mL)	25 (mg/mL)	12.5 (mg/mL)	50 (mg/mL)	25 (mg/mL)	12.5 (mg/mL)
extract of fennel	15.22	12.86	10.26	18.74	16.42	13.66	14.27	12.20	9.80

Table 3: Diameter of zone of inhibition (mm) of fennel extract.

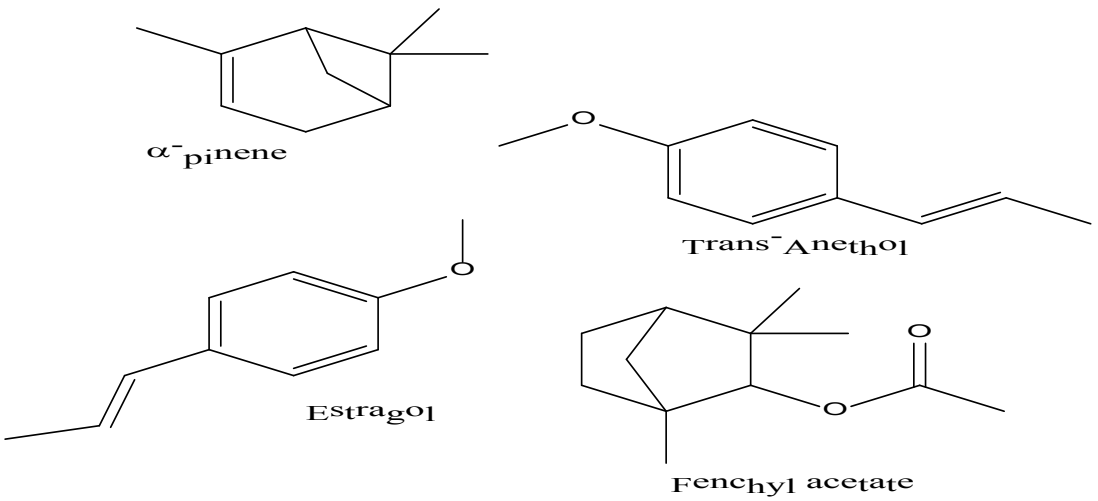


Figure 1: Chemical structures of main components of essential oils from fennel.

Antibiotics	*Escherichia coli*	*Pseudomons aeruginosa*	*Bacillus subtilis*
Amoxicillin (30 µg)	24.00	22.63	18.15
Erythromycin (15 µg)	20.33	21.27	27.44
Fluoxamine (15 µg)	33.33	23.11	38.37
Cephalexin (25 µg)	19.1	15.00	35.67

Table 4: Diameter of zone of inhibition (mm) of Antibiotics

Conclusion

This paper deals with the Environmental variation of the essential oil of fennel grown in the South West region of Iran. It should be noted that plants from this location are examined for the first time. The essential oils are made of a complex mix of chemical compounds that those are different in terms of chemical compounds. In fact, there are a variety of organic compounds such as hydrocarbons, alcohols, ketones, aldehydes, ethers, esters, oxides and other volatile oil and so on. The results show the main compound of essential oil of fennel was trans-anethol and extracts have the good antibacterial activity against all studied bacteria strains but have not any effect on *Aspergillus oryzae*. According to comparing the extract of fennel with antibiotics, we concluded the extract of fennel was not a good alternative for antibiotics.

Acknowledgments

I would like to thank Dr Alireza Salimi Beni, Dr Asad Masoumiasl for their extraordinary support in this thesis.

References

1. Alanis A (2005) Resistance to antibiotics: are we in the post-antibiotic era? Arch Med Res 36: 697-705.
2. Ayaz M, Subhan F, Ahmed J, Khan AU, Ullah F, et al. (2015) Sertraline enhances the activity of antimicrobial agents against pathogens of clinical relevance. J Biol Res (Thessaloniki) 22: 4.
3. Sharma D, Narasimhan B, Kumar P, Judge V, Narang N, et al. (2009) Synthesis, antimicrobial and antiviral evaluation of substituted imidazole derivatives. Eur J Med Chem 44: 2347-2353.
4. Livermore DM (2011) Discovery research: the scientific challenge of finding new antibiotics. J Antimicrob Chemother 66: 1941-1944.
5. Ayaz M, Subhan F, Ahmed J, Khan AU, Ullah F, et al. (2015) Citalopram and venlafaxine differentially augments antimicrobial properties of antibiotics. Acta Polon Pharm Drug Res 72: 1269-1278.
6. Pelczar M, Chan E, Krieg N (2006) Microbiology today. New York: McGraw Hill, pp: 298-433.
7. Bhaskarwar B, Itankar P, Fuluke A (2008) Evaluation of antimicrobial activity of medicinal plant Jatropha podagrica (Hook). Rom Biotechnol Lett 13: 3873-3877.
8. Piccaglia R, Marotti M (2001) Characterization of some Italian types of wild fennel (Foeniculum vulgare Mill). J Agric Food Chem 49: 239-244.
9. Díaz-Maroto MC, Pérez-Coello MS, Esteban J, Sanz J (2006) Comparison of the volatile composition of wild fennel samples (Foeniculum vulgare Mill.) from Central Spain. Journal of Agricultural and Food Chemistry 54: 6814-6818.
10. Rather MA, Dar BA, Sofi SN, Bhat BA, Qurishi MA (2012) Foeniculum vulgare: a comprehensive review of its traditional use, phytochemistry, pharmacology, and safety. Arabian Journal of Chemistry.
11. Telci I, Toncer OG, Sahbaz N (2006) Yield, essential oil content and composition of Coriandrum sativum cultivars (var. vulgare Alef. and var. microcarpum DC.) grown in two different locations. J Essent Oil Res 18: 189-193.
12. Bilia AR, Flamini G, Taglioli V, Morelli I, Vincieri FF (2002) GC–MS analysis of essential oil of some commercial Fennel teas. Food Chem 76: 307-310.
13. Barazani O, Cohen Y, Fait A, Diminshtein S, Dudai N, et al. (2002) Chemotypic differentiation in indigenous population of Foeniculum vulgare var. vulgare in Israel. Biochem Syst Ecol 30: 721-731.
14. Senatore F (2000) Oli essenziali. Provenienza, estrazione ed analisi chimica. Roma.
15. Araujo MEM (2009) Foeniculum vulgare: important biological activities of an aromatic plant. Recent Prog Med Plants 25: 21-39.
16. Figueredo G, Chalchat JC, Al Juhaimi FY, Özcan MM (2012) Effect of harvest years on chemical composition of essential oil of bitter fennel (Foeniculum vulgare subsp. piperitum) leaves. Asian J Chem 24: 2228-2230.
17. Mona Y, Kandil MAM, Hend MF, Swaefy MF (2008) Effect of three different compost level on fennel and Salvia growth character and their essential oil. Res J Agric Biol Sci 4: 34-39.
18. British Pharmacopeia (1988) HMSO. London, UK, pp: 137-138.
19. Duca E, Duca M (1979) Microbiologie Medicala Did. si Ped., Bucuresti, p: 56.
20. Prescott JF, Baggot JD, Walker RD (2000) Antimicrobial susceptibility testing and interpretation of results. In: Antimicrobial Therapy in Veterinary Medicine, Iowa State University Press, Ames, IA, USA.
21. Raman N, Sakthivel A, Rajasekaran K (2007) Synthesis and spectral characterization of antifungal sensitive Schiff base transition metal complexes. Mycobiology 35: 150-153.
22. Smibert RM, Kreig NR (1994) Phenotypic Characterization. In: Gerhardt P (ed.), Methods for General and Molecular Bacteriology. American Society for Microbiology, Washington DC, USA, pp: 607-654.
23. Viuda-Martos M, Mohamady MA, Fernández-López J, Abd ElRazik KA, Omer EA, et al. (2011) In vitro antioxidant and antibacterial activities of essentials oils obtained from Egyptian aromatic plants. Food Control 22: 1715-1722.
24. Telci I, Demirtas I, Sahin A (2009) Variation in plant properties and essential oil composition of sweet fennel (Foeniculum vulgare Mill.) fruits during stages of maturity. Industrial Crops and Products 30: 126-130.
25. Cakir A, Kordali S, Zengin H, Izumi S, Hirata T (2004) Composition and antifungal activity of essential oils isolated from Hypericum hyssopifolium and Hypericum heterophyllum. Flavour and Fragrance Journal 19: 62-68.